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Fifteenth Annual Report

The International Centre of
Insect Physiology and Ecology

Z. T. Dabaghi

1987

Fifteenth Annual Report



The International Centre of
Insect Physiology and Ecology

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Contents

Governing Council iii
1987 ICIPE Donors v
Foreword ix

Core Programmes

Crop Pests 3
Livestock Ticks 39
Medical Vectors 55
Tsetse 65

Research Support Units

Chemistry and Biochemistry 79
Histology and Fine Structure 97
Sensory Physiology 109
Biostatistics and Computer Services 119
Outreach and Training 123
Social Science Interface Research Project 133

Administration and Information 139
1987 Seminars Hosted by ICIPE 143
1987 Conferences Attended by ICIPE Staff 144
1987 Publications by ICIPE Staff 146
1987 Personnel 148

Foreword

The most important task in which the International Centre of Insect Physiology and Ecology (ICIPE) was engaged for the first 15 years of its functional existence was to build up ICIPE's base line for research excellence in (a) insect science; (b) scientific relevance to ICIPE's long-term goal of developing integrated pest management (IPM) technologies for the resource-poor communities in the tropical developing world; and (c) the education and training of a new-style scientific leadership in tropical insect science and pest management, through postgraduate training and postdoctoral enrichment programmes. By the end of 1986, we felt that in all three areas, the ICIPE had indeed arrived.

In that year, we underwent a thorough-going external review of the ICIPE's strategic plans, core research programmes and other associated programme activities, as well as the management style and substance of the ICIPE. In the same year we launched a major Task Force to review in-depth the strategic needs of insect science postgraduate training in Africa, and the special role that the ICIPE-inspired university consortium in this area — the so-called African Regional Postgraduate Programme in Insect Science (ARPPIS) — could best continue to play in this area as pace-setter and quality control mechanism for the 1990s. It was also in this year that the ICIPE invented the idea of the **in-depth research review by external assessors (IRREA)**, in which each restricted research discipline at the ICIPE undergoes an intensive external review by world renowned specialists in that discipline every three years or so, in order to assure us of the quality, scope and relevance of the particular programme activity, and to examine our strategic plans for the area in the succeeding medium-term period. We believe that this new idea is a powerful one, in terms of its possibilities for enhancement of our credibility.

Indeed, for R & D institutions such as the ICIPE, serving a demanding public such as the resource-poor rural tropical community, the question of credibility is all-important. For instance, will the seemingly sophisticated research that the ICIPE is engaged in really address the critical pest management issues that are the daily elements of the living burden of this rather neglected constituency? Do the pest management research issues of the tropics offer an intellectual challenge to the gifted scientist, easily lured to modern "big science" and "shiny technology"? Will the ICIPE turn out to be yet another technical assistance agency that will provide quick solutions to a few major insect problems and then shortly afterwards move its tents outside the tropics and insect science — leaving not a foot-mark on the sands of tropical time?

The building of credibility is a vitally important assignment for the ICIPE community — the members of the Governing Council, the ICIPE management, the ICIPE scientific community, and the friends of the ICIPE (the donors, the alumni and the host governments concerned).

It is our hope that the highlights of the work performed at the ICIPE during the course of the year 1987, recorded in this *Annual Report*, will help in strengthening the basis and articulation of this credibility. It was a most productive year, in terms of scientific research, technology development, and the dissemination of scientific information; and we feel a new excitement in the air — as we prepare to start our 18th year of scientific activity crucial to the development of new-style IPM of a sustainable nature.

THOMAS R. ODHIAMBO, *Director, ICIPE, Nairobi*

CROP PESTS RESEARCH PROGRAMME

Bionomics and Applied Ecology

- 1.1 Incidence of target insect pests and crop losses caused by them **4**
- 1.2 Pheromonal trapping and monitoring of the stem borers *C. partellus* and *B. fusca* **5**
- 1.3 Factors governing diapause termination in *B. fusca* **6**
- 1.4 Effects of different proportions of sorghum/cowpea intercrop rows on crop borer incidence **6**
- 1.5 Effect of intercropping sorghum and cowpea on the population patterns of the stem/pod borer complex **7**
- 1.6 Genetics of *Chilo partellus*: studies on its chromosomes **7**

Plant Resistance to Insect Pests

- 1.7 Sorghum resistance to stem borers **8**
- 1.8 Maize resistance to stem borers **10**
- 1.9 Effect of maize cultivar and planting density on *C. partellus* oviposition in the field **11**
- 1.10 Cowpea resistance to aphids **12**
- 1.11 Bases of resistance of cowpea to the pod borer *M. testulalis* **13**
- 1.12 Genetics of plant resistance to insect pests **13**

Biological Control

- 1.13 Parasitoids for the bio-control of stem borers **15**
- 1.14 Behavioural studies on *Dentichasmias busseolae* **16**
- 1.15 Role of parasitoids in natural populations of *M. testulalis* **17**
- 1.16 Effect of solar radiation on *Nosema* sp. sprayed on sorghum **17**
- 1.17 Evaluation of bacterial pathogens as pest control agents **18**

Insect Mass-Rearing Technology

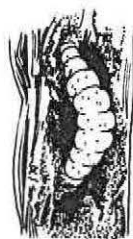
- 1.18 Rearing target insects and their natural enemies **20**

ICIPE-IRRI Research Project

- 1.19 Project on rice leafhopper and rice brown planthopper **21**

ICIPE-IITA Research Project

- 1.20 Programme of cowpea improvement **24**



1

Crop Pests Research Programme

The main goal of the Crop Pests Research Programme (CPRP) is to develop for resource-poor small-scale farmers in developing tropical countries strategies for managing key insect pests of major food crops. The pest management strategies being developed in CPRP are environmentally safe and technically as well as economically feasible for these farmers. Adoption of such pest management strategies will reduce food losses caused by various insect pests and thus increase food production.

To achieve its goal, CPRP is conducting research in four sections: (1) Bionomics and Applied Ecology (BAE), (2) Plant Resistance to Insect Pests (PRIP), (3) Biological Control (BC) and (4) Insect Mass-Rearing Technology (IMRT).

*The crops CPRP is studying include sorghum, maize, cowpea, banana, cassava and rice. The pests of these crops under study include the stem borers of sorghum and maize, (*Chilo partellus*, *Busseola fusca*, *Eldana saccharina* and *Sesamia calamistis*), the sorghum shootfly (*Atherigona soccata*), the cowpea pod borer (*Maruca testulalis*), the aphid (*Aphis craccivora*), the banana weevil (*Cosmopolites sordidus*), the cassava green spider mite (*Mononychellus spp.*), the rice brown planthopper (*Nilaparvata lugens*) and the rice leafhoppers (*Cnaphalocrocis medinalis* and *Marasmia patnalis*).*

The research on the pests of sorghum, maize, cowpea, banana and cassava is being carried out at ICIPE's Mbita Point Field Station, located on the shores of Lake Victoria, in western Kenya. Work on rice pests is being done by the ICIPE team based at the International Rice Research Institute (IRRI), in the Philippines, under an ICIPE-IRRI Collaborative Project. Similarly, work on cowpea-based farming systems entomology is being carried out by an ICIPE team based at the International Institute of Tropical Agriculture, in Ibadan, Nigeria.

The components of pest management being developed in CPRP belong to four main categories: (1) manipulating cultural practices, including (a) intercropping host-nonhost or inter-varietal crop combinations to reduce pest attack, (b) adjusting planting time in relation to pest attack and (c) disposing of crop residues; (2) using plant resistance to protect crops from pest attack; (3) using natural enemies (parasitoids, predators, pathogens) of the pests to control the pests biologically; and (4) manipulating pest behaviour for indirect pest control.

Work on the above in the CPRP sections during the year has involved both elaborating on technology and conducting pilot trials on farmers fields under their management. The progress made in these areas is briefly given below.

BIONOMICS AND APPLIED ECOLOGY

To generate basic and relevant information essential for the development of pest management strategies, the Bionomics and Applied Ecology Section (BAE) has been investigating the distribution, population biology, development, reproduction and behaviour of crop borers.

The other research activities of BAE include the design and development of methodologies for assessing on-farm yield losses caused by the borers; development of pheromone-based trapping methods for monitoring borer populations; manipulation of the behavioural responses of the borers; use of intercropping and other cultural practices, such as disposal of crop residues and adjustment

of planting dates; and use of genetic methods as a means to suppress pest populations and reduce consequent yield losses.

1.1 INCIDENCE OF TARGET INSECT PESTS AND CROP LOSSES CAUSED BY THEM

K. V. Seshu Reddy

1.1.1 Population patterns of stem borers of sorghum and maize

Studies on the incidence and infestation levels of the stem borers of sorghum and maize continued during the long rains of 1987 at Mbita Point Field Station (MPFS), as well as at Ungoye, a new field research site. At both locations the incidence of stem borers on sorghum started 3 weeks after crop emergence (WAE), whereas that on maize started 3 WAE at MPFS and 5 WAE at Ungoye. In general there was a gradual increase in the percentage of plants infested by the borers as the crops grew, reaching a peak at harvest.

Among all the stem borers, *Chilo partellus* was the first to infest both sorghum and maize crops, followed by *Busseola fusca* at MPFS and Ungoye. However, at MPFS *Eldana saccharina* infested maize 7 WAE and sorghum 9 WAE. All these stem borers continued infesting both crops until harvest.

The incidence and density of stem borers of sorghum and maize at MPFS and Ungoye are given in Table 1.1. The mean percentage of the incidence of stem borers on sorghum was 56.8 and 46.8, and that on maize 41.3 and 11.4, at MPFS and Ungoye, respectively. It is evident that the stem borer *C. partellus* is predominant at MPFS on both sorghum and maize, whereas *B. fusca* is important at Ungoye. However, very low populations of *E. saccharina* and *Sesamia calamistis* were recorded on sorghum and maize only at MPFS.

1.1.2 Distribution and infestation of banana weevil

In 1987 the banana and its key borer pest, *Cosmopolites sordidus* (Germ.), were added to the CPRP list of target crops and their pests. Preliminary surveys conducted in South Nyanza District, western Kenya, showed that the borer *C. sordidus* is widely distributed and is a major problem on bananas grown by subsistence farmers. The

percentage of plants infested by the borer ranged from 44 to 88 and the mean number of adults and grubs per plant ranged from 1 to 6 and 1 to 5.5, respectively. During the survey, in addition to *C. sordidus*, two other species—*Temnoschoita nigroplagiata* Qued. and an unidentified weevil—were also found damaging the banana plants.

1.1.3 Grain yield losses in cowpea due to insect pests

Grain yield losses in sorghum and maize caused by their stem borers and the methodology standardized for their assessment have been presented in previous ICIPE annual reports. In 1987 the grain yield losses in cowpea caused by its insect pests were assessed. The cultivar chosen was ICV 2 and the pests studied were the aphid *Aphis craccivora* and the pod borer *Maruca testulalis*.

These studies were conducted at MPFS for two seasons, the short rains of 1986/1987 and long rains of 1987. The experiment was arranged in a Completely Randomized Block Design (CRBD), with three replications. Each experiment involved the following five treatments: (1) artificial infestation of cowpea plants within nylon net cages with 10 aphids per plant 10 days after plant emergence (DAE); (2) artificial infestation of cowpea plants within nylon net cages with 10 neonate pod-borer larvae per plant 20 DAE; (3) natural infestation with different pests occurring in the test area; (4) chemical protection of plants by the application of Furadan granules at planting, and thereafter weekly sprays of Dimethoate for eight weeks; and (5) mechanical protection of plants from pests by growing the plants in cages. At harvest, the grain yields from all these treatments were compared.

The results (Table 1.2) show that there were significant variations in grain yield per plot between seasons and among treatments, higher yields being obtained during the long rains. In general, the control (caged) plots registered maximum grain yields, which differed significantly from all the other treatments except the plot protected with insecticides during the long rains of 1987.

Aphid-infested plants had the lowest yields, about 2.5 g per plot, an equivalent of 4.2 kg per ha during the short rains. The aphid attack on cowpea resulted in very high grain yield loss if the plants were as young as 10 days, with a population of 10 or more aphids per plant. An average of 92.2% grain yield loss occurred because of aphid infestation, compared to 26.2% due to the pod borer *M. testulalis* when infested 20

Table 1.1 Incidence and density of stem borer populations in sorghum and maize at Mbita Point Field Station (MPFS) and Ungoye, long rains, 1987

	MPFS		Ungoye	
	Sorghum	Maize	Sorghum	Maize
Incidence of borers (%)				
Range	0-100	0-96	0-80	0-30
Mean	56.8 ± 8.68	41.3 ± 0.26	46.8 ± 0.25	11.4 ± 0.26
Stem-borer population (larvae and pupae)/10 plants				
<i>Chilo partellus</i>	5.3	3.8	2.5	0.88
<i>Busseola fusca</i>	1.2	0.25	4.0	0.93
<i>Eldana saccharina</i>	0.56	0.72	0	0
<i>Sesamia calamistis</i>	0.03	0.03	0	0
Total	7.09	4.8	6.5	1.81

Table 1.2 Cowpea grain yields and yield losses caused by insect pests at Mbita Point Field Station

Treatment	Short rains 1986		Long rains 1987	
	Mean seed wt. (g)/plot	% grain yield loss	Mean seed wt. (g)/plot	% grain yield loss
Aphids: 10/plant at 10 DAE*	2.50 ^c	99.60	177.30 ^c	84.80
Maruca: 10/plant at 20 DAE*	480.00 ^{ab}	26.87	871.50 ^{ab}	25.51
Natural infestation by all pests	330.50 ^b	49.60	644.07 ^b	44.90
Complete insecticidal protection	416.63 ^b	36.50	1143.0 ^a	2.30
Control (caged)	656.27 ^a	0	1169.9 ^a	0

Within a column, mean values bearing the same superscript letters are not significantly different at the 5% level by Duncan's multiple range test.

* DAE: days after emergence.

DAE, and 47.3% due to natural infestation by various insect pests. Even on the plots where insecticide was applied at weekly intervals, there was an average yield loss of 19.4%.

Therefore, the grain yield losses in cowpea, as in any other crop, depend on the density of the pest population present at various crop growth stages and on the phenological stage of the plant when infestation occurs.

1.2 PHEROMONAL TRAPPING AND MONITORING OF THE STEM BORERS

C. PARTELLUS AND *B. FUSCA*

G. C. Unnithan and K. N. Saxena

Investigations aimed at developing pheromonal trapping methods for monitoring and suppressing the populations of the cereal stem borers *C. partellus* and *B. fusca* were continued during the year. Information on a few factors influencing the efficiency of certain traps was given in the 1986 ICIPE Annual Report. Additional factors were studied in 1987 and the populations of the males of these species were monitored during the year.

1.2.1 *Chilo partellus*

Studies on certain factors that influence the performance of pheromone traps for *C. partellus* were presented in the 1986 ICIPE Annual Report. Further investigations were undertaken on the effects of the trap surface area, the location of the pheromone trap in relation to the crop, the relationships among the density of males and trap catches, the attraction range of the traps and the fluctuations in the male populations at the study sites. In all the experiments reported on here, two virgin one-day-old females were used as the pheromonal source in the trap.

The number of *C. partellus* males caught in water traps was not affected by the surface area of the trap since the catches in the traps with 700 sq cm and 2800 sq cm were not significantly different (2.6 ± 0.4 and 2.2 ± 0.3 , respectively). The pheromone trap catches were influenced by the location of the traps in relation to the crop and the direction of the wind. The numbers of males trapped by the water traps, one each placed to the east, north, west and south of a crop plot at

about 2-m distance, were 18.2, 9.9, 6.0 and 6.5, respectively. In the trap placed within the crop plot, the number of males caught was 6.9. At the field site where these experiments were conducted, the wind blows east to west during the early morning hours; this appeared to be the reason for catching a significantly higher number of males in the trap placed east of the crop. The flight activity of *C. partellus* males, in response to the female pheromone, started only after 0100 hr and reached a peak between 0200 and 0300 hr. More than 90% of the total number of males in the pheromone traps were caught between 0100 and 0400 hr.

Experiments in the screenhouse involving the release of different numbers (10, 20, 30, 40 and 50) of marked males and their recapture in the pheromone traps revealed a positive correlation between the number released and the number recaptured ($r = 0.853$). However, the proportions of the males recaptured remained more or less the same, an average of about 20%.

To determine the effective range of the pheromone trap, marked males were released at a distance of 5, 10, 20 and 40 m from the pheromone trap. The proportion of the males recaptured when released 5 m from the trap was significantly higher than that of those recaptured when released 20 or 40 m from the trap (Table 1.3), indicating that the trap may be used for monitoring males in small plots.

Table 1.3 Relation between distance from the pheromone trap to site of release and the number of released *C. partellus* males recaptured

Distance to release site (m)	No. released	% recaptured (mean \pm SD*)
5	10	20.00 \pm 5.35 ^a
10	10	13.75 \pm 7.44 ^{ab}
20	10	10.00 \pm 7.56 ^b
40	10	10.00 \pm 7.56 ^b

In the last column, mean values bearing the same superscript letters are not significantly different from each other.

* SD: standard deviation.

Using water and sticky traps baited with virgin females, the fluctuations of *C. partellus* males at MPFS and at the

farmer's field in Lambwe (Ogongo) were monitored. This information will be used for timing the evaluation of synthetic pheromone when it becomes available and for determining if there is any relation between the pheromone trap catches and the lunar phase, temperature, etc. During the 1987 long rainy season at MPFS, the male populations showed two peaks, about eight weeks apart, indicating that the borer completes two generations during the cropping season. However, males were caught in low numbers throughout the season. In the farmer's field also, two peaks of emergence of males, about eight weeks apart, were noticed.

1.2.2 *Busseola fusca*

The relative efficiency and persistence of *B. fusca* synthetic pheromone (supplied by the Overseas Development and Natural Resources Institute, UK) and virgin females in attracting and trapping males were examined using water traps, and the fluctuations in the adult populations in a farmer's field during the long rainy season were monitored using funnel-type traps with the synthetic pheromone lure.

B. fusca synthetic pheromone, dispensed from polythene vials, was effective in attracting males. However, for the initial 10-day period, the traps with a single 1-day-old virgin female as lure attracted significantly more males than those with 1 and 5 mg synthetic pheromone as lures. For the subsequent 15-day period, the catches in the virgin-female traps and the synthetic-pheromone (5-mg) traps were not significantly different. Polythene vials loaded with 5 mg of synthetic pheromone remained attractive for the entire test period of 30 days.

The efficiency of different loadings of the pheromone in attracting the males was also determined. The doses tested (1, 2 and 5 mg) for one month did not differ significantly in their attractancy. Catches of males (mean \pm the standard error) in the traps lured with 1, 2 and 5 mg of synthetic pheromones were 4.5 ± 0.5 , 4.5 ± 0.5 and 6.4 ± 0.9 , respectively.

Fluctuations of *B. fusca* male populations in a sorghum field during the long rainy season showed two peaks, a major peak about 12 weeks after the crop emerged and a minor peak about 7 to 8 weeks later. The emergence of the first generation *B. fusca* seemed to last about 4 weeks. Most of the second generation *B. fusca* larvae go into facultative diapause, and the minor peak of male catch seems to represent the emergence of adults that have completed development without an intervening diapause.

1.3 FACTORS GOVERNING DIAPAUSE TERMINATION IN *B. FUSCA*

T. Okuda

The maize stem borer *Busseola fusca* undergoes diapause during the off-season (July/August–February/March) as last-instar larva for about 6 to 8 months in dry sorghum and maize stems and stubbles. Factors determining diapause termination in this insect were investigated during the year.

During the early phase of diapause (3 to 4 months), none of the environmental factors tested, such as moisture and temperature, could terminate the diapause. In late diapause, the larvae became sensitive to water and pupated after

artificial wetting. Water contact seemed to be an important factor for the termination of late diapause, since neither drinking water nor feeding on fresh sorghum stems as young as 6 weeks induced the diapause termination.

Diapausing larvae collected from the field during the 1987 short rainy season (November–December) pupated under artificial wetting in the laboratory. However, the incidence of pupation in the field was as low as that in dry conditions in the laboratory, in spite of exposure to precipitations over 10 mm. To interpret the apparently contradictory data, late diapausing larvae were introduced into wet conditions for 1, 3, 5, 7 and 9 days. These larvae were then transferred to dry conditions and the incidence of pupation observed. Larvae not exposed to water served as the control. Exposure to water for 1 and 3 days had no apparent effect on the larvae. The incidence of pupation was slightly higher (25% of larvae pupated) after 5 days exposure to water. The highest level of pupation was achieved after 7 to 9 days continuous exposure to water. A characteristic of the short rainy season is that rain usually falls for a short period during the night. With sunshine and high temperatures during the day, the atmosphere and soil do not maintain a high moisture content, as they do in the long rainy season. We thus concluded that during the short rainy season diapausing larvae in the field are not exposed to enough moisture (for more than one week) to pupate. Only in the long rainy season are conditions sufficiently and continuously moist to induce diapause termination in *B. fusca*.

Chilling late diapause larvae at -10° C for 5 minutes also accelerated diapause termination and to such an extent that 100% of the treated larvae pupated, as compared with 40% in the control.

1.4 EFFECTS OF DIFFERENT PROPORTIONS OF SORGHUM/COWPEA INTERCROP ROWS ON CROP BORER INCIDENCE

K. V. Seshu Reddy and B. S. K. Masyanga

Intercropping sorghum or maize with cowpea or varieties of maize with varieties of sorghum has been reported to reduce attacks of crop borers on these crops. During the 1987 long rainy season, we examined the effects of planting the two crops in different proportions.

This study was conducted on a farmer's field located near Mbita Point Field Station (MPFS) and involved 8 treatments. Of these, 6 were intercrop treatments (sorghum:cowpea ratios [in rows] 1:1, 2:2, 1:2, 2:1, 3:1 and 1:3) and 2 were the monocrops of sorghum and cowpea. Observations on the incidence of crop borers were taken at 2-week intervals.

In all treatments involving sorghum, the damage by the stem borer *C. partellus* started 2 weeks after crop emergence (WAE) and that of *B. fusca* 10 WAE. Another stem borer, *E. saccharina*, was observed only 16 WAE. The pod borer *Maruca testulalis* attacked the cowpea crop 6 WAE. In general the number of sorghum stem-borer larvae increased with the plants age. The mean number of *C. partellus* larvae plus pupae per sample increased from 0.91 at 2 WAE to 6.9 at 16 WAE, while *B. fusca* rose from 1.48 to 3.04 at 16 WAE.

However, in the case of *Maruca*, the number of larvae per sample decreased by about 69% from 11.1 to 3.4 between 6 and 8 WAE.

The proportion of 3 rows of sorghum to 1 row of cowpea was most effective in reducing the damage by *C. partellus* on sorghum: damage was reduced by 32%. Alternating 2 rows of sorghum with 2 rows of cowpea reduced *B. fusca* damage by 20% compared to the monocrop of sorghum. Damage caused by the pod borer *M. testulalis* was reduced by 46% in a 1:3 sorghum/cowpea ratio compared to the cowpea monocrop. These studies show that reduced levels of attack of crop borers on intercrops of sorghum and cowpea depend on the ratio between the rows of the two crops.

1.5 EFFECT OF INTERCROPPING SORGHUM AND COWPEA ON THE POPULATION PATTERNS OF THE STEM/POD BORER COMPLEX, WITH SPECIAL EMPHASIS ON *C. PARTELLUS*

E. Minja

Another aspect of intercropping that BAE investigated was the effect of planting sorghum and cowpea at different times. This aspect was studied at the Mbita Point Field Station and on a farmer's field on Rusinga Island. The sorghum cultivar Serena and the cowpea cultivar Ex-Luanda were grown as intercrops in three patterns: sorghum sown simultaneously with cowpea, sorghum sown two weeks before cowpea and sorghum sown two weeks after cowpea. Eggs and larval and pupal populations were monitored regularly, with special attention to *C. partellus*.

The data from the experiments show that intercropping reduced the population build-up of the borers in the early stages of plant growth and that at the end of the season the sorghum monocrop had a significantly ($P < 0.05$) higher number of stem-borer larvae than the sorghum sown simultaneously with cowpea (Table 1.4).

A lower rate of population build-up occurred when the two crops were planted at different times than when they were planted simultaneously but by harvest the infestation level was almost identical. Future experiments will examine the possible cause of reduction of stem/pod borers in the sorghum/cowpea intercrops compared to their monocrops.

1.6 GENETICS OF *CHILO PARTELLUS*: STUDIES ON ITS CHROMOSOMES

V. A. O. Okoth

Genetic research on *Chilo partellus* at CPRP aims to generate information for the use of F1 sterility in the control of this pest. F1 (or inherited) sterility is a genetic method that has been deployed for the control of a number of lepidopteran pests. The radiation doses used for inducing F1 sterility are so adjusted that when the released males and females interbreed, no progeny are produced, but when they outcross with insects in nature, the egg-hatch is decreased and the progeny produced are semi-sterile or sterile. Our initial study of the genetics of *C. partellus* was focused on karyotyping, first because the display of a full chromosome complement in the insect would permit rapid recognition of any aberration in chromosome number or morphology due to irradiation; and second because a knowledge of basic chromosome number was considered important in monitoring sterile insect releases in the field.

The chromosome analysis was undertaken using the squash technique. Various tissues of *C. partellus*—from the brain, salivary glands, fat bodies and gonads (testes and ovaries)—were fixed in a mixture of glacial acetic acid and 95% ethanol (1:3). Each fixed tissue was then immersed in 1 M HCl acid for 5 minutes to dissociate its cells, stained with a drop of 2% lacto-aceto-orcein (synthetic), and then squashed under a coverslip on a microscope glass slide. The preparations were examined under a Leitz Dialux 20 EB microscope and photographed, when necessary, with an automatic camera.

The results showed that dividing testicular cells were most suitable for chromosome analysis in *C. partellus*. Chromosomes were easily counted at metaphase of the first maturation division in the spermatocyte of the fifth-instar male larvae and pupae. Haploid chromosome number (n) in the male was found to be 21. In the fifth-instar larvae, the testes contained spermatocytes, including metaphase I cells. Spermatocyte formation continued in the pupae, where all stages of primary and secondary spermatocytes, spermatids and maturing spermatozoa were seen.

These investigations revealed the following facts. (1) Meiosis in the male *C. partellus* is essentially completed at the time of adult eclosion. (2) Larvae in the fourth or earlier

Table 1.4 Effect of the time of planting sorghum and cowpea on the sorghum stem borer larval population (average of three replicates in selected weeks) 1986/87

Sorghum cropping pattern	Mean number of stem borer larvae per plant (Weeks after sorghum plant emergence)								Mean of weeks 3—16 inclusive
	3	4	5	6	13	14	15	16	
Sorghum monocrop	1.07	1.67	1.67	1.27	2.73	4.53	5.97	4.60	2.54 ^a
Simultaneously	1.26	0.60	0.93	0.87	2.13	1.67	4.73	2.67	1.73 ^b
14 days before cowpea	0.27	0.40	1.60	1.60	3.13	3.30	4.80	2.87	1.97 ^{ab}
14 days after cowpea	0	0.20	0.20	2.13	3.80	3.00	4.20	3.00	1.94 ^{ab}

In the last column, mean values bearing the same superscript letters are not significantly different ($P > 0.05$).

instars should be used for affecting spermatogonia through male irradiation. (3) Young fifth-instar larvae are to be irradiated for affecting early spermatocytes. (4) Young to mature pupae are to be irradiated to affect all stages of spermatocytes, spermatids and spermatozoa. (5) The adults are to be treated to affect mature sperms. (6) Male fifth-instar larvae and male pupae are probable candidates for induction of F1 sterility in *C. partellus* through irradiation.

Our future work will examine the doses of radiation and development stages (pupa or larva) that are appropriate for induction of F1 sterility. We will also study the competitiveness of irradiated and non-irradiated male *C. partellus* caged with non-irradiated females in the laboratory, greenhouse and field.

PLANT RESISTANCE TO INSECT PESTS

The main goal of the Plant Resistance to Insect Pests (PRIP) Section is to develop strategies for effective use of insect resistance in sorghum, maize and cowpea as a component of the management of their insect pests, particularly borers. To achieve this goal, studies have been made in the following broad areas.

1. Evaluation of levels of resistance and its components in diverse germplasm material or cultivars to the target pests. *This study has been undertaken with three objectives: (1) to identify germplasm/cultivars that are resistant to the pests and that have other desirable characters, including good yield, so that they can be cultivated; (2) to identify cultivars that have a high level of pest resistance but lack other desirable characters so that they can serve as sources for developing pest-resistant varieties; (3) to provide a broad-spectrum pool of material with diverse resistance levels and components so as to select appropriate ones for developing varieties that would combine pest resistance with other desirable characters.*

2. Elucidation of mechanisms of resistance to crop borers. *This aspect has been studied to identify factors that impart resistance or susceptibility to different germplasm material/cultivars against the stem borers. The factors studied include: (1) the colonizing responses of the insects to plants and (2) plant characters (physical, chemical) that determine these responses. Information on these factors*

would assist the plant breeders in incorporating resistance characters with other desirable characters in the varieties that are to be developed.

3. Genetics of resistance. *This study is yielding information on the ways crop cultivars inherit resistance components to the pests. This information, together with that on the factors that impart resistance, would also assist in planning breeding strategies for development of varieties with desirable characters.*

1.7 SORGHUM RESISTANCE TO STEM BORERS

K. N. Saxena

1.7.1 Evaluation for resistance/susceptibility

The evaluation of various crop cultivars for resistance to borers is undertaken by the PRIP Section in two main stages. The first stage involves tests on cultivars planted in single-row replicated plots and infested with the borers naturally or artificially. The second stage involves tests on cultivars planted in multiple-row replicated plots and infested naturally or artificially. These evaluations are being carried out first at ICIPE's Mbita Point Field Station, and then, with reference to certain promising lines, on farmers' fields and national agricultural research stations in various ecological zones.

The methods and parameters developed or standardized in the project and the borer resistance levels in several sorghum cultivars, as evaluated in the first and second stages at the field station, have been given in previous annual reports. On the basis of these tests, a few sorghum lines were taken up for multi-locational evaluation on farmers' fields and at national agricultural stations as described below.

Multi-locational evaluation. Ten sorghum lines, listed in Table 1.5, were taken for these tests. Of these lines, IS 18520 (Serena) served as the tolerant check for comparison with other lines. Among the others, IS 1044, IS 4405 and DKV 2 were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), IS 1044 having been consistently most resistant to the stem borers in previous tests. The accessions LRA 53 and LRA 59, obtained from collections from the Semi-Arid Food Grain Research and Development Consultative Advisory Committee (SAFGRAD), showed moderate resistance as well as good

Table 1.5 Ratio of the number of larvae plus pupae per 10 plants for a test cultivar to that for the check IS 18520 at harvest at different locations

Sorghum line	MPFS	Lambwe	Ungoye	Busia	Katumani	Kampi ya Mawe
IS 18520	1.00 (24.0 ± 6.8*)	1.00 (13.3 ± 1.6*)	1.00 (10.3 ± 1.8*)	1.00 (6.0 ± 1.5*)	1.00 (8.7 ± 3.3*)	1.00 (4.3 ± 2.0*)
IS 1044	0.72	0.91	0.01	0.47	0.49	1.86
DKV 2	1.17	1.04	0.97	2.13	1.69	2.56
86LRA 53	1.83	1.62	1.04	1.42	0.64	1.40
Mb 1	1.44	1.02	1.43	1.60	0.89	1.47
Mb 3	1.26	1.50	1.62	1.98	0.72	1.48
Mb 6	1.50	1.87	2.14	2.42	1.30	1.56
Mb 11	1.74	1.96	1.61	2.60	1.38	1.00

* Number (mean ± standard error) of larvae plus pupae per 10 plants of IS 18520 is given within parentheses as the reference check.

yield and other desirable characters. Mb 1, 3, 6 and 11, obtained from the farmers in the Mbita Division of western Kenya, showed tolerance to the borers and good yields as well as resistance to birds. SABINA, 1576 and 2KX-17 were obtained from the National Dryland Farming Research Station, Katumani (Machakos).

These sorghum lines were evaluated at the following locations.

- ICIPE's Mbita Point Field Station (MPFS), located in western Kenya on the shores of Lake Victoria, at an altitude of about 1170 m, and with semi-arid warm conditions and one regular rainy season, the annual rainfall being 950 mm.
- Ungoye field site of the MPFS, about 35 km from Mbita and having a climate similar to that at Mbita.
- A farmer's field at Lambwe (Ogongo), about 20 km from Mbita and at almost the same altitude as that at Mbita but with two regular rainy seasons.
- National Agricultural Research Sub-Station at Alupe (Busia), about 300 km from MPFS, at an altitude of 1220 m and with two rainy seasons, the annual rainfall being 1191 mm.
- National Dryland Farming Research Station at Katumani (Machakos), about 70 km from Nairobi, at an altitude of 1601 m, with two rainy seasons but almost semi-arid conditions, the annual rainfall being 726 mm.

At each of the above locations, the test cultivars were planted in multiple-row plots (10 × 10 m plots at Lambwe and 5 × 5 m plots at other locations). Ten cultivars were planted at MPFS, but at other locations one or the other cultivar was omitted or added, as shown in Tables 1.5 to 1.7.

Infestation of the plants was allowed to occur from natural populations of the insect so that information could be gathered not only on the resistance of the cultivars but also on the population levels of the pests at the test locations. The parameters measured were those described in previous annual reports: population density of the larvae and pupae of stem borers in the plants, foliar damage and stem tunnelling at different developmental stages of the crop, including harvest and yield of grain per plant.

The results (Tables 1.5 to 1.7) show that the species of stem borers, the population density of their larvae and pupae, the levels of damage caused by them and the grain yield differed from one cultivar to another at each location as well as from one location to another for certain cultivars.

1.7.2 Mechanisms of resistance/susceptibility in sorghum to stem borers

This aspect has been under study with reference to the resistance of the following sorghum cultivars to *C. partellus*: (1) the susceptible IS 18363, IS 18463 and IS 2146; (2) the tolerant check IS 18520 (Serena); and (3) the resistant IS 4660, IS 2205 and IS 1044, the last one being most resistant. Work has shown that differences in the insects' colonization responses to the above cultivars can contribute in varying degrees to the resistance or susceptibility of the cultivars: (1) oviposition, (2) larval orientation (arrest, attraction), (3) feeding, (4) food utilization and nutrition and (5) development. Low levels of most of these responses to the sorghum IS 1044 render it most resistant and high levels of responses to IS 18363 render it most susceptible, with the resistance of IS 18520 falling between these two cultivars.

Table 1.6 Ratio of the percentage of stem tunnelled for a test cultivar to that for the check IS 18520 at harvest at different locations

Sorghum line	MPFS	Lambwe	Ungoye	Busia	Katumani	Kampi ya Mawe
IS 18520	1.00 (52.9 ± 3.9*)	1.00 (18.4 ± 3.2*)	1.00 (30.0 ± 5.0*)	1.00 (9.2 ± 2.6*)	1.00 (7.4 ± 4.5*)	1.00 (15.9 ± 2.8*)
IS 1044	0.18	0.48	0.43	0.17	0.39	1.67
DKV 2	0.85	1.10	0.86	1.49	1.18	1.03
86LRA 53	0.73	1.38	0.64	1.62	0.65	0.67
Mb 1	0.92	0.65	0.87	1.39	0.89	0.97
Mb 3	0.61	1.14	1.04	1.52	0.74	1.07
Mb 6	0.46	0.89	0.86	1.15	0.91	0.86
Mb 11	0.64	0.83	0.62	1.33	0.99	0.96

* Mean values of the percentage of stem length tunnelled in IS 18520 are given within parentheses to serve as the reference check.

Table 1.7 Ratio of grain yield of a test cultivar to that of IS 18520 (check) at different locations

Sorghum line	MPFS*	Lambwe	Ungoye	Busia	Kampi ya Mawe
IS 18520	1.00 (68.6 ± 0.6†)	1.00 (81.0 ± 3.5†)	1.00 (60.0 ± 2.8†)	1.00 (86.9 ± 2.8†)	1.00 (8.4 ± 8.4†)
IS 1044	1.21	0.82	0	0.49	2.24
DKV 2	1.40	1.33	0	0.99	2.25
86LRA 53	1.40	1.47	0	0.91	1.85
Mb 1	1.15	1.13	1.18	0.98	4.13
Mb 3	1.33	1.15	1.68	1.12	4.95
Mb 6	1.40	1.27	0.89	1.03	3.76
Mb 11	1.33	1.30	1.24	0.64	2.36

* All plants were covered by a 3.5-m-high fish net (28-mm mesh) to protect the crop from birds but allow insects to pass through.
† Actual grain yield (mean ± standard error) for IS 18520 is shown within parentheses to serve as the reference check.

The role of larval orientation and feeding, as presented in previous annual reports, was with reference to the first-instar larvae, which feed on leaves within the whorl. The role of the same behaviour of third- or fourth-instar larvae, which come out of the whorl and bore into the stem to feed on its tissues, was examined in 1987. The cultivars for which these studies were undertaken included the highly susceptible IS 18363, the tolerant IS 18520 and the highly resistant IS 1044. The results are summarized below.

Larval 'arrest' of fourth-instar larvae on different sorghum cultivars. The larvae feeding and developing within the leaf whorl of sorghum plants move out of the whorl in late third or early fourth instar. The proportion of those remaining on a plant would reflect its suitability for larval arrest, whereas its unsuitability in this respect would be reflected in the proportion of the larvae leaving the plant. A low level of arrest of the larvae on a cultivar would contribute to its resistance while a high level of this response would contribute to the cultivar's susceptibility. A comparison of this response among the three cultivars showed that the larval arrest on IS 18520 was quite high (70–73.3%) but very low (30%) on the resistant IS 1044. Such a low suitability of the latter cultivar for retaining the larvae from the fourth instar upwards would contribute to its resistance against *C. partellus*.

Larval attraction in fourth-instar larvae to different sorghum cultivars. The fourth-instar larvae that leave a plant may arrive on other plants, their arrival being determined by their attraction to the plants. To understand whether or not the sorghum cultivars differ from one another in their attractancy for the larvae, two series of experiments were undertaken, one in the laboratory and the other in the field.

The laboratory tests were conducted on a grid (35 × 25 cm) of wire (1 mm in diameter) by a previously published method (Saxena, K. N., Khattar, P. and Goel, S., *Experientia*, 1977). Such tests with the leaves of the three target sorghum cultivars showed that the larval attraction to the leaves of all these plants was almost equally high when each was offered against a blank control in a no-choice situation.

The larvae were attracted to the leaves of the susceptible IS 18363 and tolerant IS 18520 cultivar much more than to their visual stimuli, whereas the larval response to the resistant IS 1044 leaves was almost the same as that to its visual stimuli. The larvae were thus attracted to the susceptible plants in response to their volatiles more than to their visual stimuli. The dried leaves of the three cultivars had as low an attractancy for the larvae as had the visual stimuli, suggesting the loss of the volatile attractants on drying.

Unlike the leaves, the stems of the three cultivars differed in their attractancy, the larval attraction to IS 1044 being much less than that to the other two. Thus, the lower attractancy of IS 1044 stems compared to IS 18363 or IS 18520 stems for fourth-instar larvae would contribute to its relatively higher resistance to the stem borer.

1.8 MAIZE RESISTANCE TO STEM BORERS

J. K. O. Ampofo

Maize cultivars from different sources have been under study for both the levels and the mechanisms of resistance to stem

borers. The results of our evaluations of maize lines and information on certain mechanisms, particularly colonization responses of the stem borer *C. partellus*, have been presented in previous annual reports. In 1987 further work on these aspects was undertaken, as briefly presented below.

1.8.1 Evaluation of maize cultivars for development of multiple-borer resistance

From July to December 1985, 105 maize accessions were received from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico, and evaluated for resistance to *C. partellus* at Mbita Point Field Station. Seeds from the resistant selections were sent back to CIMMYT for incorporation into a multiple-borer resistance pool. Selections from this pool were received by ICIPE in 1987 for testing against *C. partellus* at Mbita.

From this pool, 199 accessions were planted in single-row plots and artificially infested at the whorl stage 3 to 4 weeks after plant emergence (WAE). The plants were rated for: (1) foliar damage (on a 1–9 scale) prior to harvest, (2) incidence of streak disease, (3) days to flowering, (4) plant height, (5) height of the first ear, (6) root lodging and (7) lodging due to borer attacks.

The data on the above parameters are summarized in Table 1.8. Of the accessions tested, 37 showed a foliar damage rating of 3 or less, reflecting their high resistance to leaf-feeding by *C. partellus*. All these plants were tagged and infested again at the flowering stage. The plants were then rated for stem breakage and those that showed this symptom were also discarded. Plants showing good levels of resistance to both foliar damage and stem breakage were selected and harvested for seed. The number of accessions belonging to this category was 6: no. 59, 70, 75, 86, 113 and 183. The incidence of disease, particularly streak virus, which is predominant at Mbita, was quite low in these accessions. Their other agronomic characteristics are also indicated in Table 1.8. Seeds from these plants will be used for the next season's evaluation for infestation and damage levels. Some of this material has also been sent to CIMMYT to be incorporated into another cycle of a multiple-borer resistant pool, which is being developed as part of an ICIPE/CIMMYT collaborative venture. Such material will be tested at ICIPE as well as at other international and national agricultural centres for resistance to various borers.

1.8.2 Mechanisms of maize resistance to stem borers

The role of different colonizing responses of *C. partellus* in determining the resistance or susceptibility of seven cultivars of maize has been given in previous annual reports. During 1987 the role of larval orientation was further investigated, as summarized below.

Larval orientation towards resistant and susceptible maize cultivars. Seven maize lines (Inbred A, OH 43, ICZ1-CM, ICZ2-CM, MP 701, MP 702 and MP 704) were tested in a circular arrangement in a screenhouse. There were 5 plants of each line in the arrangement. One hundred neonate first- and third-instar larvae were released in the centre of the circle. The plants were dissected after 48 hours and the number of larvae recovered from each recorded.

The results on neonate larvae were confounded by ant predation and were therefore discarded. However, there was

Table 1.8 Maize selections showing low levels of foliar damage and their subsequent performance against further infestation by stem borers

Acc. no.	Foliar damage (1-9)	Incidence of streak disease	Days to 50% flowering	Plant height (cm)	Ear height (cm)	Lodging	
						Root	Borer
113	1.7	Moderate	58	175.3	108.7	13.3	0
65	2.7	Moderate	57	133.1	86.6	13.3	13.3
175	3.0	Moderate	56	175.0	110.0	0	20.0
70	1.9	Moderate	59	132.3	97.3	0	0
59	2.3	Moderate	58	139.7	82.9	0	0
92	2.6	High	58	152.6	79.2	0	6.7
51	3.0	Moderate	58	168.2	109.5	6.7	6.7
149	2.3	Moderate	57	193.1	106.2	6.7	0
129	1.8	Moderate	57	200.9	110.2	6.7	20.0
158	2.5	Moderate	56	179.4	95.1	0	6.7
17	2.7	Moderate	56	193.7	125.1	6.7	13.3
157	2.8	High	56	142.7	86.0	13.3	26.7
16	2.3	High	57	153.0	87.6	20.0	20.0
183	2.9	Moderate	59	145.2	89.8	0	0
112	2.9	High	58	117.3	59.1	33.3	6.7
152	2.8	Moderate	52	178.1	105.9	26.7	0
168	2.8	Moderate	57	170.5	103.3	6.7	13.3
67	2.8	Moderate	57	144.2	93.3	6.7	13.3
54	2.9	Moderate	57	155.6	93.4	0	6.7
75	2.2	Low	56	173.7	95.2	0	0
21	2.6	Moderate	57	180.5	112.2	13.3	13.3
45	2.9	Moderate	55	147.8	74.3	6.7	6.7
18	2.7	High	56	140.1	79.6	13.3	13.3

a good recovery of the third-instar larvae, but their percentage differed from one cultivar to another. The less the percentage of larvae recovered from a cultivar, the less would be the cultivar's attraction and, hence, the greater would be the contribution to the resistance of the cultivar. The maximum larval recovery and, hence, attraction was made on Inbred A. Such a high attraction for this line would therefore contribute to its high susceptibility, which has been reported. But larval attractancy of another susceptible cultivar, OH 43, was almost one-third that of Inbred A, suggesting that this characteristic could not impart overall resistance to the cultivar. In other words, larvae cannot effectively find OH 43 plants, but when larvae happen to be on those plants, as for example when emerging from eggs laid on the plants, the larvae cause heavy damage.

The remaining cultivars have been shown to be resistant to the borer, and their larval attractancy ranged from one-fourth to one-half that of Inbred A. Thus the low larval attraction of these cultivars would contribute to their overall resistance.

C. partellus larval response to infested and uninfested maize plants. This aspect was studied to understand how larval establishment and development on different maize cultivars may be influenced by prior infestation and damage by the borer. For this study, seven cultivars were grown in a screenhouse: OH 43, ICZ1-CM, ICZ2-CM, MP 701, MP 702, MP 704 and Inbred A. They were infested 3 weeks after plant emergence (WAE) by placing 2 neonate larvae in each whorl; control plants were uninfested. At 4 WAE, 10 neonates were placed in the whorls of the infested as well as the uninfested plants. At this stage there were visible foliar damage symptoms on the previously infested plants. Ten days after the second infestation, the plants were dissected

and all the larvae that were recovered were recorded and their weights taken.

The results show that the larval recovery from both sets of plants was identical. The larvae thus showed no marked preference for the infested or uninfested plants.

The weights of the larvae recovered from the previously infested plants were higher (9.7 mg per larva) than those of the larvae from the previously uninfested plants (6.7 mg per larva). This suggests that previous infestation had no detrimental effect on new colonizing larvae, but rather seemed to help the larvae to grow.

1.9 THE EFFECT OF MAIZE CULTIVAR AND PLANTING DENSITY ON *C. PARTELLUS* OVIPOSITION IN THE FIELD

S. Firempong

C. partellus has been reported to show differences in oviposition on different maize cultivars. However, there is scarce information on the effects of different planting densities of these cultivars on oviposition and egg distribution by the insect. This aspect was therefore studied using three maize cultivars: Inbred A (susceptible), MP 702 (moderately resistant) and MP 704 (highly resistant). These were planted at three densities—low, medium and high—with between-row spacings 1 m, 0.75 m and 0.50 m, respectively. There were 9 subplots, each measuring 6 m × 10 m. Each subplot was further divided into 3 to make 3 replicates. Thus, the number of plants per 60 sq m was 189, 252 and 378 at the low, medium and high plant densities, respectively. The numbers of egg masses laid by the females

of the natural population in the field were recorded twice a week for 11 weeks, beginning 2 weeks after planting (WAP).

The results (Table 1.9) show that each cultivar had 2 peaks of oviposition occurring between 3–5 and 7–10 WAP. As shown in Table 1.9, a significantly greater number of eggs was laid on Inbred A than on MP 704 or MP 702. This difference in oviposition occurred during the second peak oviposition. Regarding the effect of plant density, the oviposition on each cultivar remained statistically identical for all three densities tested.

Table 1.9 *C. partellus* oviposition on three maize cultivars at three planting densities

Cultivar	Mean no. (\pm standard error) egg masses/ 35 plants in 12 weeks		
	Low density	Medium density	High density
<i>Entire period</i>			
Inbred A	1.50 \pm 0.20 ^a	1.50 \pm 0.27 ^a	1.69 \pm 0.20 ^a
MP 702	0.42 \pm 0.08 ^b	0.63 \pm 0.09 ^b	0.69 \pm 0.12 ^b
MP 704	0.44 \pm 0.09 ^b	0.56 \pm 0.09 ^b	0.61 \pm 0.10 ^b
<i>7–10 weeks after planting</i>			
Inbred A	2.17 \pm 0.32 ^a	2.58 \pm 0.17 ^a	2.83 \pm 0.22 ^a
MP 702	0.75 \pm 0.19 ^b	0.76 \pm 0.06 ^b	2.83 \pm 0.22 ^b
MP 704	0.58 \pm 0.24 ^b	0.75 \pm 0.08 ^b	0.83 \pm 0.11 ^b

Within a column and between columns, mean values bearing the same superscript letters are not significantly different ($P > 0.05$).

Least significant difference over entire period = 0.22; 7–10 weeks after planting = 0.85.

The pattern of egg mass distribution was found to be random on all the cultivars at the three densities. Using the data on egg mass distribution, an optimum sampling plan for egg masses was determined for the three cultivars, based on Taylor's Power Law. The sampling plan was aimed at determining the smallest sample size that would assure workers the desired reliability when evaluating cultivars in the field for resistance to the moth's oviposition.

Table 1.10 Laboratory and field evaluation of cowpea cultivars

Cultivar	Aphid population build-up		% dead seedlings	Field population score (55 DAP†)	ORSI‡
	7 DAI*	14 DAI*			
ICV 1	28.75 \pm 11.00	159.33 \pm 89.33	55.5	3.89 \pm 0.60	1.00
ICV 2	82.75 \pm 27.05	70.25 \pm 19.84	50.0	3.83 \pm 0.30	1.17
ICV 3	62.50 \pm 27.54	113.25 \pm 84.42	85.7	3.27 \pm 0.68	1.28
ICV 4	30.00 \pm 2.89	67.50 \pm 37.50	50.0	3.73 \pm 0.13	0.86
ICV 5	151.50 \pm 39.94	399.20 \pm 99.67	100.0	3.87 \pm 0.60	2.22
ICV 6	130.50 \pm 19.97	254.00 \pm 70.47	100.0	3.38 \pm 0.66	1.91
ICV 7	50.25 \pm 37.26	178.00 \pm 69.42	100.0	3.73 \pm 0.52	1.39
ICV 8	153.25 \pm 14.53	155.23 \pm 96.85	63.6	3.35 \pm 0.09	1.71
ICV 9	129.00 \pm 25.81	133.75 \pm 41.28	50.0	3.63 \pm 0.37	1.47
ICV 10	168.00 \pm 30.17	65.00 \pm 18.35	14.3	2.45 \pm 0.60	1.34
ICV 11	85.20 \pm 19.67	19.33 \pm 10.31	37.5	2.57 \pm 0.73	0.94
ICV 12	10.50 \pm 2.22	28.33 \pm 14.40	11.1	2.51 \pm 0.86	0.37
ICV 13	58.00 \pm 12.02	199.75 \pm 27.12	37.5	3.64 \pm 0.85	1.08
ICV 14	108.00 \pm 47.08	371.50 \pm 34.02	50.0	2.88 \pm 0.33	1.56

* DAI: days after infestation.

† DAP: days after planting.

‡ ORSI: overall resistance/susceptibility index.

1.10 COWPEA RESISTANCE TO APHIDS

S. Firempong

1.10.1 Evaluation of cowpea cultivars for resistance to *Aphis craccivora*

Fourteen cowpea cultivars improved by ICIPE were evaluated in a screenhouse and in the field for resistance to the cowpea aphid, *Aphis craccivora*. The screenhouse tests involved determining (1) the response of the cultivars to heavy aphid infestation and (2) the build-up of aphid populations on the cultivars.

The study of the responses of the cultivars involved planting single rows of each cultivar in aluminium traps and infesting them with 20 adult apterate aphids one week later. Fifteen days after infestation, the cultivars were evaluated for the percentage of plants surviving. The results were compared with uninfested seedlings grown concurrently. For the study of population build-up of the aphid, ten newly born nymphs were placed on one-week-old seedlings growing in ten-litre plastic buckets. Seven days later the first population count was taken and a second one was taken one week later.

The screenhouse experiments were followed by the field study, in which the cultivars were evaluated on a 1–5 scale for their degree of infestation under natural conditions.

The results of these experiments are shown in Table 1.10. These are further condensed in the Overall Resistance/Susceptibility Index (ORSI) to differentiate between resistant and susceptible cultivars, with ICV 1 as the susceptible check. On this scale, the three most resistant cultivars are ICV 12, 11 and 4, and the three most susceptible ones are ICV 5, 8 and 14.

1.10.2 Mechanisms of resistance in cowpea to *Aphis craccivora*

The role of non-preference and antibiosis in cowpea resistance to aphids was examined in the screenhouse, using six selected cultivars. Non-preference studies were done under both no-choice and choice situations. No-choice tests involved recording the percentage of alate aphids that settled

and reproduced when 100 alates were caged overnight on 20 seedlings. In the choice situation, the six cultivars were planted in a latin block design and the number of alates that settled and reproduced on each cultivar was recorded.

Studies on antibiosis involved rearing cohorts of apterate aphids on different cultivars and comparing them in respect of the number of aphids surviving to reproduce, the fecundity, the duration of reproduction, the net reproductive rate, the gross reproductive rate, the intrinsic rate of increase, the finite rate of increase and the doubling time.

The non-preference studies revealed that the cultivars IT82D-812 and ICV 12 were the least preferred ones for colonization and TVu 946 and IT81D-1137 were the most preferred.

The antibiosis tests showed that ICV 12 was the most resistant cultivar: aphids reared on it suffered the highest mortality, took longest to become adults and had the lowest fecundity and the shortest duration of reproduction. ICV 1 was the most susceptible: aphids reared on it showed the best performance in each of the above parameters.

1.11 BASES OF RESISTANCE OF COWPEA TO THE POD BORER *M. TESTULALIS*: LARVAL FEEDING IN RELATION TO POD AGE, SIZE AND MOISTURE CONTENT

S. H. O. Okech and T. O. Tayo

The role of some factors, particularly certain colonizing responses of the cowpea pod borer *Maruca testulalis*, to different cultivars in determining their susceptibility or resistance to the pest has been given in previous annual reports. In 1987 studies were continued on the feeding responses of the pest larvae to the susceptible and resistant cowpea cultivars. The effects of pod age, size and moisture content were studied using three cultivars: TVu 946 (resistant), ICV 2 (moderately resistant) and VITA 1 (susceptible). Fourth-instar larvae were fed for 24 hours on 3-, 6-, 9- and 12-day-old pods. The sizes of the pods and seeds were measured and the moisture content of the pod parts were determined. Transverse sections of the pods were made by hand and the relative thickness of the tissues in the pods was measured using a calibrated micrometer in five sections of each pod age.

The results show that the quantity of the ingested food (mg fresh weight) varied with the age of the pod (Table 1.11). The consumption was lowest in 3-day-old pods (25–42 mg) and

increased with pod age up to 9 days, when consumption was 190–285 mg. Thereafter, the consumption from 12-day-old pods decreased to 127–190 mg. However, this amount was still higher than the consumption of 6-day-old pods (107–122 mg). The quantities ingested from 3- and 6-day-old pods were not statistically different among the cultivars. However, more 9-day-old pods of ICV 2 were consumed than pods of VITA 1 and TVu 946. For 12-day-old pods, consumption of VITA 1 and ICV 2 was higher than that of TVu 946.

The length and girth of 3-day-old pods was similar in all varieties. However, as the pods advanced in age, their length and girth increased and became different among the cultivars in the order VITA 1 > ICV 2 = TVu 946.

The total cross-sectional dimension of 6-day-old pods of TVu 946 was 64% that of VITA 1 and the thickness of the pericarp and parenchyma cross-sections of these pods of TVu 946 were 69% and 61%, respectively, of those of VITA 1. Similar trends were recorded for 12-day-old pods. The corresponding values for ICV 2 were 84%, 100% and 81% of those in VITA 1. Since *M. testulalis* bores into the pod before settling for feeding, those pods with wider girth (cross-section), such as VITA 1, would be more convenient for feeding than TVu 946.

The percentage of moisture content (PMC) of the pods increased from 3- to 9-day-old pods. However, moisture content was highest in VITA 1 and least in TVu 946. The PMC of the husk increased for up to 12 days in VITA 1 but declined after 9 days in other varieties, as was the case for the whole pod. The whole pod PMC in VITA 1 was 13% higher than in TVu 946 for 12-day-old pods, while the PMC of the 3-, 6- and 9-day-old pods was higher by about 5% in VITA 1 than in TVu 946. Thus, there seems to be a positive correlation between the quantity of the food consumed and the moisture content of the pod.

1.12 GENETICS OF PLANT RESISTANCE TO INSECT PESTS

R. S. Pathak

The main objective of the studies on the genetics of plant resistance to insect pests is to discover appropriate breeding methods and strategies to develop insect-resistant cultivars. The type of gene action involved in the inheritance of resistance helps determine the particular breeding method to improve the resistance level and to incorporate the resistance genes into an agronomically acceptable cultivar. The results

Table 1.11 Quantity of food ingested by fourth-instar *M. testulalis* larvae from pods of three cowpea cultivars

Cultivar	Susceptibility	Quantity ingested (mg fresh wt) in 24 hr (Age of the pod [days])			
		3	6	9	12
VITA 1	S	25.25 ± 7 ^a	121.75 ± 17 ^a	190.23 ± 48 ^b	162.30 ± 32 ^b
ICV 2	MR	42.33 ± 4 ^a	91.00 ± 18 ^a	285.50 ± 23 ^a	192.00 ± 20 ^a
TVu 946	R	30.80 ± 4 ^a	107.00 ± 13 ^a	210.25 ± 9 ^b	127.30 ± 1 ^b

S: susceptible; R: resistant; MR: moderately resistant.

Within a column, mean values bearing the same superscript letters are not significantly different at the 5% level by Duncan's multiple range test.

of the experiments carried out in 1987 involved new sources of resistance of maize and sorghum to the stem borer *C. partellus*.

1.12.1 Gene effects for maize resistance to *Chilo partellus*

Two resistant maize cultivars—ICZ1-CM and MP 704—were crossed with the susceptible parent Inbred A. The resistant parent was used as the P₁ in each cross. Six populations—P₁, P₂, F₁, F₂, BCP₁ and BCP₂—were evaluated in a randomized complete block design with four replications during the short rainy season of 1986/87 and the long rainy season of 1987. One-row plots each of P₁, P₂ and F₁; three-row plots each of BCP₁ and BCP₂ and six-row plots of F₂ were planted in each replication. The row width was 75 cm and plants were spaced 30 cm apart. Each plant was infested with egg batches containing 50 blackhead-stage eggs of *C. partellus* in the fourth week after emergence. Each plant was evaluated for three damage parameters: leaf-feeding (1-9 scale), the percentage of dead-heart per plot basis (four weeks after artificial infestation) and the percentage of stem-tunnel length at harvest. Percentage data were transformed into arc sine before statistical analysis.

Significant differences were detected among the resistant and susceptible parents for each damage parameter in all the three crosses (Table 1.12).

The following gene effects were estimated according to the six-parameter model:

[m] = mean effect

[d] = additive gene effect

[h] = dominance gene effect

[i] = additive × additive type of gene interaction

[j] = additive × dominance type of gene interaction

[l] = dominance × dominance type of gene interaction

The additive-dominance genetic model involving three parameters ([m], [d] and [h]) was used to explain the inheritance of the resistance measurements in crosses that showed non-significance of scale tests.

The estimates of additive gene effect [d] were highly significant for leaf-feeding, dead-heart and stem-tunnel length in all three crosses. These results suggest that there was additive gene action in the expression of these resistance characters (Table 1.13). Dominance gene effect [h] was highly significant for dead-heart in all the crosses, while it was significant in only one cross in the case of leaf-feeding and stem-tunnel length. The estimates of dominance effects were greater than those of additive effects, particularly regarding dead-heart.

Highly significant epistasis (gene interaction) was present for dead-heart in all crosses, while it was significant for leaf-feeding in one cross. For dead-heart, additive × dominance [j] effects and dominance × dominance [l] effects were more important than the additive × additive [i] effects, while for leaf-feeding, [i] and [l] effects were more important than [j] effects.

From these results it may be concluded that both additive [d] and non-additive (dominance [h] and epistasis [i, j, l])

Table 1.12 Generation means for measurements of resistance to *C. partellus* in three maize crosses

Damage parameter	Cross	P ₁	P ₂	F ₁	F ₂	BCP ₁	BCP ₂	LSD 5%*
Leaf-feeding (1-9)	Cross 1	3.70	4.90	3.80	4.75	3.48	4.33	0.641
	Cross 2	2.14	2.69	2.25	2.52	2.42	4.61	0.359
	Cross 3	2.42	3.67	3.43	3.41	3.01	3.67	0.411
% dead-heart (arc sine)	Cross 1	0.67	44.27	0.68	27.73	2.69	17.10	4.371
	Cross 2	0.81	62.30	0.81	20.48	8.73	25.96	11.556
	Cross 3	0.90	62.50	0.84	13.00	2.71	14.01	9.128
% stem-tunnel length (arc sine)	Cross 1	20.00	29.50	26.15	24.73	21.85	25.30	5.256
	Cross 2	39.87	61.22	51.00	46.45	50.38	46.50	8.991
	Cross 3	26.88	65.35	33.85	38.90	30.88	41.47	8.608

Cross 1 = ICZ1-CM × Inbred A; Cross 2 = ICZ2-CM × Inbred A; Cross 3 = MP 704 × Inbred A.

* LSD: least significant difference.

Table 1.13 Estimates of gene effects for *C. partellus* resistance of three maize cultivars crossed with Inbred A

Damage parameter	Cross	Mean effect [m]	Additive [d]	Dominance [h]	Ad. × Ad. [i]	Ad. × Dom. [j]	Dom. × Dom. [l]
Leaf-feeding (1-9)	Cross 1	4.75**	-0.85**	-3.90**	-3.40**	-0.25	4.00**
	Cross 2	2.44**	-0.27**	-0.51	—	—	—
	Cross 3	3.28**	-0.62**	-0.38	—	—	—
% dead-heart (arc sine)	Cross 1	27.72**	-14.41**	-93.11**	-71.32**	7.39**	78.02**
	Cross 2	20.48**	-17.23**	-43.28**	-12.53	13.51**	7.86
	Cross 3	13.00**	-11.29**	-49.40**	-18.55	19.50**	50.18**
% stem-tunnel length (arc sine)	Cross 1	29.35**	-4.75**	-15.30	—	—	—
	Cross 2	42.58**	-10.68**	7.08	—	—	—
	Cross 3	38.90**	-10.58**	-23.17**	-10.90	8.65	26.13

Cross 1 = ICZ1-CM × Inbred A; Cross 2 = ICZ2-CM × Inbred A; Cross 3 = MP 704 × Inbred A.

** Significant at the 1% level.

gene effects were important in the inheritance of dead-heart, while the gene effect for leaf-feeding and stem-tunnel length was predominantly additive. A recurrent selection procedure would be appropriate for increasing the level of resistance in a population. Heterosis breeding could be exploited in F_1 crosses showing increases in yield and resistance.

1.12.2 Gene effects for sorghum resistance to *C. partellus*
Studies were undertaken to determine the inheritance of resistance to the stem borer *C. partellus* in a cross involving the resistant cultivar IS 1044 and the susceptible cultivar IS 18363. The resistant parent IS 1044 was used as the P_1 in the cross. The six populations— P_1 , P_2 , F_1 , BCP_1 and BCP_2 —were grown in a randomized complete block design with four replications. One-row plots each of P_1 , P_2 and F_1 ; three-row plots each of BCP_1 and BCP_2 and five-row plots of F_2 were grown in each replication. The spacing of 75 cm between rows and 15 cm between plants was retained. Each plant was infested with egg batches containing 40 blackhead-stage eggs of *Chilo partellus* in the fourth week after emergence. The damage parameters of leaf-feeding (1–9 scale), percentage of dead-heart and percentage of stem-tunnel length were measured to indicate the level of resistance or susceptibility. The leaf-feeding and dead-heart were evaluated in the fourth week after artificial infestation, and stem-tunnel length was measured at harvest time.

Significant differences were observed between the susceptible and resistant parent for all damage parameters (Table 1.14). Estimates of additive [d] gene effect were highly significant for all damage parameters (Table 1.15). This indicated that additive gene effect was important in the inheritance of these damage parameters. Dominance [h] and epistasis [i, j, l] gene effects were important only for leaf-feeding. These results suggest that transgressive segregation for a higher resistance level may result from this cross through a recurrent selection programme.

Table 1.14 Generation means for measurements of resistance of *C. partellus* in a sorghum cross between IS 1044 (resistant) and IS 18363 (susceptible)

Damage parameter	P_1	P_2	F_1	F_2	BCP_1	BCP_2	LSD 5%*
Leaf-feeding (1–9)	2.23	6.93	4.13	3.68	3.15	6.35	0.890
% dead-heart (arc sine)	1.28	27.63	4.17	4.92	0.03	15.00	7.764
% stem-tunnel length (arc sine)	40.10	62.03	46.13	46.68	46.43	55.25	7.105

* LSD: least significant difference.

Table 1.15 Estimates of gene effects for *C. partellus* resistance of sorghum cultivar IS 1044 crossed with IS 18363

Damage parameter	Mean effect [m]	Additive [d]	Dominance [h]	Ad. × Ad. [i]	Ad. × Dom. [j]	Dom. × Dom. [l]
Leaf-feeding (1–9)	3.68**	–3.2**	3.85**	4.3**	–0.85	–5.90*
% dead-heart (arc sine)	4.05**	–13.77**	3.35	—	—	—
% stem-tunnel length (arc sine)	34.41	–10.96**	37.34	—	—	—

* Significant at the 5% level; ** significant at the 1% level.

BIOLOGICAL CONTROL

*The research in the Biological Control (BC) Section is aimed at developing strategies for using natural enemies of certain major arthropod pests of sorghum, maize, cowpea and cassava. The natural enemies under investigation include parasitoids, predators and pathogens. Previous work done by BC on these natural enemies has been presented in earlier annual reports. The natural enemies identified as promising for the control of crop borers include *Pediobius furvus*, *Dentichasmias busseolae* and *Trichogramma sp.* among the parasitoids and *Nosema sp.* among the pathogens. The work carried out on these natural enemies in 1987 is reported below.*

1.13 PARASITIDS FOR THE BIO-CONTROL OF STEM BORERS

G. W. Oloo

1.13.1 Biotic potential of *Trichogramma spp.*

In the *ICIPE 1986 Annual Report* we reported examining aspects of the biology and behaviour of selected parasitoid species with a view to finding suitable hosts for mass production and to obtaining information on their performance under cage experiments. The main emphasis in 1987 was on determining the biotic potential of various strains of *Trichogramma* collected from various areas of Kenya. The biological criteria for this comparative study were the net reproductive rate, the intrinsic rate of increase and the finite population growth rates. Those promising strains will then be evaluated further for performance under field conditions on the basis of their survival, functional (and numerical) responses, dispersal capacity and rate of parasitization. This will be the last step before beginning mass-rearing and then releasing the parasitoid showing a high bio-control potential.

The objective of the research reported upon below was to determine the biotic potential of various local species/strains of *Trichogramma* in Kenya for borer management.

Trichogramma species/strains used in the study were obtained by exposing freshly laid eggs of *Chilo partellus* (from our Insect Mass-Rearing Technology Section) in fields of maize, sorghum and wild banana. After a two-day exposure, the eggs were collected and maintained in the laboratory to recover egg parasitoids. On emergence, the parasitoids (*Trichogramma* spp.) were transferred to a growth chamber set at a constant temperature of 27° C, which is the average temperature in the field where the target pest, *Chilo* spp., occurs. Each mated *Trichogramma* female was provided with a batch of *Chilo* eggs and 20% sucrose syrup for nourishment. Mortality was recorded every day until the last female died. Progeny production, sex ratio and generation time were also recorded. These data were used to estimate the net reproductive rate, intrinsic rate of increase and finite population growth rate.

Four strains of *Trichogramma* were collected: one from Mtwapa, on the Kenya coast; one from Rusinga Island, near Mbita Point Field Station (MPFS); one from Lambwe Valley, 20 km from MPFS; and one from Homa Bay area, 40 km from MPFS. Of these, the Homa Bay strain died off, the Mtwapa strain had the highest finite growth rate (51.6 per female per generation), the Rusinga strain had the lowest growth rate (22.0 per female per generation) and the Lambwe strain was close to the Mtwapa strain (48.7 per female per generation). These results (Table 1.16) show that the Mtwapa

Table 1.16 Biotic potential of three strains of *Trichogramma* (Ex-Lambwe, Ex-Mombasa and Ex-Rusinga) at 27° C, 49% RH

Strain	Net reproductive rate (R_0)*	Intrinsic rate of increase (r_m)	Finite population growth rate (†)
Ex-Rusinga	24.06	0.35	1.41 (22.0)‡
Ex-Lambwe	49.43	0.43	1.54 (48.7)
Ex-Mombasa	51.39	0.44	1.55 (51.6)

* Number of times the generation will multiply per generation.
 † Number of individuals added to the population per female per day. Mean generation time is 9 days.
 ‡ Number of increase per female per generation.

Table 1.17 Temporal pattern of female attractiveness in *D. busseolae*

Age (hrs)	Total males tested	No. males reacting	No. males reaching test chamber	No. males reaching control
<i>Control (hosts only)</i>				
36 HBE	50	13	5	8
24 HBE	80	14	5	9
12 HBE	80	33	23*	10
1 HBE	80	41	34*	7
	80	62	56*	6
<i>At emergence</i>				
1 HAE	80	65	61*	4
6 HAE	80	71	69*	2
24 HAE	80	69	64*	5
48 HAE	80	67	59*	8
72 HAE	80	47	39	8
	80	15	6	9

HBE: hours before emergence; HAE: hours after emergence.

* Values are significantly different from control at the 0.05 level of probability.

strain has a relatively high biotic potential. We are now mass-producing this strain for field tests on survival, dispersal capacity and parasitization rate on Rusinga Island.

1.14 BEHAVIOURAL STUDIES ON *DENTICHASMIAS BUSSEOLAE* HEINRICH (HYMENOPTERA:ICHNEUMONIDAE)

J. W. Bahana

1.14.1 Evidence of a sex pheromone in *D. busseolae*

Reported observations on mating in the parasitoid *D. busseolae* showed that males were able to mate one day after emergence and females were receptive immediately after emergence. To examine the mating behaviour of *D. busseolae* in relation to the possible presence of a sex pheromone, observations were undertaken using individual *C. partellus* pupae parasitized ten days previously and reared at room temperature. The pupae were subjected to olfactometer tests. The test apparatus consisted of a Y-tube with each branch connected to an air pump. Two-day-old males were placed in one branch leading to the stem of the Y-tube, while parasitized *C. partellus* pupae or virgin females were placed in the other branch of the tube. The controls consisted of unparasitized pupae and the blank chamber, respectively. The presence of a sex pheromone was tested periodically by assaying parasitized pupae on a 12-hour schedule until the morning that a parasitoid emerged. The virgin females collected from such assayed pupae were similarly assayed for 6 hours during the next 5 days until they no longer elicited response from males.

The history of each assayed pupa was kept and notes made of the responses of the males and of the sex of the progeny that finally emerged from the pupa. The relative attractiveness of the assayed individuals was scored by counting the numbers of males that entered the test chamber at the end of a 30-minute observation period.

1.14.2 Adult age and mating

The effect of the age of both males and females on mating was tested in the laboratory by observing the mating behaviour of pairs of various age combinations of the

parasitoids. The observations were made from 0800 to 1200 hr, which has been shown to be the high mating period.

The influence of mating activity on the parasitoid population structure was further tested by setting up a series of situations in which equal numbers of virgin and inseminated females were presented with hosts each day for the parasitoids' life-span. Parasitized hosts were held until emergence of offspring. Their sex ratio was then noted.

Table 1.17 shows the female attractiveness to males in *D. busseolae*. The table shows that males started to react to the presence of the females 36 hours before they emerged from the host pupae. Female attractiveness started waning 2 days after emergence and females older than 4 days were not attractive to males at all. The directed response to the parasitized host pupae and the behaviour displayed in the olfactometer indicated that pheromonal attractants were being used.

The effect of mating on the sex ratio of the progeny is summarized in Table 1.18. The results show that hosts parasitized by uninseminated females produced only male offspring. On the other hand, inseminated females produced both male and female offspring. This phenomenon, called 'Arrhenotoky', or facultative parthenogenesis, is a common occurrence in many parasitoid species.

Table 1.18 Effect of age and insemination state of the sex ratio in *D. busseolae*

Age (days)	Virgin females		Inseminated females	
	No. progeny	Sex ratio (M:F)	No. progeny	Sex ratio (M:F)
0-7	8	1:0	9	1:3.1
8-14	18	1:0	19	1:3.1
15-21	16	1:0	17	1:2.7
22-28	10	1:0	11	1:1.0
29-35	6	1:0	7	1:1.0
36-42	6	1:0	7	1:0.6
43+	4	1:0	4	1:0.2

1.15 ROLE OF PARASITOIDS IN NATURAL POPULATIONS OF *M. TESTULALIS*

J. B. Okeyo-Owuor

Previous studies at the ICIPE Mbita Point Field Station (MPFS) and on farmers' fields in Lambwe have revealed the existence of a rich fauna of *M. testulalis* parasitoids at these sites. It was observed that most of the parasitoids attacked the pupal stage of the pest. To date no egg parasitoid has been found and only two larval parasitoids have been recovered.

In 1987 studies were initiated with a two-fold objective: first, to isolate and identify any new parasitoids attacking the pest, and second, to determine the level of parasitism in natural populations of *M. testulalis*. Studies were conducted on cowpea crops planted at three sites—MPFS, farmers' fields in Lambwe and a field site at Ungoye.

The results at MPFS and Lambwe revealed that the parasitoids previously identified on the pest—*Tetrastichus sesamiae*, *Antrocephalus* sp., *Braunsia kriegeri*, *Apanteles*

sp. and *Bracon* sp., all belonging to the order Hymenoptera as well as a Dipteran (Tachinidae)—were continually present and parasitizing *M. testulalis* in the field. And three new parasitoids—*Invrie* sp. (Chalcididae), *Macrocentrus* sp. and *Echthromorpha variagata*—were found to parasitize *M. testulalis* at MPFS. As was observed earlier, *Antrocephalus* sp. was the most prevalent, constituting 83% of the total parasitism, with the others contributing only 16.3%. It was also found that *M. testulalis* mortality due to parasitism was low, being 11.7%. Mortality due to egg parasitism was not observed, while larval parasitism was merely 1.4%. The highest parasitization occurred in the pupae (10.3%).

Antrocephalus sp. (Chalcididae, Hymenoptera) was first recorded as a parasitoid of *M. testulalis* at MPFS and in Lambwe fields in the 1983 long rains. Its identification at the Commonwealth Institute of Entomology went only as far as the genus level for the materials sent in 1983 and 1987, suggesting that this parasitoid is a new species of the genus, requiring taxonomic description and species naming. Among *M. testulalis* parasitoids, *Antrocephalus* sp. is the most prevalent. Scarce information exists on the biology and even rearing technique of this parasitoid. Hence, studies on its biology were initiated at the MPFS laboratory to facilitate development of a suitable rearing technique.

The results showed that both male and female parasitoids live for 20 to 60 days, depending on the presence of food. Adult parasitoids emerged from the host pupae during the day starting from 0800 hr, with the highest number (54.6%) emerging between 1400 and 1500 hr. Mating occurred immediately after adult emergence and females were observed to mate only once. During oviposition the female pierced the host pupa with its ovipositor. Only one egg was laid per pupa and the developmental period from egg to adult parasitoid took 14.5 days in *M. testulalis* and 9.8 days in *C. partellus*. The reasons for the variation observed on this developmental period in the two host species need further investigation.

1.16 EFFECT OF SOLAR RADIATION ON *NOSEMA* SP. SPRAYED ON SORGHUM

M. O. Odindo

Inactivation of microbial control agents by the ultraviolet component of sunlight is a major drawback in the use of micro-organisms for pest control. In the present study, we investigated the effect of sunlight on a microsporidian pathogen, *Nosema* sp., when sprayed on sorghum infested with *C. partellus*.

Sorghum (var. Serena) was planted in a screenhouse in a plot of 8 rows, each row having a mean of 13 plants and replicated 3 times. Four weeks after plant emergence all tillers were removed so as to leave only the main stem. Rows 1 to 7 were then infested with neonate *C. partellus* larvae at the rate of 20 larvae per plant. The sorghum was left alone for 4 days so that larvae could stabilize on the plants.

When all the infested plants showed signs of infestation (foliar damage score of 1 and 2 on a scale of 0–09), rows 2 to 7 were then inoculated with an aqueous suspension of the microsporidian pathogen *Nosema* sp. (concentration $3.1 \times$

10⁶ spores/ml) in the following schedule: Row 2, 0800 hr; Row 3, 1000 hr; Row 4, 1200 hr; etc.; to Row 7, 1800 hr.

Weekly notes were taken on foliar damage, rate of plant growth and occurrence of dead-hearts. At three stages of the plant growth, the level of solar radiation was measured using a Licor (R) Quantum meter 185 within the plant canopy, in the shade, and in the sun in the screenhouse and the sun outdoors. Temperatures were also taken of the plant canopy, of the screenhouse and of the leaves.

At 15 weeks after plant emergence, all plants were harvested and dissected, and observations were made on sorghum head formation, larval infestation and larval mortality.

All the plants that were infested showed foliar damage by the third day after first-instar larvae had been introduced into the leaf funnel, at the foliar score of 1 (Table 1.19). However, further foliar damage stopped in most of the plants by the application of *Nosema* sp. (Table 1.19). Dead-heart formation remained high only in the infested non-inoculated control rows, where 23.3% plants had this damage by the fifth week after infestation. The highest level of dead-heart formation in the inoculated plots at this time was 11.2% in the rows treated at 1000 hr.

The time of application of the pathogen did not contribute significantly to the level of control achieved. For example, by the time of harvest (15 weeks after planting), the number of dead plants in the plots infested but not inoculated was 62.2%. Similarly, plots infested but treated with *Nosema* at 0800, 1200 and 1400 hr had 19.4%, 21.6% and 13.5% plant death, respectively.

Similarly, although the control plot had the lowest percentage of plants with fully formed heads (27%), all the treatments had a high percentage of fully developed plants, varying from 63.9% in plants treated at 0800 hr to 83.8% in plants treated at 1200 hr. Once again the level of control achieved was not correlated with the time of treatment of plants. This factor was also evident where the number of infestation and emergence holes per plant or the number of larvae and pupae recovered per plant were considered.

The level of solar radiation in the screenhouse increased gradually from 637 $\mu\text{Em}^{-2}\text{s}^{-1}$ at 0800 hr to reach a maximum 1767 $\mu\text{Em}^{-2}\text{s}^{-1}$ at 1400 hr. Radiation was considerably higher outside than inside the screenhouse. For example, at 0800 hr the radiation outdoors was already at 1450 $\mu\text{Em}^{-2}\text{s}^{-1}$.

However, both the open shade and the plant canopy had consistently lower radiation. At 1400 hr radiation in the plant canopy was 83 $\mu\text{Em}^{-2}\text{s}^{-1}$, or only 4.7% of that recorded in the open.

Temperature was high throughout the experimental period. However, the leaf temperature was up to 5° C lower than the ambient temperature, particularly in the morning.

Results obtained in this experiment suggest that sunlight is not likely to limit the use of *Nosema* sp. in controlling *C. partellus*. This is likely to favour the use of this pathogen in the protection of sorghum and maize from infestation by the spotted stalk borer.

1.17 EVALUATION OF BACTERIAL PATHOGENS AS PEST CONTROL AGENTS

M. Brownbridge

The ability of bacterial pathogens to control a wide variety of crop pest species is well documented. Little work has been performed, however, on the potential of these agents for pest control purposes by the poor farmers of East Africa. Bacteria such as *Bacillus thuringiensis* (Berliner)(*B.t.*) also are pathogens that can be mass-produced at relatively low cost on artificial media and that are environmentally safe (non-toxic) to non-target organisms. As such, they fit well into ICIPE's mandate to conduct research to elucidate safe methods for suppressing pest populations. The current research programme was therefore initiated to evaluate this potential and to provide candidate organisms for possible incorporation into a pest management programme. The target pest species have been *Chilo partellus* and *Spodoptera exempta*, with the bulk of the research work orientated towards the control of the borer. The results presented briefly here are from the research work performed with *C. partellus* only.

Three approaches to the problem were considered: (1) evaluation of a number of currently available commercial formulations based on various *B.t.* strains, (2) screening identified *B.t.* strains obtained from several stock collections for toxic activity to *C. partellus* and (3) isolation of indigenous strains of entomopathogenic bacteria from soil samples collected from a variety of geographic and ecological zones in Kenya.

Table 1.19 Foliar damage in sorghum infested with first instar *C. partellus* larvae and treated with *Nosema* sp. at various times of the day

Time after infestation (days)	Mean foliar damage*							
	Control†	0800	1000	Time of inoculation		1600	1800	Control‡
				1200	1400			
0	0	0	0	0	0	0	0	0
4§	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0
11	3.5	1.7	1.2	1.7	1.3	1.7	1.0	0
18	5.5	1.0	1.7	1.0	1.0	1.0	1.0	0
25	6.5	1.3	1.3	1.3	1.0	1.0	1.0	0
32	7.5	1.7	1.3	1.0	1.3	1.0	1.0	0

* Scale for scores on foliar damage: 0-9.

† Plants infested but not treated with pathogen.

‡ Plants not infested.

§ Day of inoculation.

1.17.1 Evaluation of commercial formulations

Four commercial preparations were tested against *C. partellus*: Thuricide, San 415, Certan (all Sandoz products) and Dipel 2X (Abbot). Thuricide, San 415 and Dipel 2X are based on the *B.t. kurstaki* strain, and the fourth preparation, Certan, on *B.t. aizawai*.

These preparations were used first to find out if a product was available straight away for incorporation into, and evaluation in, a pest management programme. Second, the preparations provided a standard reference point of activity to which the activity of other bacterial preparations might be compared, which also facilitated strain comparisons for both laboratory and field experiments. Third, the preparations served as standards of stable and known toxicity that could be used in a series of laboratory experiments where a product of reproducible toxicity was required.

All of the products based on *B.t. kurstaki* were active against *C. partellus*. Certan, however, appeared to be totally inactive. Dipel 2X proved to be most active against all instars of *C. partellus* (Table 1.20), but for logistical reasons, San 415 was adopted as the reference standard.

Table 1.20 LC₅₀ values of three commercial preparations based on *B.t. kurstaki* vs. L2—L5 stage *C. partellus* larvae (LC₅₀ values expressed in ITUs/gram of artificial diet)

Product	LC ₅₀ value (ITU/g)			
	L2	L3	L4	L5
Thuricide	590	2620	2370	1870
San 415	940	2910	2790	1310
Dipel 2X	560	1590	1460	980

It was demonstrated that different larval instars required different doses to induce a 50% mortality response (LC₅₀). The second and fifth instars appeared to be the most susceptible to the bacterial treatments, while the third- and fourth-larval instars required significantly higher doses in their LC₅₀ response. This experiment was performed largely to demonstrate the different responses of larval instars to each of the products, and to get more feedback on the required dose level for control purposes in the field where, for all practical purposes, the first-, second- and third-instar larvae are the targeted stages. Thus, the timing of any application with regard to the developmental stage of the larvae on the plant, and the concentration used, would seem to be critical to the degree of control that would be afforded.

The effects of sub-lethal doses of the bacteria on larval development were also studied because in the field the larvae may not be exposed to a lethal dose of the bacterium before entering the stem. The dose taken in, however, could have an adverse effect on the biology and subsequent development of the larva. Results of the series of experiments performed indicated that one five-day exposure of larvae to a sub-lethal dose of *B.t.*, when compared to a control batch, retarded the development of the exposed larvae (in terms of weight gain), increased larval mortality over the experimental period and reduced the pupation rate, with adults emerging from less than 5% of these pupae.

1.17.2 Screening identified *B.t.* strains

Several of the identified strains of bacteria were shown to be toxic to *C. partellus* larvae (Table 1.21). The relative toxicity of each of these strains will be determined in future experimental work and the most pathogenic considered for field evaluation.

Table 1.21 *B. thuringiensis* strains toxic to *C. partellus* larvae

Strain	H-Serotype	Strain	H-Serotype
<i>B.t. tolworthi</i>	H-9	<i>B.t. galleriae</i>	H-5a 5b
<i>B.t. entomocidus</i>	H-6	<i>B.t. japonensis</i>	H-23
<i>B.t. toumanoffi</i>	H-11a 11b	<i>B.t. morrisoni</i>	H-8a 8b
<i>B.t. kurstaki</i>	H-3a 3b	<i>B.t. dendrolimus</i>	H-4a 4b
<i>B.t. subtoxicus</i>	H-6	<i>B.t. sotto</i>	H-4a 4b
<i>B.t. kenya</i>	H-4a 4c	<i>B.t. alesti</i>	H-3a
<i>B.t. thuringiensis</i>	H-1	<i>B.t. wuhanensis</i>	—
<i>B.t. aizawai</i>	H-7		

Interestingly, some of the *B.t. aizawai* strains tested were very active against *Chilo* larvae, whereas a number of the strains were practically innocuous (also indicated by the lack of activity of Certan for *Chilo* larvae). This was also seen with *B.t. thuringiensis* and *B.t. alesti*. Each of the strains tested was isolated from different source material, and the results indicate both an inter- and intra-strain specificity in host activity.

1.17.3 Local isolation and screening of bacteria

Several crystal-forming *B.t.* strains were isolated from soils collected from various geographic regions in Kenya. The active isolates held are presented in Table 1.22.

Table 1.22 *B. thuringiensis* strains, toxic to *C. partellus*, isolated from material collected in Kenya

Strain code	Source and type of isolation material
MF 1-1, -3, -4	Mfangano Island, soil
MF 2B-3	Mfangano Island, mud
MF 3A-1	Mfangano Island, soil
MF 4B-2	Mfangano Island, soil
MF 9-3	Mfangano Island, mud
BU 5-14	Busia, soil
BU 5-3/7	Busia, soil
BU 8-22	Busia, soil
M 32-1	Mombasa, mud
M 40-1,-6,-8	Mombasa, soil
M 44-1, -2, -3, -4, -6, -7, -8	Mombasa, soil
L 1-1 through L 1-12	Lambwe Valley, armyworm frass

The survey was initiated to obtain isolates with the potential of higher activity towards the pest species, which might possess other desirable characteristics. Also, strains isolated in a tropical region may be better adapted and suited for the control of pests in such regions than exotic varieties.

The work has provided a number of viable candidate strains that may be valuable in a pest control programme. These strains are currently under in-depth toxicity studies to elucidate the most pathogenic isolates, which may then be

considered for preliminary field evaluation. The host range in terms of the pest species outlined in the ICIPE mandate also remains to be determined.

The results obtained from the experimental work performed to date indicate that certain *B.t.* strains have a high toxicity to—and hence have much promise for the control of—*C. partellus*. Work is now under way to quantify these effects and take the most potent strains to the field for further critical appraisal.

INSECT MASS-REARING TECHNOLOGY

1.18 REARING TARGET INSECTS AND THEIR NATURAL ENEMIES

R. S. Ochieng

In 1987 the research efforts of the Insect Mass-Rearing Technology Section (IMRT) were concentrated on the predatory mite of the green cassava mite. Effort was also put on the development of an artificial diet for the production of the maize stalk borer *Busseola fusca*.

1.18.1 Artificial diet for a Phytoseiid mite

In 1986 a diet, ICD 286, was developed on which the phytoseiid mite *Neoseiulus teke* (originally named *Amblyseius teke*) developed and reproduced. To date 45 generations have been produced. This is the first time an artificial diet that can support the development of a phytoseiid mite for several generations has been developed. However, like many insects bred entirely on an artificial diet, the behaviour of *N. teke* changed: it failed to recognize the host after a few generations.

In 1987 our efforts were concentrated on reversing this behaviour so that the artificially reared predator could recognize its prey. A liquid diet, ICD 387, has been developed that has been able to do this. Phytoseiid mites at generation 40 could recognize their host (prey). Thus far five generations from generation 40 have been able to change from an artificial diet to prey and from prey to an artificial diet without difficulties.

Table 1.23 Development and reproduction of the predacious mite, *N. teke*

Biological criterion	Developmental period (days)	
	Artificial diet	Natural diet
Egg	2	2
Larva	0.6	0.5
Proto-nymph	1	1
Deuto-nymph-adult	1	1
Pre-oviposition period	4	4
Total life cycle (egg-adult)	8.6	8.5
Fecundity (eggs/female)	28	35.5
Longevity (adult female)	25	26
Frequency of egg laying (eggs/day)	2	2
Duration of egg laying	16	20

The data (Table 1.23) show development and reproduction of the predacious mite (*N. teke*) on liquid diet ICD 387 as compared with development on prey after feeding on an artificial diet. This is a significant step in the study of the behaviour of insects reared on an artificial diet. It is also significant in that it will now enable mass-production of the predacious mite for releases to control mites of the genus *Tetranychus*.

1.18.2 Artificial diet for the maize stalk borer, *Busseola fusca*

As stated earlier, it had become necessary to develop an artificial diet for rearing the maize stalk borer. The economic status of *B. fusca* is becoming more significant in areas around Mbita Point Field Station.

Data on diet B2, on which *B. fusca* developed for three generations without undergoing diapause, is shown in Table 1.24. The development of the borer on this diet was quite good. However, the continued production and the development of mass-production methods were hindered because the fourth generation failed due to infertility. The cause of this infertility is not yet known, but it is probably due either to a nutrient imbalance or to inbreeding. However, this diet was able to maintain the continued development of *Busseola* larvae throughout the year without the larvae going into diapause when fresh larvae were introduced.

Table 1.24 Diet B2 for rearing *B. fusca*

Ingredient	Amount
<i>Fraction A</i>	
1. Bean powder (Roscooco)	170 g
2. Sorghum leaf powder	140 g
3. Brewer's yeast (Yestermin)	48 g
4. Ascorbic acid	4.8 g
5. Sorbic acid	1.5 g
6. Methyl-paraben	3 g
7. Formalin 40%	3 ml
8. Distilled water	600 ml
9. Vitamin mix (Vanderzant)	4 g
10. Wesson's salt	3 g
11. Benlate	1 g
<i>Fraction B</i>	
12. Agar Agar Tech. No. 3	25 g
13. Distilled water	1000 ml

1.18.3 Rearing natural enemies

The unit has taken over from the Biological Control Section the mass production of the three natural enemies identified as having the greatest potential in our biological control programme for the crop borers. These three are: egg parasitoids, *Trichogramma* sp., and two pupal parasitoids, *Pediobius furvus* and *Tetrastacus sesamiae*. Methods for mass production are now being developed.

1.18.4 IMRT Nairobi branch

The Nairobi branch of the Insect Mass-Rearing Technology Section is basically a service and supplies unit.

In 1987 the unit mass-reared and supplied target insects to ICIPE's research programmes and units, as well as to

university colleges, other research institutes and high schools. The stem borer *Chilo partellus* was supplied to Tsetse, Histology and Fine Structure, and Sensory Physiology; the housefly, *Musca domestica*, to Chemistry and Biochemistry and the Crops Pests Biological Control Section; mosquitoes, *Aedes aegypti*, to the Crop Pests Biological Control Section; and the stem borer *Eldana saccharina* and armyworm, *Spodoptera exempta*, to Chemistry and Biochemistry and Histology and Fine Structure. The unit also maintained colonies of rabbits, rats, mice and hamsters both for research and for maintaining haematophagous insects.

Also in 1987 the unit began experimenting with rearing and establishing colonies of *G. pallidipes* and *G. fuscipes*.

ICIPE-IRRI RESEARCH PROJECT

1.19 RESEARCH ON RICE LEAFFOLDER AND BROWN PLANTHOPPER

Z. R. Khan

1.19.1 Artificial diet for rice leaffolder

The rice leaffolder is a major pest of rice. Presently only natural field populations or greenhouse colonies of rice leaffolders sustained on rice leaves are available for research. Larvae obtained this way are often variable in size and infected with fungal diseases and other pathogens. To provide a dependable laboratory population, we developed an artificial diet for rearing a sizeable colony of the rice leaffolder *Cnaphalocrocis medinalis* (Figure 1.1). This is the first report of a successful rearing of the pest on a semi-synthetic diet. The artificial diet contains the following: media gel, ground wheat germ, soy protein, ground pinto beans, sorbic acid, methyl paraben, aureomycin, ascorbic acid, torula yeast, casein, formaldehyde and Vanderzant vitamin mix.

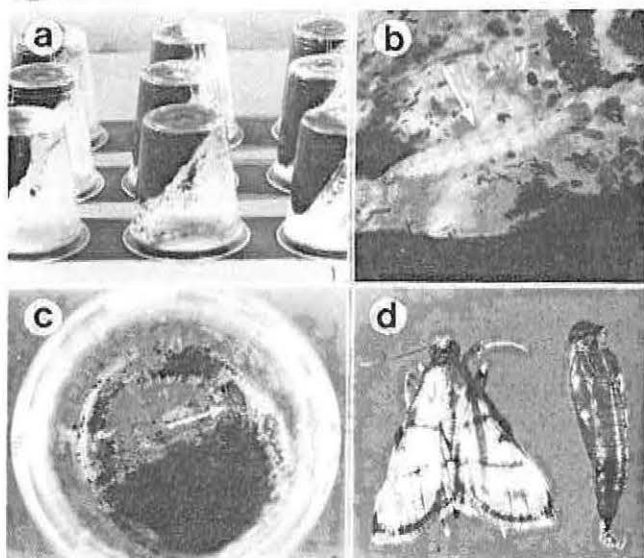


Figure 1.1 Rearing rice leaffolder *Cnaphalocrocis medinalis* on an artificial diet: (a) inverted clear plastic cups with artificial diet, (b) a leaffolder larva feeding on the diet, (c) a leaffolder pupa pupated in the plastic cup and (d) a pupa and an adult of the leaffolder developed on the diet.

The period of larval development on this diet (14–21 days, with an average of 16.2 days) was almost the same as that on IR 36 plants (17 days). The percentage of larvae completing development on the diet was also quite high (93%). The average weight of pupae was 21.02 mg, as compared with 17.8 mg on IR 36 plants. The average life-spans of males (8.3 days) and females (9.6 days) were similar to those reared on susceptible rice plants (7.8 days and 9.3 days, respectively). The average fecundity of females reared on the artificial diet (182 eggs per female) was also similar to that of females reared on IR 36 rice plants (163 eggs per female). We have reared the leaffolder for several generations on the artificial diet.

1.19.2 Allozyme variations in two rice leaffolder species

We studied the allozyme variation in 14 randomly chosen loci of the two rice leaffolders, *Cnaphalocrocis medinalis* (Guenée) and *Merasmia patnalis* Bradley, using starch gel electrophoresis. The two species were remarkably different in esterase locus in the larval and pupal stages, as shown by differences in the isozymes resolved. There was, however, a dramatic change in the adult stage, when the two species showed similar allozymes. For isocitric dehydrogenase, which showed two distinct zones, one was found to be expressed only in the adult (IDH-1) and not in the larval and pupal stages of the two species. One striking difference between the two species with respect to this locus was the existence of allelic differentiation. *M. patnalis* was differentiated for IDH^{B4} and *C. medinalis* for IDH¹⁰⁰. Another allozyme locus that showed remarkable differences between the two species was the acid phosphatase locus. *M. patnalis* showed slower migrating and *C. medinalis* faster moving isozymes.

1.19.3 Feeding behaviour of rice leaffolder on susceptible and resistant rice plants

The feeding behaviour of the rice leaffolder was recorded electronically on susceptible and resistant rice plants. Waveforms for spinning and feeding were distinctly different on leaf blades of susceptible and resistant plants. On susceptible IR 36, the insect readily folded the leaf blade and did sustained feeding (Table 1.25 and Figure 1.2). In contrast, the insect became restless on the resistant TKM 6 plant, which consequently reduced its effective feeding period. Monitoring feeding behaviour electronically is an important technique in screening rice varieties for insect pests of the chewing type.

Table 1.25 Means of electronically recorded events during 60-minute feeding by *C. medinalis* larvae on susceptible (S) and resistant (R) rice varieties.

Variety	Spinning (min)	Feeding (min)	Movement (min)	Rest (min)
TKM 6 (R)	25.3	10.8	19.8	4.1
IR 36 (S)	16.7	27.1	13.3	1.9
Difference	8.6**	-16.3**	6.5**	2.2 ^{ns}

** Significant ($P < 0.01$).

^{ns}: not significant.

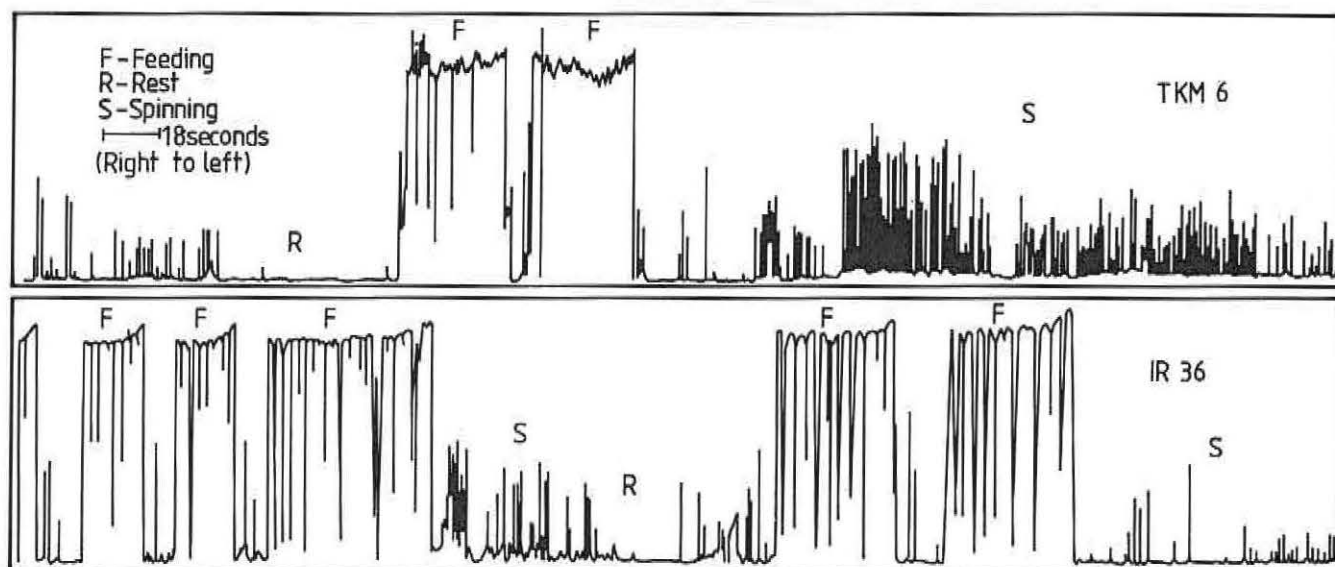


Figure 1.2 Electronically recorded waveforms during *C. medinalis* feeding on resistant TKM 6 and susceptible IR 36 rice plants.

1.19.4 Settling and feeding preferences and growth of rice leaffolder on wild rices

Wild rices are valuable sources of resistance to several insect pests and diseases of rice. We evaluated four wild rices—*Oryza australiensis*, *O. nivara*, *O. punctata* and *O. perennis*—for their resistance to the rice leaffolder *Cnaphalocrocis medinalis*. In a choice test, *C. medinalis* larvae showed a strong non-preference for settling and feeding on *O. australiensis*, *O. perennis* and *O. nivara* compared with the susceptible IR 36 rice variety (Table 1.26). A comparison of the insect's settling and feeding on wild rices and resistant TKM 6 rice variety showed that none of the wild rices were less preferred than TKM 6. The insect preferred *O. australiensis* to the TKM 6 plant for settling and feeding. First-instar larvae caged on *O. perennis* showed

significantly lower weight after 15 days of infestation than other rice varieties and wild rices.

1.19.5 Effect of nitrogen levels on the incidence of rice leaffolders on rice varieties

In a field trial at Lian, Batangas, five levels of nitrogen fertilizer (0, 30, 60, 90 and 120 kg per ha) were applied on rice varieties susceptible (IR 36), moderately resistant (IR 5865-26-1) and resistant (TKM 6) to leaffolders to study the incidence of rice leaffolders. The leaffolder damage on IR 36 and IR 5865-26-1 plants increased significantly with an increase in the application of nitrogen, whereas on TKM 6 plants, the increase in nitrogen fertilizer showed no significant increase in damage.

Table 1.26 Settling response of *Cnaphalocrocis medinalis* larvae on leaf cuts of susceptible IR 36 or resistant TKM 6 plants versus wild rices in a choice test.

Plant	Larvae settled (%)	Difference	Plant	Larvae settled (%)	Difference
IR 36	87.5		TKM 6	41.6	
<i>Oryza australiensis</i>	12.5	75.0**	<i>O. australiensis</i>	58.4	-16.8**
IR 36	65.3		TKM 6	61.1	
<i>O. nivara</i>	34.7	30.6**	<i>O. nivara</i>	38.9	22.2 ^{ns}
IR 36	70.8		TKM 6	51.4	
<i>O. perennis</i>	29.2	41.6**	<i>O. perennis</i>	48.6	2.8 ^{ns}
IR 36	51.4		TKM 6	45.8	
<i>O. punctata</i>	48.6	2.8 ^{ns}	<i>O. punctata</i>	54.2	-8.4 ^{ns}
IR 36	83.3		TKM 6	16.7	
TKM 6	16.7	66.6**	IR 36	83.3	-66.6**

** Significant ($P < 0.01$).

^{ns}: not significant.

1.19.6 Responses of rice-infesting and grass-infesting populations of brown planthopper to rice plants and *Leersia hexandra* grass

Recently a population of brown planthopper, *Nilaparvata lugens*, was found thriving on *Leersia hexandra*, a weed grass common in rice fields. We studied orientational and settling responses, feeding behaviour, metabolism of ingested food, growth, adult survival, egg production, oviposition and the hatchability of eggs of the two *N. lugens* populations on TN 1 rice plants and *L. hexandra* grass. TN 1 plants were most suited for the establishment of the rice-infesting population, while *L. hexandra* was a favourable host for the *Leersia*-infesting population. Individuals derived from one host did not thrive on the other host because of the significant reduction in feeding, food assimilation, growth, longevity and fecundity.

1.19.7 Biochemical aspects of host selection in brown planthopper populations infesting rice and grass plants

Plant volatiles extracted as steam distillates from TN 1 rice plants and *L. hexandra* grass significantly affected host selection behaviour and establishment of rice- and grass-infesting *N. lugens*. The odour of TN 1 extract attracted rice-infesting *N. lugens*, whereas that of *L. hexandra* extract attracted grass-infesting *N. lugens*. TN 1 extract was more toxic than the extract of *L. hexandra* to the first instars and newly emerged females of grass-infesting *N. lugens*. Similarly, *L. hexandra* extract was more toxic than TN 1 extract to the first instars and newly emerged females of rice-infesting *N. lugens*. Ingestion and assimilation of food by females of rice-infesting *N. lugens* were less on TN 1 plants treated with *L. hexandra* extract than on plants treated with acetone or TN 1 extracts. Intake and assimilation of food by grass-infesting *N. lugens* were also significantly reduced on *L. hexandra* plants treated with TN 1 extracts.

Gas chromatography analyses of steam distillates from TN 1 rice versus *L. hexandra* plants indicate several differences. Analysis of free sugars, soluble proteins and amino acids revealed that TN 1 plants were richer in sugars, proteins and amino acids than *L. hexandra* plants. It was concluded that rice-infesting *N. lugens*, adapted to feed on higher concentrations of sugars and amino acids, do not prefer nutritionally poor *L. hexandra* grass, and that grass-infesting *N. lugens* do not prefer nutritionally rich TN 1 rice plants.

1.19.8 Feeding behaviour of brown planthopper biotypes on resistant and susceptible rice varieties

The feeding activity of biotypes 1, 2 and 3 of *N. lugens* was monitored on TN 1 (no resistance gene), Mudgo (*bph* 1 gene) and ASD 7 (*bph* 2 gene) using an electronic monitoring device. Electronically recorded waveforms corresponding to planthopper probing, salivation and ingestion differed significantly on the leaf sheaths of susceptible and resistant rice plants. All three biotypes probed readily and fed longer on their respective susceptible varieties than on resistant varieties. The quality of food ingested and assimilated was also significantly higher on susceptible than on resistant plants.

1.19.9 Technique to purify biotype 1 population of brown planthopper

Since the biotype 1 population of the brown planthopper (BPH) is maintained on TN 1 rice plants susceptible to all three biotypes, the purity of biotype 1 is always questioned. We designed a simple technique to obtain a homogenous population of biotype 1 from single pairs of males and females selected according to their reduced ingestion and assimilation of food from resistant Mudgo and ASD 7 rice varieties. To ensure the homogeneity of the population, progenies from such pure populations of biotype 1 were monitored for three generations for their nymphal growth and adult longevity and fecundity on resistant and susceptible rice varieties.

1.19.10 Abilities of brown planthopper populations to transmit grassy stunt and ragged stunt viruses to *Leersia hexandra* and rice plants

Studies were conducted to compare the efficiency of the grass-infesting *N. lugens* population with the rice-infesting biotype 1 population to transmit grassy stunt virus (GSV) and ragged stunt virus (RSV) diseases to rice and *Leersia hexandra* grass. Grass-infesting *N. lugens* transmitted both GSV and RSV to rice as well as to *L. hexandra* grass. 52% of rice plants showed a positive transmission of GSV and 18% showed a positive transmission of RSV when grass-infesting *N. lugens* was used as a vector. *Leersia* grass also proved to be a reservoir for both GSV and RSV. *L. hexandra* plants developed the typical symptoms of longitudinal swelling in the veins and wavy leaf margins due to RSV. GSV did not cause any visible symptom, although by using the ELISA technique (enzyme-linked immunosorbent assay), we detected the virus in the infected plant. For recovery test of the viruses using the grass-infesting brown planthopper, GSV and RSV were transmitted back to rice; the percentage of such transmission was about 13%.

ICIPE-IITA RESEARCH PROJECT

The ICIPE/IITA Collaborative Research Programme on Cowpea Improvement was established in October 1985 to provide the national programmes, and ultimately the farmers, in East Africa with cowpea germplasm that will give improved yields with minimal or no insecticide application.

Primary focus was given to identifying and developing widely adapted 'field resistant' cowpea lines by conducting variety trials with insecticide sprayed and unsprayed treatments over a wide range of agro-ecologies and by locating breeding nurseries in key environments. Emphasis was placed on evaluating accessions. Over 100 different lines were included in replicated tests and 12 000 progeny rows visually evaluated during 1987. Lines identified as field resistant would have three main uses: (1) as material directly useful to the national programmes in the region (if agronomically elite and widely adapted), (2) as parents in the hybridization programme and (3) as material for further investigation into the basis/mechanisms of

resistance. A total of seven sites, including five agro-ecological zones and spanning altitudes from 10 to 1500 metres above sea level, were used. Important agronomic studies were conducted on whether a specific breeding programme is needed to develop germplasm optimally suited to intercropping and on the role of early planting in reducing seed yield losses due to pests. A summary of multilocational variety trials and agronomic investigations undertaken in 1987 is given in Table 1.27. The activities of the breeding programme in 1987 are given in Table 1.28.

1.20 ICIPE-IITA COLLABORATIVE RESEARCH PROGRAMME ON COWPEA IMPROVEMENT

J. Ehlers

1.20.1 Identification of 'field resistant' cowpea lines

Results from trials conducted in six environments with insecticide-protected and unprotected treatments suggest that the line TVx3343-Olj possesses a high level of 'field resistance' to the major pests observed in all locations—flower

Table 1.27 Summary of experiments conducted in 1987 by the ICIPE/IITA Collaborative Research Programme on Cowpea Improvement

	No. entries	Reps	Treatments	Locations*						
				A	B	C	D	E	F	G
<i>First season trials</i>										
ICIPE/IITA early maturity	12	4	Spray +/-			X	X	X	X	X
ICIPE/IITA medium maturity	12	4	Spray +/-			X	X	X	X	X
ICIPE/IITA preliminary	27	2	Spray +/-	X	X	X				
ICIPE/IITA dual purpose	16	4	Spray -	X	X	X				
ICIPE/IITA soybean	15	4	Spray -		X	X	X	X†	X†	X†
Correlation of performance	12	3	Inter/Mono Spray +/-			X				
Thrips assessment	6	4	Spray +/-	X	X					
Date of planting	2	4	Spray +/-	X	X					
<i>Second season trials</i>										
Correlation of performance	16	4	Inter/Mono Spray +/-	X	X		X			X†
Date of planting	2	4	Spray +/-	X	X					
Intercrop/density	2	4	Spray +/-		X					

* A: Mbita Point Field Station, B: Ogongo, C: Homa Bay, D: Busia, E: Katumani, F: Kampi ya Mawe, G: Mtwapa.

† Crop failure due to drought.

Table 1.28 Summary of breeding work in 1987 by the ICIPE/IITA Collaborative Research Programme on Cowpea Improvement

First rains	No. families	Second rains	No. families
F4-Ogongo	900	F5-Ogongo*	20†
		F5-Ogongo§	36†
F4-Mtwapa	800	F5-Mtwapa	400
F3-Ogongo	1920	F4-Ogongo	1332
F3-Kampi ya Mawe	1220	F4-Ogongo	215
F3-Katumani	700	F4-Ogongo	76
F2-Ogongo	41-B†	F3-Ogongo	471
			15-B
Totals	7540		4606

* Early genetic test.

† Number of entries, 2-replicate test.

‡ Bulk populations (1 ha).

§ 2-replicate SPS test.

|| Crop failure to drought.

thrips (*Megalurothrips sjostedti*) and pod-sucking bugs (mainly *Clavigralla tomentosicollis* and *Nezara viridula*). Loss in seed yield due to pests for this line averaged about 60% (Tables 1.29, 1.A4, 1.A6 and 1.A12). These results were obtained with trials of 12 to 16 of the most promising lines identified and therefore are not comparisons with susceptible checks. In most locations TVx3343-Olj also produced the highest seed yields under unsprayed conditions. This line is intermediate in flowering time and days to maturity compared with the other lines in these tests, and we do not believe the line avoids insect damage as a result of early flowering and maturing. ICV 1 and Katumani 80 also appear to have greater than average field resistance to pests, although the former line is very early to flower and mature and may avoid pest damage in some environments. Katumani 80 is highly resistant to the cowpea aphid, *Aphis craccivora*.

Detailed work is needed to verify and quantify these observations and determine the magnitude of resistance to

Table 1.29 Summary of seed yields (kg/ha) obtained without protection from insect pests for six improved cowpea lines and one local cultivar at seven environments in Kenya in 1987

Line	First season				Second season			
	Homa Bay*	Busia*	Mtwapa*	Homa Bay†	Busia	Ogongo	Mbita	Mean
TVx3343-O1j	1850(09)	300(56)	650(29)	1660(33)	680(33)	170(65)	2530	1120(37)
Katamani 80	2250(19)	140(66)	870(17)	720(55)	440(46)	200(63)	1600	890(44)
ICV 1	1960(46)	320(38)	250(66)	900(47)	540(32)	240(51)	1560	824(47)
Machakos 66	1750(32)	90(71)	310(53)	950(51)	570(41)	20(82)	1720	770(55)
IT83D-442	1640(42)	120(67)	430(56)	180(74)	430(53)	80(66)	1920	690(60)
IT82D-812	180(51)	70(71)	160(52)	220(75)	160(65)	70(71)	1440	330(64)
Kisumu market	1710(49)	70(73)	70(80)	90(80)	180(65)	30(80)	1130	470(71)
Means	1500(41)	160(61)	330(56)	570(62)	470(51)	100(69)	1760	700(57)

The percentage of loss in seed yield due to insect pests for each line is given in parentheses.

* Results combined for early and medium multilocation trials.

† Results from monocrop treatment of correlation of performance study.

individual insect pests and the basis of any resistance identified.

1.20.2 Information on production constraints in Kenya

Excellent information was obtained from multilocal trials on production constraints of the major cowpea-growing areas of Kenya. In the western half of the country, insect pests were judged to be the major production problem, while in the eastern half, drought, at least in 1987, was judged to be the major yield-limiting factor. Seed yield losses averaged 53% at Homa Bay, 66% at Mtwapa and 76% at Busia during the first season, and 69% at Ogongo and 51% at Busia during the second season. A total of seven environments, covering six agro-ecological zones, from 10 to 1500 metres above sea level, were used. Key environments, or hotspots, for field screening for particular pests and diseases were identified as follows: (1) *the Maruca pod borer* at the Coast Agricultural Research Station in Mtwapa in the first season, (2) *thrips* at the Alupe Agricultural Research Substation in Busia in both the first and second season, (3) *foliar diseases* at the Alupe Agricultural Research Substation in Busia in the first season, (4) *yield potential* at the Agricultural Research Substation in Homa Bay in the first season, (5) *drought* at a farmer's field in Ogongo in the second season, (6) *drought* at the Agricultural Research Substation in Kampi ya Mawe in the first season, and (7) *cold* at the National Dryland Farming Research Station in Katumani in the first season.

1.20.3 Early planting reduces yield losses due to pests

Date-of-planting trials were conducted at two locations in both the first and second seasons of 1987. Early planting resulted in significantly less seed yield losses due to pests and higher unprotected seed yields at two locations (Figure 1.3). Unfortunately, yield could not be determined on the two second-season experiments because of bird feeding, but pre-harvest observations suggest similar results would have been obtained. These results should be confirmed and then on-farm studies conducted to investigate the feasibility of early planting.

1.20.4 Intercropping reduces seed yield losses due to pests

In four trials conducted comparing seed yield losses due to pests under monocropping and intercropping with maize,

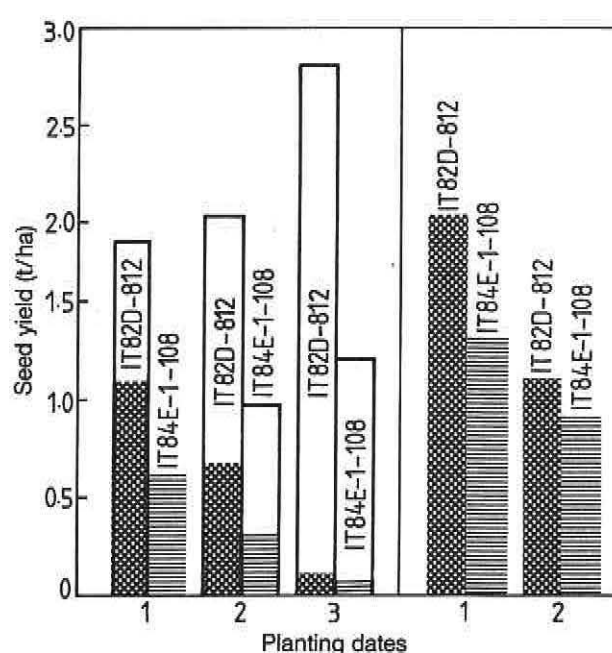


Figure 1.3 Effect of date of planting on seed yields and seed yield losses due to pests for two cowpea cultivars in two experiments conducted in Kenya in 1987.

losses were less under intercropping, although the difference was significant in only one case (Table 1.30). These results are similar to those of other workers and suggest that intercropping can help reduce pest damage. However, intercropping when used alone as a pest control strategy may not provide an economic level of protection in most locations and seasons.

1.20.5 Correlation of yields under monocropping and intercropping

The great majority of farmers in Africa grow cowpeas in association with a staple cereal, usually millet, sorghum or maize. However, most plant improvement programmes conduct varietal evaluations in monocropped trials, and it is questionable whether optimum genotypes for intercropping are identified from such trials. To provide information on the necessity of conducting yield tests under intercropping, which is more costly and less convenient (particularly in the

Table 1.30 Effect of intercropping (with maize) on percentage of seed yield loss due to insect pests in four experiments conducted in Kenya in 1986—87

Year	Experiment		No. of entries	Percentage of loss			Significant
	Season	Location		Monocrop	Intercrop	Difference	
1986	2nd	Ogongo	12	70.1	62.6	7.5	no
1987	1st	Homa Bay	12	61.8	54.6	7.2	no
1987	2nd	Ogongo	16	69.4	43.8	25.6	yes
1987	2nd	Busia	16	51.2	51.0	0.2	no

Analysis done with arcsine transformed data values.

case of preliminary evaluations when the number of lines being tested is large), correlations of seed yield and seed yield rank under monocropping and intercropping (with maize) for sets of twelve or sixteen genotypes were studied in five trials. Significant, positive simple-linear correlations (ordinary r) and Spearman's Rank Correlations were observed in six and seven, respectively, of nine possible comparisons (Table 1.31). In three of the nine comparisons the top-yielding variety under monocropping was also the top under intercropping, and in six of nine times the top-ranked variety under monocropping was among the top four entries under intercropping. Still, important differences in ranking between the two cropping systems existed in some experiments and these results suggest caution in using monocropping to evaluate materials for intercropping. Preliminary evaluations, where many accessions are screened for agronomic merit or pest resistance, can probably be done under monocropping. Given equal land and monetary resources, twice as much material can be evaluated under monocropping, and the advantage of looking at more material in the early stages of the evaluation process may

outweigh the disadvantages of possibly not detecting a line especially well suited to intercropping. Visual evaluations are more easily done, and for many characters genotypic differences are more apparent under monocropping than under intercropping. More information is needed on this subject.

1.20.6 Promising dual-purpose genotypes identified

Among 15 dual-purpose lines evaluated at three locations for leaf and seed yield, TVx1948-04f, Machakos 66 and IT83S-742-11 gave the highest combined leaf and seed yields (Table 1.A8). Machakos 66 has given good seed yields over a wide range of environments (Tables 1.A3, 1.A5 and 1.A11) and shows good resistance to foliar diseases. Unfortunately, TVx1948-04f is not preferred because of its narrow leaves, and IT83S-742-11 is not preferred because of its seed colour and rough seed coat. The latter two lines should be good parents from which to develop superior dual-purpose genotypes. IT83S-742-11 is highly resistant to the cowpea aphid, *Aphis craccivora*.

Table 1.31 Relative performance ranking under monocropping and intercropping and correlation of performance for 12 or 16 cowpea lines in 7 experiments performed in 1986 and 1987 in Kenya

Monocrop rank	Intercrop rank									
	Ogongo		Homa Bay		Busia		Ogongo		Mbita	
	S	U	S	U	S	U	S	U	U	
1	1	3	6	4	7	1	1	3	4	
2	3	1	5	7	6	3	3	5	3	
3	2	7	1	5	13	2	8	6	10	
4	4	4	4	1	2	9	2	15	2	
5	7	2	2	3	10	15	5	11	9	
6	8	5	7	2	14	13	14	2	5	
7	12	8	3	11	16	7	11	8	14	
8	6	6	8	9	12	5	7	9	12	
9	11	11	10	12	11	8	12	10	6	
10	5	9	9	6	8.5	10	9	1	1	
11	10	12	11	8	8.5	12	6	4	11	
12	9	10	12	10	1	4	4	12	13	
13					4	6	15	14	8	
14					3	14	13	16	15	
15					5	16	16	13	7	
16					15	11	10	7	16	
Correlation†										
Rank	0.74**	0.84**	0.77	0.57*	-0.14	0.52*	0.63**	0.40	0.52*	
Means	0.80**	0.75**	0.49	0.54*	-0.07	0.56*	0.66*	0.28	0.67*	

S and U refer to insecticide sprayed and unsprayed treatments, respectively.

* Significant at the 0.05 level of probability; **Significant at the 0.01 level of probability.

† Rank: Spearman's Rank Correlation Coefficient; means: simple linear correlation of entry means.

1.20.7 Germplasm development activities

Over 12 000 four-metre rows of F3, F4 or F5 generation breeding materials were grown in nurseries at five locations in 1987 (Table 1.28). These progenies were developed from more than 200 crosses between local and exotic lines and represent the largest source of genetic diversity available for use by breeders in East Africa. The main nursery site at Ogongo (1300-m altitude) covered more than six acres during the first season and included a specially planted pest 'screening nursery' with 'spreader rows' planted two weeks early at the edge of each tier in rows perpendicular to the materials under evaluation. This was done to allow pest populations to build up and distribute themselves over the field. About 900 F4 progenies and 260 germplasm accessions from Kenya were screened in this way. The whole of the nursery was grown with minimal insecticide application (one light spray at early pod-fill and one at late pod-fill to ensure adequate planting seed quality). Selection was conducted at maturity for seed yield, pod set and seed damage; these are the characters affected directly by flower thrips and pod-sucking bugs (the major pests) and were judged to be relatively efficient selection criteria.

1.20.8 Selection for specific adaptation

Two acres of nursery materials were grown at Mtwapa (near Mombasa, with a hot, humid climate and sandy soils), at Kampi ya Mawe (arid, 600-m altitude, between the coast and Nairobi) and at Katumani (cool, arid, 1500-m altitude, near Machakos). Severe droughts were encountered at the latter two sites during the first season of 1987, which we hope will allow us to select drought-resistant/tolerant progenies.

1.20.9 Flower thrips and *Maruca* larvae populations monitored

During the first season of 1987, flower thrips and *Maruca* pod-borer larval populations were monitored on five IITA lines and one local line for four weeks beginning one week before flowering. No significant differences were noted among lines for numbers of *Maruca* larvae in flowers at any sampling date or in total numbers (Table 1.A7). Significant differences in thrips numbers per flower were noted at the third sampling date, two weeks after the onset of flowering, and in mean numbers of thrips over sampling dates (Figure 1.4, Table 1.A7). The highest thrips populations were observed on the line TVx3866-04f which, interestingly, achieved the second highest (and statistically not different from the highest line) unprotected seed yield. These results suggest that maximum differences in thrips numbers among lines occur approximately two weeks after flowering and that TVx3866-04f may be a better-than-average host but may also possess better-than-average tolerance to flower thrips.

1.20.10 Pest incidence on promising lines under intercropping and monocropping

Populations of flower thrips (*Megalurothrips sjostedti*), *Maruca* pod borer (*Maruca testulalis*) and cowpea aphid (*Aphis craccivora*) were monitored on ICIPE/IITA project trials involving 16 promising cowpea lines at two locations under monocropping and intercropping (with maize) by Dr. Suleman Okech, a Postdoctoral Fellow at ICIPE, during the second season of 1987. Cultural operations, transport to field

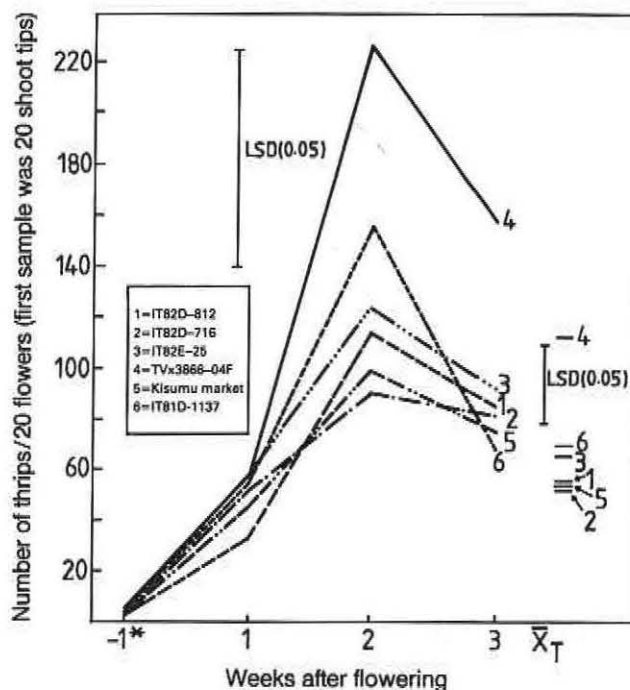


Figure 1.4 Populations of adult flower thrips on six cowpea lines at four sampling dates at Mbita Point Field Station, first season, 1987.

sites, technical assistance and casual labour were all supplied by the ICIPE/IITA Cowpea Improvement Programme. At this time, a summary of the data is not available.

1.20.11 Good-yielding soybean lines identified

TGx989-1E and TGx536-02D were identified as promising soybean lines for mid-altitude (900- to 1400-m) areas of western Kenya based on trials conducted at three locations (Table 1.A13).

1.20.12 Distribution of germplasm to national programmes

Numerous F4 and F5 progenies developed by the programme and selected IITA lines were distributed to national programmes in East Africa and Botswana. About 500 lines were sent to Tanzania (Mr. Joseph Mligo), Ethiopia (Mr. Amare Abebe) and Somalia (Mr. M. Handule). About 1000 lines were sent to Kenya (Mr. E. C. Ngugi) and Botswana (Ms. Mmasera Manthe) and 800 IITA lines were shipped to Makerere University (Dr. Sam Kyamanywa), in Uganda. Several F3 bulk populations were sent to Dr. Levi Akundabweni, of the Crop Science Department of the University of Nairobi, for genetic studies.

1.20.13 Training

Three ICIPE/IITA field assistants attended an IITA Regional Course on Cowpea/Soybean Research and Production, held in Addis Ababa from August to September 1987.

1.20.14 Conclusions and recommendations

A good body of information has been generated so that varietal recommendations to national programmes in the region can be made. ICV 1, a derivative of Katuli 107,

Machakos 66 and HB35/4/ID (the latter two lines are from the Katumani National Dryland Farming Research Station, in Machakos, Kenya) have given stable yields over a wide range of environments. Their performance has been documented not only by the ICIPE/IITA Collaborative Research Programme, but also by ICIPE-PRIP scientists and the national programme in Kenya. One additional year of testing these materials under the farmers conditions (intercropping with maize or sorghum, low density planting and no insecticide use) should be done. Katumani 80 (which is very similar to ICV 12) is highly resistant to cowpea aphid and also has given dependable yields and shown moderate field resistance to insect pests. However, it appears to be highly susceptible to bacterial blight infection and therefore would be unsuitable in many locations in which severe outbreaks of the disease occur, which include most cowpea-growing areas of Kenya.

The following lines are recommended for further testing in advanced, multilocal trials: TVx3343-0lj, IT82E-16, IT82E-25, IT83D-442, IT85F-2120, IT83S-682-7 and IT83S-850 (the latter for high-density planting in highly productive environments). IT83D-666 is especially vigorous in the coastal region (on sandy, poor soils and under high temperatures, however, it has shown susceptibility to bacterial blight in some environments and its black seeds may discourage its use by farmers). IT83S-850 is susceptible to scab (*Elsinoe phaseoli*) when environmental conditions are favourable for the pathogen. IT83D-442 is a highly productive line when protected from insect pests and when planted at moderate to high density on good soils in areas with 40 days of reliable rainfall.

The following lines have shown in more than one experiment better-than-average field resistance to insect pests: TVx3343-0lj, TVx3866-04f, IT82E-25, ICV 1 and Katumani 80. Entomologists should take a close look at these lines.

In determining priorities for host-plant-resistance breeding, more information is needed to determine the relative economic significance of different pests of cowpeas in East Africa. Visual observations suggest that flower thrips and pod-sucking bugs are the major economic pests of cowpeas in Kenya (western Kenya in particular).

By 1989 many of the breeding lines developed by the programme will be sufficiently inbred and then more in-depth entomological investigations may be initiated on materials that appear to have noteworthy field resistance or other characters of interest (such as plant architecture and fibrous pods).

An extremely large amount of genetic variability has been assembled and created through hybridization. To make use of the potential of the material will require the input of a competent breeder working in concert with entomologists.

1.20.15 Appendix: Details and results of individual trials in the first season, March-June, 1987

ICIPE/IITA early maturity multilocation yield trial.

Entries: 12

Reps: 4

Exp. design: RCB with split plots

Plot size: 4 rows, 4-m length

Treatments: 2—no protection and complete protection from pests

Locations: 5—Homa Bay, Busia, Mtwapa, Katumani, Kampi ya Mawe

This trial, which included twelve promising local and exotic cowpea lines (eight lines from IITA), was conducted at five locations in major cowpea-growing agro-ecologies of Kenya. The experimental design was a Randomized Complete Block with split plots and four replications. Insecticide protected and unprotected treatments formed the main plots, with

Table 1.A1 Seed yields, 100 seed weight and days to 50% flowering for 12 early maturity cowpea lines across five locations in Kenya, first season, 1987

Entry	Location (kg/ha)										Means (kg/ha)		Seed weight (g/100)	Days to flower
	Homa Bay		Busia		Mtwapa		Katumani		Kampi ya Mawe		P	U		
	P	U	P	U	P	U	P	U	P	U				
IT82E-16	2921	2346	829	332	1648	107	416	396	221	232	1207	683	13.4	44.7
ICV 1	3115	1958	600	316	1434	251	576	217	510	75	1276	581	11.5	40.3
HB35/4/1D	2876	2603	608	337	1197	320	525	345	214	82	1083	737	11.0	39.9
IT82E-25	2916	2055	804	254	1162	386	231	186	541	194	1131	615	11.9	45.7
IT83D-666	2797	1905	441	166	1853	389	362	141	418	116	1174	543	18.1	42.5
IT83D-442	3196	1638	708	122	1447	427	406	190	250	193	1202	514	16.4	42.3
ICV 5	2619	2012	684	257	938	39	452	338	442	194	1027	568	14.0	44.6
IT84E-1-108	2639	1764	484	134	1258	379	507	302	258	196	1029	555	24.5	41.0
IT82D-885	2924	1336	714	111	1260	148	278	217	289	144	1093	391	17.8	44.7
IT83D-356-1	2370	1192	584	173	1244	229	231	153	343	183	954	386	15.3	44.5
UCR 239	2120	1504	473	118	994	213	501	369	293	86	876	458	10.4	43.9
IT83S-850	2372	1328	418	164	808	375	333	196	332	111	853	435	18.2	43.3
Means	2379	1803	612	207	1270	272	401	254	342	151	1072	541	15.2	43.1
LSD* (0.05) entries		565		183		359		142		255		148	3.4	1.1

P: protected; U: unprotected.

Protected plots sprayed weekly, three times from early flowering using the Electrodyn sprayer and Cymbush Super insecticide.

* LSD: least significant difference.

entries as subplots. Cymbush Super (cypermethrin + dimethoate) was applied three times with the Electrolyd sprayer at 10-day intervals from early flowering. Excellent control of pests was observed at every location, although spraying at the Busia location would probably have resulted in slightly higher seed yields, since some thrips damage was evident at the time the first spray was applied.

Good growing conditions occurred at Homa Bay and Busia, while severe drought greatly affected yields at Kampi ya Mawe and Katumani, where virtually no maize and very little sorghum was harvested from local farms. Yields were only moderate in the protected plots at Mtwapa due to a combination of periodic droughting and the low fertility of the sandy soils.

In general, diseases were of minor importance, although bacterial blight (*Xanthomonas* sp.) and septoria leaf spot (*Septoria vignae*) were present on some lines at Busia. The top-performing variety overall was IT82E-16, followed closely by ICV 1 and HB35/4/1D (Table 1.A1). IT83D-666 was particularly well adapted to the coastal environment. Seed yield losses due to pest attack averaged about 50% across locations and entries (Table 1.A2). The lowest losses were observed on HB35/4/1D and IT83S-850, while the highest losses occurred on IT83D-442, IT83D-885 and IT83D-356-1. Interestingly, damage by *Maruca testulalis* to pods, measured at Mtwapa (the one location where damage was high), was highest on the two red-podded entries, IT83D-666 and ICV 5, which was contrary to the expectation of many cowpea workers. Significant differences in pod-sucking bug damage scores were not observed but are presented for interest and for comparison with work done by others.

ICIPE/IITA medium maturity multilocation yield trial.

Entries: 12

Reps: 4

Exp. design: RCB with split plots

Plot size: 4 rows, 4-m length

Treatments: 2—no protection and complete protection from pests

Locations: 5—Homa Bay, Busia, Mtwapa, Katumani, Kampi ya Mawe

Many of the environmental observations made on the early trial also pertain to this trial because the trials were planted at the same time in the same locations and in adjacent fields. The top-yielding line across locations and spray treatments was TVx3343-0lj (Table 1.A3). This was also the top-yielding line when unprotected. The line suffered less seed yield loss due to pest attack across locations and was ranked either first or second at each of the three locations for which significant differences in percentages of losses occurred (Table 1.A4). Machakos 66 and Katumani 80 were also considered promising.

More detailed investigations are warranted on TVx3343-0lj to confirm the present observations and then to explore the basis for its apparent field resistance.

ICIPE/IITA correlation of performance-intercrop/monocrop trial.

Entries: 12, 16

Reps: 3, 4

Exp. design: RCB with split plots

Plot size: 6 rows, 4-m length

Table 1.A2 Percentage loss of seed yield due to insect pests for 12 early maturity cowpea lines at three locations and the percentage of damaged pods due to *Maruca testulalis* and scores for pod-sucking bug damage at Mtwapa in Kenya, first season, April—June, 1987

Entry	Location (%)			Mean (%)	<i>Maruca</i> damage (%)	Pod-sucking damage*
	Homa Bay	Busia	Mtwapa			
HB35/4/1D	17.6	41.7	58.9	39.4	65	5.0
IT83S-850	41.5	41.2	46.2	43.0	64	4.8
IT82E-25	32.6	55.2	50.5	46.1	51	4.5
ICV 1	46.4	37.9	65.5	46.4	48	5.3
IT83D-666	30.2	47.7	61.9	46.6	83	5.5
ICV 5	25.7	43.5	78.9	49.4	71	5.8
IT82E-16	23.8	49.9	76.1	49.9	68	5.3
IT84E-1-108	34.8	57.7	56.7	49.7	69	5.0
UCR 239	32.6	64.7	61.8	53.0	59	6.3
IT83D-442	42.4	67.3	56.4	55.3	70	5.3
IT83D-356-1	44.8	56.8	65.2	55.6	64	5.0
IT83D-885	46.4	65.7	68.4	60.2	70	5.3
Mean	34.9	52.4	62.2	49.6	65	5.3
LSD† (0.05) entries		16.7		9.7	14	ns‡

Arcsine transformation used on all data values.

* Pod sucking damage rated visually, 0–9 scale.

† LSD: least significant difference.

‡ ns: entry scores not significantly different.

Table 1.A3 Seed yield, 100 seed weight and days to 50% flowering for 12 medium maturity cowpea lines at five locations in Kenya, first season, 1987

Entry	Location (kg/ha)										Means (kg/ha)		Seed weight (g/100)	Days to flower
	Homa Bay		Busia		Mtwapa		Katumani		Kampi ya Mawe					
	P	U	P	U	P	U	P	U	P	U	P	U		
TVx3343-01j	2516	1849	893	295	911	651	484	262	233	24	1095	696	14.8	41.5
Machakos 66	3071	1751	745	94	834	313	337	333	111	11	1019	500	13.5	42.3
Katumani 80	2955	2249	647	137	970	874	490	255	188	7	889	596	13.2	42.9
TVx3866-04f	2929	1032	632	155	832	384	138	199	167	10	857	519	12.6	43.6
IT84D-448	2929	1032	430	50	1187	425	150	110	33	27	916	308	18.4	44.0
IT83S-871	2395	603	403	25	1395	597	41	110	173	9	947	219	16.1	44.3
IT83S-639-4	2229	1031	947	194	239	189	318	432	359	34	779	376	15.2	43.1
Kisumu Mkt	2150	1706	667	67	978	69	48	88	252	10	966	243	15.2	48.4
IT84D-552	2884	979	383	47	1165	341	243	139	182	13	874	228	17.6	43.0
IT83S-680-9	2721	351	352	7	1025	246	0	6	247	18	929	112	15.4	44.0
IT82D-716	2494	516	565	58	816	265	0	0	0	0	775	168	14.9	43.9
IT82D-812	2870	179	500	72	498	157	161	147	161	7	682	241	14.8	42.9
Mean	2679	1107	581	100	904	376	200	173	176	14	873	364	15.2	43.6
LSD* (0.05) entries		563		145		411		180		146		136	1.3	0.9

Protected plots sprayed weekly, four times, from early flowering using the Electrodyn sprayer and Cymbush Super insecticide.

P: protected; U: unprotected.

* LSD: least significant difference.

Table 1.A4 Percentage loss of seed yield due to insect pests for 12 medium maturity cowpea lines at three locations and pod-sucking bug damage at Mtwapa, Kenya, first season, 1987

Entry	Location (%)			Mean (%)	Pod-sucking bug damage*
	Homa Bay	Busia	Mtwapa		
TVx3343-01j	8.6	55.6	28.5	30.9	4.8
Katumani 80	19.2	65.7	16.9	33.9	5.3
TVx3866-04f	31.3	62.3	40.7	44.8	5.0
IT83S-689-4	46.7	61.5	47.1	51.7	5.5
Machakos 66	32.0	70.5	52.6	51.8	4.5
IT82D-812	51.0	70.5	51.6	57.7	5.8
IT84D-448	53.4	74.1	55.0	60.8	4.8
IT82D-716	63.3	72.0	55.8	63.7	5.5
IT84D-552	61.0	74.7	58.4	64.7	5.5
IT83S-871	70.3	78.8	48.8	66.0	5.8
Kisumu Mkt	48.8	72.8	79.5	67.0	5.5
IT83S-680-9	76.8	85.8	63.7	75.4	5.0
Mean	46.9	70.4	49.9	55.7	5.3
LSD† (0.05) entries		21.0		12.1	ns‡

Arcsine transformation used on all data values.

* Pod-sucking bug damage rated visually, 0-9 scale.

† LSD: least significant difference.

‡ ns: entry scores not significantly different.

Treatments: 2—no protection, complete protection from pests, intercrop (with maize), and monocrop

Locations: 5—Homa Bay (first season), Ogongo, Busia, Mbita, (Mtwapa) (unprotected monocrop and intercrop treatments only)

The objective of this trial was to study the need for a separate breeding programme to develop varieties especially suited to intercropping. The degree to which the yield ranking of cowpea genotypes under monocropping and intercropping (with maize) are correlated was assessed as parameters that

provide information on this question. The experiments also provide information on the extent to which seed yield losses due to pests under monocropping differ from those observed under intercropping.

During the second season, the number of entries was increased from 12 to 16 to obtain a wider range of genotypes and to accommodate promising new entries (as determined from the previous season's preliminary yield trial results).

The results from the first-season trial grown at Homa Bay are presented in Table 1.A5. Good growing conditions occurred throughout the season and protected monocrop

Table 1.A5 Seed yields of 12 diverse cowpea lines when grown as a monocrop and as an intercrop (with maize), with and without insecticide protection at Homa Bay, Kenya, April–June, 1987

Entry	Protected (kg/ha)		Unprotected (kg/ha)		Mean (kg/ha)	Days to flower	Seed weight (g/100)
	Monocrop	Intercrop	Monocrop	Intercrop			
TVx3343-01j	2543	825	1662	366	1349	42	14.8
Kisumu Mkt	2474	833	85	146	885	48	13.9
At3-1/80f	2362	1145	55	179	935	45	15.3
Machakos 66	2253	858	946	360	1104	41	13.2
IT82D-812	2250	1062	219	255	946	41	13.9
TVx3866-04f	2157	808	1087	242	1074	43	11.9
IT83D-442	2067	910	176	80	808	39	15.8
ICV 5	2014	807	335	478	909	40	14.3
ICV 1	1952	735	898	530	1029	37	12.7
IT82D-889	1918	745	329	113	776	38	15.4
Katamani 80	1887	721	724	429	940	43	13.3
IT83S-850	1656	656	272	150	684	39	20.9
Mean	2128 ^a	842 ^b	566 ^{bc}	277 ^c	953	41	14.6
LSD† (0.05) entries		485			210	1	1.3
Correlation‡	$r = 0.49$		$r = 0.54^*$				

† LSD: least significant difference.

‡ Correlation between entry mean seed yields under monocropping and intercropping.

* Significant at the 0.05 level of probability.

Means followed by a different superscript are significantly different ($P < 0.05$)

yields averaged over two tons per hectare. Simple linear correlations of the means were positive and near $r = 0.50$ for both sprayed and unsprayed treatments, although only the unsprayed comparison was statistically significant. Rank correlations for both sprayed and unsprayed comparisons were statistically significant. Mean seed yield losses under intercropping were less than for monocropping (62% vs 55%), but the difference was not statistically significant (Table 1.A6).

During the second season, trials were conducted at three locations. (A fourth location was planted at Mtwapa but died from drought several weeks after emergence.) Significant positive simple linear correlations between intercrop and

monocrop entry means were observed for three of the five possible comparisons.

Date of planting trial.

Entries: 2

Reps: 4

Exp. design: RCB

Plot size: 4 rows, 4-m length

Treatments: two or three dates of planting, protected and unprotected from insect pests; planting dates isolated by five-metre strips of maize

Locations: 2—Mbita, Ogongo

The objective of these trials was to assess if seed yield losses due to insect pests could be minimized by early planting. The effect of early planting on reducing losses due to pests was clear. At Ogongo during the first season, although yield increased at the later planting dates when the crop was protected (these plantings avoided an early-season drought), yields of the unsprayed treatments decreased significantly (Figure 1.3). Unprotected seed yields were also lower at Mbita Point Field Station (MPFS) during the first season for the second planting. Since the experiment at MPFS was irrigated and diseases were not important, these results were due to heavier pest attack on the later-planted plots.

Thrips and Maruca larvae population assessment trial.

Entries: 6

Reps: 4

Exp. design: RCB

Plot size: 6 rows, 4-m length

Treatments: protected and unprotected from insect pests at Ogongo, unprotected only at Mbita; thrips populations in shoot tips and flowers assessed at weekly intervals, four times from one week before flowering at MPFS; *Maruca* larval populations in flowers assessed weekly, three times from the

Table 1.A6 Comparison of seed yield losses due to insect pests under monocropping and intercropping (with maize) for 12 diverse lines of cowpea, first season, Homa Bay, Kenya, 1987

Entry	Monocrop (%)	Intercrop (%)	Overall (%)
TVx3343-01j	33.3	47.8	40.5
Katamani 80	55.3	31.8	43.6
ICV 1	47.2	40.9	44.0
Machakos 66	50.6	45.3	47.9
TVx3866-04f	43.8	55.8	49.8
ICV 5	66.3	38.5	52.4
IT83S-850	67.9	72.7	65.3
IT82D-889	68.0	67.4	67.7
IT82D-812	75.0	61.7	68.3
Kisumu Mkt	79.5	64.7	72.1
IT83D-442	73.5	71.7	72.6
At3-/0f	81.6	66.5	74.8
Mean	61.8	54.6*	58.2
LSD† (0.05) entries	21.8		17.8

Arcsine transformation used on all values.

* The percentage of yield loss under intercropping is not significantly less than under monocropping.

† LSD: least significant difference.

onset of flowering at MPFS

Locations: 2—Mbita, Ogongo (seed yields only)

The purpose of this trial was to obtain information on flower thrips and *Maruca* larval populations, to determine if selected cowpea lines differed in the suitability as hosts to these pests, to determine the sampling time that results in the maximum expression of thrips population differences among genotypes, and to see if any relationship between yield levels and pest populations existed.

The results of this experiment are presented in Table 1.A8 and Figure 1.4. Significant differences among lines in *Maruca* larval populations were not observed at any of the three sampling dates or in total numbers. Significant differences in numbers of flower thrips were observed at the third sampling date and in total numbers of thrips. There

was, however, no apparent relationship between pest populations and unprotected seed yields.

ICIPE/IITA dual-purpose trial.

Entries: 12

Reps: 4

Exp. design: RCB

Plot size: 4 rows, 4-m length

Treatments: entries only; no protection from pests

Locations: 3—Homa Bay, Ogongo, Mbita

The purpose of this trial was to identify lines giving good leaf yields when picked using the traditional system of southwestern Kenya and giving reasonable seed yields when using no insecticides. The trial was planted and seed yields obtained at three locations, but leaf yields were obtained only

Table 1.A7 Seed yields of selected cowpea lines when grown with and without protection from pests at Ogongo, unprotected seed yields, seed weight, number of flower thrips and *Maruca* pod-borer larvae at Mbita Point Field Station (MPFS), South Nyanza, Kenya, April—June 1987

Entry	Ogongo (kg/ha)		MPFS (kg/ha)	Seed weight (g/100)	Adult thrips* (no./flower)	<i>Maruca</i> larvae† (no./flower)
	Protected	Unprotected	Protected			
IT82D-812	2787	892	776	14.8	5.9	0.33
Kisumu Mkt	2312	261	228	14.9	5.6	0.23
IT82E-25	2260	1529	1363	14.8	6.9	0.19
TVx3866-04f	2153	1326	1251	12.5	11.1	0.20
IT82D-716	1922	510	638	16.8	5.6	0.33
IT81D-1137	1676	433	399	26.8	7.0	0.29
Mean	2186	825	776	16.8	7.0	0.26
LSD‡ (0.05) entries		511	442	0.8	3.0	ns§

* Mean from four replicates from four weekly samples (the first sample comprising 15 shoot tips and the three following samples comprising 20 flowers each).

† Mean from four replicates from three weekly samples, 20 flowers per sample.

‡ LSD: least significant difference.

§ ns: not significant.

Table 1.A8 Mean leaf dry weight for four weekly pickings (traditional picking procedure), seed yield and seed weight for 15 entries over two locations in South Nyanza, Kenya, April—May 1987 (no insecticides used)

Entry	Picking (kg/ha)				Total (kg/ha)	Seed yield (kg/ha)	Seed weight (g/100)
	First	Second	Third	Fourth			
Kasino 1	98	245	381	142	847	106	17.4
TVx1948-04F	90	263	389	119	860	614	14.0
IT85F-2841	100	252	361	146	859	168	15.8
Kisumu local	99	215	313	160	788	277	15.2
At3-1/80f	105	283	271	114	773	344	15.1
IT85F-986-5	92	271	373	29	765	596	17.5
Machakos 66	165	257	311	31	765	926	13.4
IT85F-1993	90	241	394	33	758	354	19.8
IT84D-368	94	262	336	56	748	292	15.8
IT83S-742-11	101	220	351	73	744	854	13.4
IT85F-1991	101	281	301	51	734	519	19.2
IT85F-2020	101	250	315	17	683	480	20.0
Mbita local	70	170	309	117	665	113	11.0
ICV 14	67	182	331	34	614	633	13.4
ICV 6	93	204	258	46	601	506	12.2
Mean	130	320	444	104	998	452	15.5
LSD* (0.05)	15	23	45	25	60	272	1.5

* LSD: least significant difference.

at Mbita and Ogongo. At weekly intervals from 30 days after planting, four leaf pickings were made by local women using the traditional picking method of the area. Leaves were sun-dried for several days in fine mesh bags, then dried overnight in an oven before weighing. The highest leaf yields were obtained by TVx1948-04f, IT85F-2481 and by the local line, Kasino 1 (Table 1.A8). However, the highest seed yields were produced by Machakos 66, a line that also produced good amounts of high-quality, disease-free leaves. The highest total production (weight of leaves and seeds) was achieved with Machakos 66 (1691 kg per ha), followed by IT83S-742-11 (1598 kg per ha) and TVx1948-04f (1474 kg per ha). Machakos 66 can be recommended for use by farmers, although it is not altogether clear what proportion of leaves to seeds, assuming there is an upper limit to total biomass yield, most farmers would desire. Because of the heavy seed yield losses due to post-flowering pests in cowpeas in the region, demand for seed is probably greater than that for leaves in many communities. As a group, the local cultivars had the highest leaf yields at the final picking, suggesting they would have continued to produce leaves and may have achieved highest leaf yields in the trial. The low seed yields of the local lines are most likely a consequence of their later flowering and maturity, which exposes them to higher pest populations, although it has been suggested that unconscious

selection for susceptibility may have occurred, because farmers normally choose plants producing the most vegetation, which in many cases would be those genotypes producing the fewest pods.

ICIPE/IITA preliminary yield trials.

Entries: 27

Reps: 2

Exp. design: RCB

Plot size: 2 rows, 4-m length

Treatments: 2—no protection and complete protection from pests

Locations: 4—Homa Bay, Ogongo, Mbita, Mtwapa (see below) (no protected treatment at Mbita)

This trial was composed of a wide range of materials that looked promising based on single-row observations the previous season at Ogongo. Reasonably good growing conditions were encountered at all locations, although bacterial blight infestation was severe at Ogongo. This allowed rating for resistance to be done (Table 1.A9). Particularly susceptible lines included Katumani 80, IT84D-434, CB 5, IT85F-867-5 and IT85F-867; these lines suffered early defoliation and obvious yield loss. IT83D-442, IT85F-

Table 1.A9 Seed yields and other agronomic information on 27 lines of cowpea at three locations in Kenya, first season, 1987

Entry	Location (kg/ha)				Mean (kg/ha)	Seed weight (g/100)	Days to flower	Bacterial blight*
	Mbita U	Ogongo U	P	Homa Bay P				
IT83S-682-7	1340	1334	2602	3405	2170	15.5	46.3	1.0
IT85F-2674	1240	1831	3189	2264	2131	14.9	52.0	0.3
IT85F-2120	1044	1671	2904	2648	2067	15.5	45.8	0.3
KVu 285/2	372	1819	2346	3234	1943	9.1	47.8	0.8
IT84S-2155	1770	1103	2106	2627	1901	16.9	46.0	1.0
ICV 1	1376	1773	2129	2227	1876	12.0	39.0	1.0
IT85F-2076	1074	1352	2134	2695	1814	17.9	47.3	2.3
IT85F-1992	1242	839	2261	2889	1808	18.9	46.8	1.0
IT84S-2157	1799	873	1659	2458	1697	17.1	45.8	1.3
Katumani 80	1636	1404	1419	2301	1690	11.7	47.8	4.3
IT83D-442	1681	612	1112	3055	1615	15.9	41.0	3.3
IT85F-2694	796	852	1541	2786	1494	17.6	47.5	2.0
IT83D-219	823	1181	1638	2314	1489	16.2	47.3	0.3
IT85F-2805	1044	625	1487	2635	1448	11.7	46.5	1.8
KVu 356	198	972	2522	1708	1350	17.5	50.5	1.5
IT85F-867	1259	476	895	2577	1302	12.8	45.3	4.0
IT84S-2163	1167	652	1214	2173	1302	12.7	47.0	0.8
IT83S-891	923	724	1259	2009	1229	12.0	46.5	0.8
ICV 5	876	1019	772	2226	1223	12.7	43.8	2.5
IT85F-2269	640	730	1755	1690	1204	21.6	45.5	1.5
IT85F-2614	822	809	1237	1947	1204	16.0	44.8	0.5
IT85F-2227	1018	766	732	2055	1143	14.3	46.3	0.8
IT85F-867-5	1016	269	796	2311	1098	15.3	44.8	5.0
CB 5	67	400	1273	2572	1078	20.6	42.5	4.5
IT85F-2410	1063	292	1107	1847	1077	15.4	42.5	3.3
IT84S-2231-5	1114	229	760	1825	982	18.1	43.0	1.5
IT84D-434	393	170	478	1072	512	14.2	52.8	7.5
Mean	1027	917	1605	2354	1466	15.3	46.1	2.0
LSD† (0.05) entries	819		780	1056	413	1.1	1.3	1.9

U: unprotected; P: protected.

* Bacterial blight severity rated visually, 0-9 score at Ogongo.

† LSD: least significant difference.

2410, ICV 5 and IT85F-2076 showed moderate symptoms and yields may have been affected (Table 1.A9).

The top-yielding line overall was IT83S-682-7, followed by IT85F-2674 and IT85F-2120 (Table 1.A9). The top-yielding lines under unprotected conditions over two environments were IT85F-2674, ICV 1 and Katumani 80. The top-yielding entries under protected conditions were IT83S-682-7, IT85F-2120 and IT85F-2674. Protected plots were sprayed three

Table 1.A10 Agronomic performance of 31 local and exotic cowpea lines at the Coast Agricultural Research Station, Mtwapa, first season, 1987

Entry	Seed yield (kg/ha)	Seed weight (g/100)	Days to flower
UCR 243	1462	17.0	47.0
IT82E-27	1450	13.6	44.5
AT3-1/80f	1318	17.4	44.5
ICV 11	1260	13.4	41.0
IT84D-460	1188	21.4	45.0
IT84S-2246	1131	18.8	45.0
IT82D-789	1110	16.7	42.0
ICV 6	1100	11.7	39.0
IT84S-2231-15	1080	22.3	40.0
IT81D-1137	1077	22.2	44.0
ICV 12	1066	14.0	42.0
IT82D-716	1062	14.0	45.0
IT83D-442	1043	15.7	41.5
IT81D-832	1028	26.0	44.5
IT84E-124	1019	17.2	40.5
ICV 7	1003	13.3	43.5
UCR 188	985	14.9	42.5
IT81D-1069	964	21.9	46.5
UCD 7964	917	20.5	43.5
IT82D-453-2	894	17.3	45.5
UCR 187	851	12.8	43.5
TVx3236	846	12.4	46.0
IT82D-875	804	14.5	45.5
ICV 9	774	12.8	45.0
ICV 8	716	9.7	42.5
IT84D-434	683	14.6	44.5
CB 5	639	22.5	44.0
IT82D-889	590	14.8	40.0
ICV 5	562	12.9	42.5
IT84D-453	437	12.8	45.5
UCR 194	394	9.4	41.0
Mean	951	16.1	43.5
LSD* (0.05) entries	436	3.1	2.9

* LSD: least significant difference.

times at 10-day intervals from flowering with Cymbush Super and using the Electrobyn sprayer.

The purpose of the trial at Mtwapa was to look at a wide range of genotypes (and not necessarily those lines showing promise in mid-altitude locations where most of the initial evaluations were carried out). Six lines from the University of California programmes at Davis and Riverside, seven lines from ICIPE and 18 diverse lines from IITA were compared. The trial was protected by two applications of Cymbush Super applied at flowering and mid-pod-fill using the Electrobyn sprayer. UCR 243, IT82E-27, AT3-1/80f and ICV 11 were among the top-yielding lines (Table 1.A10).

ICIPE/IITA soybean variety adaptation yield trial.

Entries: 15

Reps: 4

Exp. design: RCB

Plot size: 4 rows, 4-m length

Treatments: entries only; no protection from pests

Locations: 6—Ogongo, Homa Bay, Busia, Mtwapa, Katumani, Kampi ya Mawe

Severe drought, which reduced cowpea yields to between 350 and 450 kg per ha in protected plots at Kampi ya Mawe and Katumani, resulted in a soybean crop failure at these locations. The soybean crop also failed at Mtwapa, probably because of periodic droughting on poor, sandy soils and intense heat. Plants at Mtwapa were very stunted, being only about 15 cm after more than 80 growing days. Good growth occurred at the other three locations, although a late-season drought at Busia probably reduced yields of the later maturing entries on this trial.

Over the locations where crops were obtained, TGx989-1E and TGx536-02D gave the highest yields, 1550 and 1493 kg per ha, respectively (Table 1.A13). No diseases were noted and nodulation was good on all promiscuous varieties. However, yields probably were affected at the Ogongo location by pod-sucking bugs, in particular *Clavigralla tomentosicollis* and *Nezara viridula*. A crop of unsprayed cowpeas was planted adjacent to this experiment and pod-sucking bugs appeared to have migrated onto the soybeans at the maturity of the cowpeas. Heavy pod-sucking bug damage was noted on pods that developed relatively late (high on the plant) and was more pronounced on late-maturing entries.

Table 1.A11 Seed yields of 16 cowpea genotypes when grown under monocropping and intercropping (with maize) and when protected and not protected from insect pests at two locations and unprotected at a third location during the second season, Kenya, 1987

Entry	Busia (kg/ha)				Ogongo (kg/ha)				Mbita (kg/ha)		Days to flower (g/100)	Seed weight
	Protected		Unprotected		Protected		Unprotected		Protected	Unprotected		
	Mono	Inter	Mono	Inter	Mono	Inter	Mono	Inter	Mono	Inter		
IT83D-442	1513	708	432	257	428	81	80	33	1929	663	39	16.9
ICV 1	1026	720	542	219	661	84	244	59	1563	427	37	13.1
Katamani 80	1302	611	435	227	730	58	203	54	1604	494	43	12.5
Machakos 66	1134	677	568	283	617	105	22	50	1719	809	43	14.0
IT82E-25	1570	683	437	135	460	58	102	31	2085	500	42	13.2
TVx3343-01j	1191	677	682	350	828	88	170	53	2535	722	42	12.9
TVx3866-04f	1325	676	487	109	595	67	88	39	1917	416	43	13.7
IT83S-682-7	1132	872	260	237	676	91	79	84	2063	747	42	16.1
KVu285/2	1116	742	286	171	698	78	41	17	833	209	44	10.3
IT85F-2674	1218	464	318	206	761	136	72	55	1289	358	42	16.2
IT85F-2120	1338	634	352	227	848	155	84	39	2544	713	42	15.5
IT82D-812	1055	781	161	200	673	78	69	31	1440	531	42	15.6
Kisumu Mkt	1331	814	193	108	758	102	31	25	1125	544	46	15.2
ICV 5	1211	661	285	268	620	94	167	19	1781	484	39	15.9
IT82E-16	1204	667	557	305	838	125	94	63	1740	561	42	15.4
IT83D-666	1017	589	242	119	488	50	64	22	2042	517	39	19.6
Mean	1230	686	468	257	667	91	101	42	1763	543	42	14.8
LSD† (0.05)				226				165		394	2	1.4
Correlation‡	$r = 0.07$		$r = 0.56^*$		$r = 0.66^*$		$r = 0.28$		$r = 0.67$			

* Significant at the 0.05 level of probability.

† LSD: least significant difference.

‡ Correlation between monocrop and intercrop mean yields.

Table 1.A12 Percentage of loss due to insect pests under monocropping and intercropping (with maize) for 16 cowpea lines at two locations in Kenya, second season, 1987

Entry	Location (%)					
	Ogongo		Busia		Means (%)	
	Monocrop	Intercrop	Monocrop	Intercrop	Monocrop	Intercrop
TVx3343-01j	65.1	32.9	30.5	33.3	47.8	33.1
ICV 1	50.6	34.5	31.5	52.7	41.1	43.6
Katamani 80	62.6	17.5	46.4	44.2	54.5	30.8
IT82E-16	71.3	42.9	40.8	42.2	56.0	42.6
IT83S-682-7	70.4	21.7	58.0	54.5	64.2	38.1
IT82E-25	59.3	35.6	53.8	60.4	56.5	48.0
IT83D-442	66.3	51.1	53.2	41.6	59.7	46.4
IT85F-2674	72.8	43.5	59.3	41.1	66.1	42.3
Machakos 66	82.4	49.3	40.6	44.7	61.5	47.0
TVx3866-04f	71.2	41.7	47.4	62.9	59.3	52.3
ICV 5	63.2	62.5	58.9	46.6	61.0	54.5
IT85F-2120	73.9	56.1	55.7	49.0	64.8	52.6
IT83D-666	75.1	40.1	58.4	61.7	66.8	50.9
IT82D-812	71.3	50.3	64.7	54.4	68.0	52.4
KVu285/2	76.3	63.3	56.0	60.4	66.1	61.9
Kisumu Mkt	79.5	57.1	64.9	67.1	72.2	62.1
Mean	69.4 ^a	43.8 ^b	51.2 ^b	51.0 ^b	60.3 ^a	47.4 ^b
LSD* (0.05) entries		25.9		18.0		15.7

Arcsine transformation used on all data values.

* LSD: least significant difference.

Means followed by a different superscript letter are significantly different ($P < 0.05$).

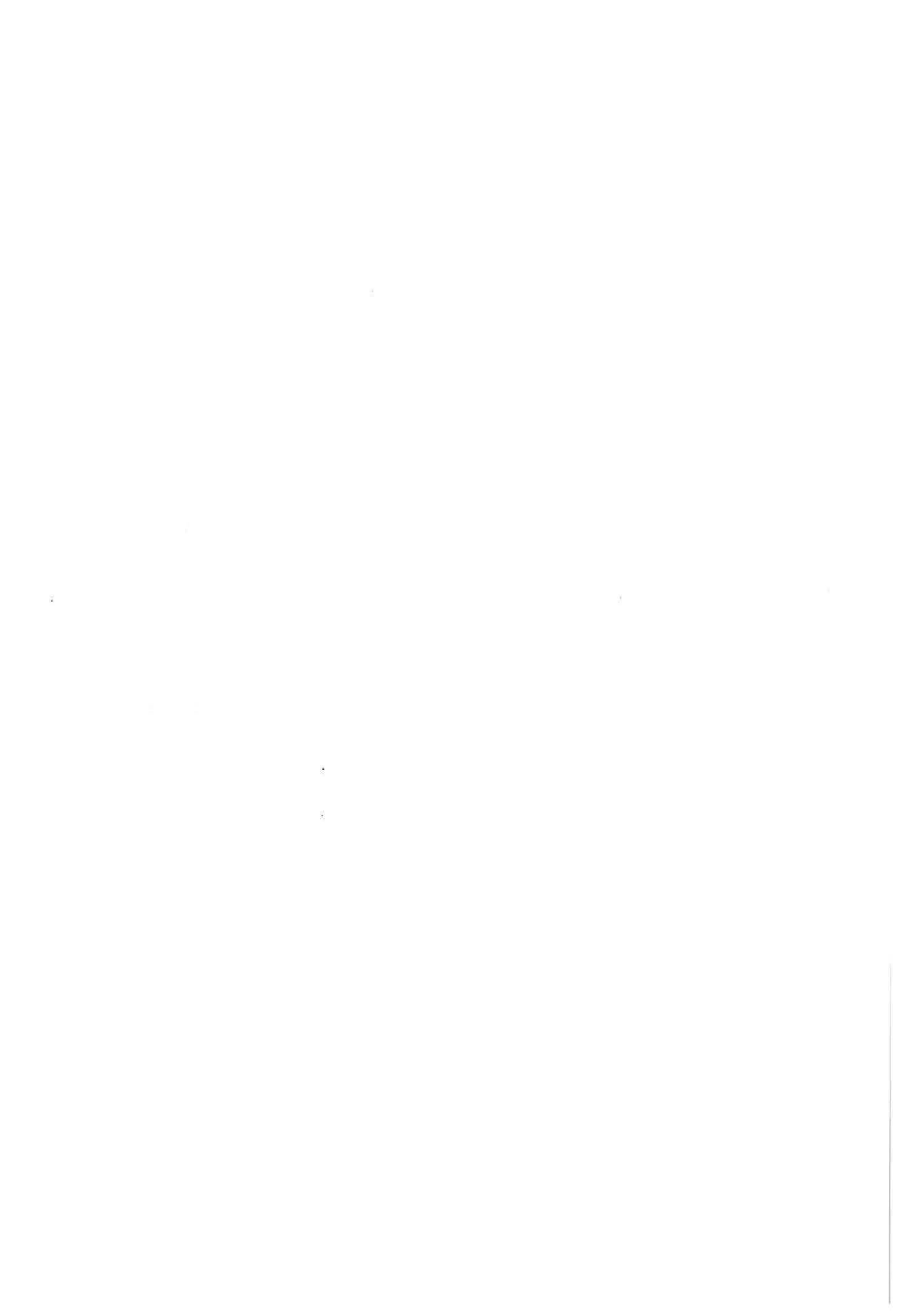
Table 1.A13 Seed yield and days to flower for 15 soybean lines at three locations in Kenya, first season, 1987

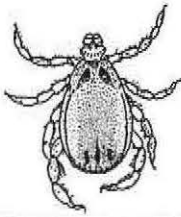
Entry	Location (kg/ha)			Mean (kg/ha)	Days to flower
	Ogongo	Homa Bay	Busia		
TGx989-1E	1680	1877	1093	1550	56.0
TGx536-02D	1947	1750	781	1493	35.0
TGx539-5E	1401	1233	956	1197	39.3
TGx987-1E	1221	932	877	1010	39.0
TGx854-36D	1096	941	977	1004	35.8
TGx989-5E	794	758	1416	989	36.0
TGx993-1E	1161	683	926	923	36.3
TGx981-1E	762	832	1149	914	36.0
TGx814-21E	1312	663	677	884	41.0
TGx311-41D	1107	572	867	849	36.0
Jupiter	514	776	931	740	38.3
TGx814-26D	1088	406	683	726	42.0
TGx99-2E	1088	734	325	716	36.8
TGx984-4E	604	150	722	492	38.8
TGx954-94E	401	295	412	369	41.8
Mean	1078	840	853	924	37.9
LSD* (0.05) entries	595	478	550	308	3.7

* LSD: least significant difference.

LIVESTOCK TICKS RESEARCH PROGRAMME

- 2.1 The presence of antibodies in naive cattle and rabbits to fully fed homogenate of *Rhipicephalus appendiculatus* ticks **40**
- 2.2 Immunization of rabbits by tick infestation and salivary gland antigens: lack of booster effects **42**
- 2.3 The induction of artificial immunity in sheep to *Rhipicephalus appendiculatus* antigens **42**
- 2.4 Correlation of host resistance with intradermal response **43**
- 2.5 Tick population modelling **43**
- 2.6 Survival of *Rhipicephalus appendiculatus* on the ground **44**
- 2.7 Ecological studies on *Amblyomma variegatum* **44**
- 2.8 Population dynamics of livestock ticks on Rusinga Island **45**
- 2.9 Tsetse and trypanosomiasis survey on Rusinga Island **47**
- 2.10 The population of *Rhipicephalus appendiculatus* and associated species on cattle in western Kenya **48**
- 2.11 Development and survival of *Rhipicephalus appendiculatus* laboratory and local field strains in the field **48**
- 2.12 Tick-host relationship on Rusinga Island **49**
- 2.13 Resistance induced by the laboratory and field-derived strains of *Rhipicephalus appendiculatus* **49**
- 2.14 Cattle productivity on Rusinga Island **49**
- 2.15 Cattle disease survey on Rusinga Island **50**
- 2.16 Selecting Boran cattle for tick resistance **51**





2

Livestock Ticks Research Programme

The Livestock Ticks Research Programme (LTRP) studies the problems caused by ticks of livestock, with the prime objective of developing cost effective outreach programmes that will suppress and control tick population levels. The programme's three main areas of study are the development of a vaccine against the most common tick species in East Africa, the strategic use of minimal amounts of acaricide and the examination of the inherited and acquired characteristics conferring tick resistance.

In 1987 our laboratory studies concentrated on identifying, purifying and testing antigens for biological activity when used as vaccines against ticks. We focussed attention on the midgut as the most promising source of antigens, and a range of proteins and protein mixtures has been prepared from this source, some of which have been tested and shown to have anti-tick activity. In the field the effects of vaccinated sheep on paddock-pasture tick levels have been shown to be very significant.

*The collection of data necessary for building a model suitable for use as a tick management tool has been intensified. Survival and development studies on *Rhipicephalus appendiculatus* and *Amblyomma variegatum* are being carried out in parallel with the electronic collection of climate data in three biotypes; two complementary sites have been identified for similar studies. Tick population studies on Rusinga Island and at Lolgorien were continued, and ecological studies on *R. appendiculatus* and host resistance have quantified the level of host resistance in an endemic non-controlled environment. This has led to the development of a first-generation resistance index for the rapid assessment of host immunity status.*

The studies on Rusinga Island were again this year reinforced by social economic studies. The latter are now providing a valuable interface between the programme scientists and the agricultural community and are helping to define and influence tick management strategies.

A major study into the phenotypic expression of tick resistance was begun at the Boran National Stud Farm, at Rumuruti. Tick population burdens were quantified monthly and the animals ranked for resistance to the major tick species. Ranking was shown to be repeatable on an individual animal basis from month to month, and a simple visual assessment that can be used for culling is being developed. Several physical characteristics did not correlate with resistance, contrary to reports of other studies. There was a correlation between resistance ranking and response to the intradermal injection of antigen. Bulls and heifers were selected to form two breeding herds that will become the basis of studies on the genetics of host resistance, in collaboration with Texas A&M University.

2.1 THE PRESENCE OF ANTIBODIES IN NAIVE CATTLE AND RABBITS TO FULLY FED HOMOGENATE OF *RHIPICEPHALUS APPENDICULATUS* TICKS

A. O. Mongi, P. B. Capstick, L. H. Otieno,
T. R. Odhiambo, C. A. Aganyo and R. M. Newson

This study was undertaken following the observation that when ticks were fed on tick-naive rabbits, the latter showed immediate skin reactions accompanied by serous exudate, and the ticks themselves demonstrated reduced engorgement weights. These effects are similar to those shown by rabbits immunized with tick-derived immunogens.

Precipitating antibodies have been reported after haematophagous arthropod infestations. No detailed information exists on the isolation and characterization of the antigens, or on any methodology for differentiating these antigens from tick antigens that provoke a protective immune response in the host against feeding ticks. In this study we attempted, therefore, to examine naive bovine and rabbit sera for antibodies against soluble whole *R. appendiculatus* tick extract. Attempts were also made to isolate, purify and characterize those antigens recognized by the naive animal sera. In addition, we studied antigens from other haematophagous arthropods such as tsetse, stomoxys and tabanid flies.

Precipitating antibodies were detected by immunodiffusion in sera of 10 tick-naive cattle and 30 tick-naive rabbits with soluble whole-tick antigen extract. In addition,

the tick-naive animal sera also reacted with antigen extract prepared from tsetse fly *Glossina pallidipes*, stomoxys and tabanid flies (Figure 2.1). The precipitins in each case appeared to be the same. Sera from rabbits immunized with solubilized whole-tick antigen extract also gave precipitating antibodies when tested against the three-fly antigen preparations.

The elution profile of the dissociated and dialyzed antigen-antibody complexes obtained from the agarose gel precipitates fractionated on the Sephacryl S-200 column is shown in Figure 2.2. Three main protein peaks were detected by absorption at 280 nm. SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analysis of each of the resolved fractional peaks is shown in Figure 2.3. Sixteen protein subunits were demonstrated and are presented in Table 2.1. It was also observed that some of the protein subunits recognized from the whole-tick extract by the naive animal sera correspond to those tick midgut protein subunits recognized by rabbit IgGs immune to successive tick infestations presented (Table 2.2). In all cases, the 70 000, 58 000 and 30 000 dalton proteins were readily detected.

It was concluded that these antibodies may be detected by immunodiffusion; this could be a useful simple technique with which to screen experimental animals. Such a simple technique may also help researchers select animals from the field that have not been exposed to ticks or other ectoparasites. This finding emphasizes the need for careful selection of antigens to be used against feeding ticks. It is possible that these non-specific antibodies may affect the evaluation of antigens for immunization.

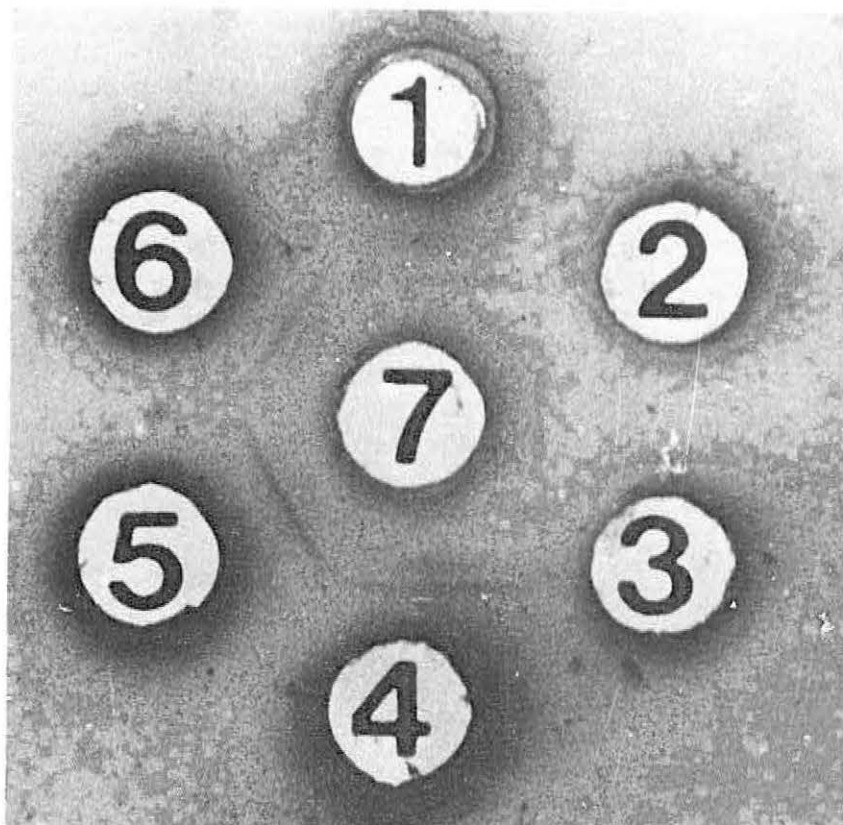


Figure 2.1 Immunodiffusion patterns of whole-antigen extract derived from *R. appendiculatus* ticks, tsetse (*Glossina pallidipes*), tabanid and stomoxys flies placed in peripheral wells 1, 6, 5 and 4, respectively, against naive rabbit sera to ticks placed in the central well. Wells 2 and 3 were filled with physiological buffered saline.

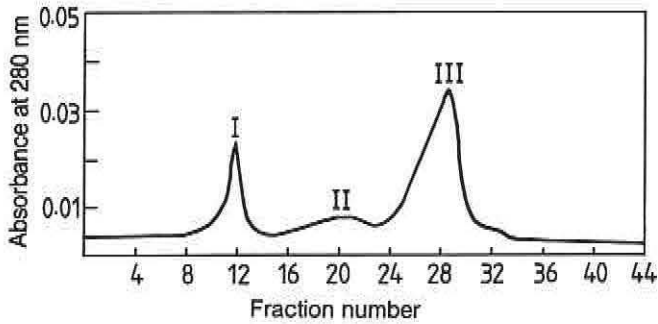


Figure 2.2 Chromatographic presentation of an elution profile of antigen-antibody immunoprecipitate dissociates passed through a 1.6×70 cm column of Sephacryl S-200. The column was eluted at a flow rate of 15ml/h with 0.15-M PBS pH 7.5; 2-ml fractions were collected and the eluted proteins detected at 280 nm.

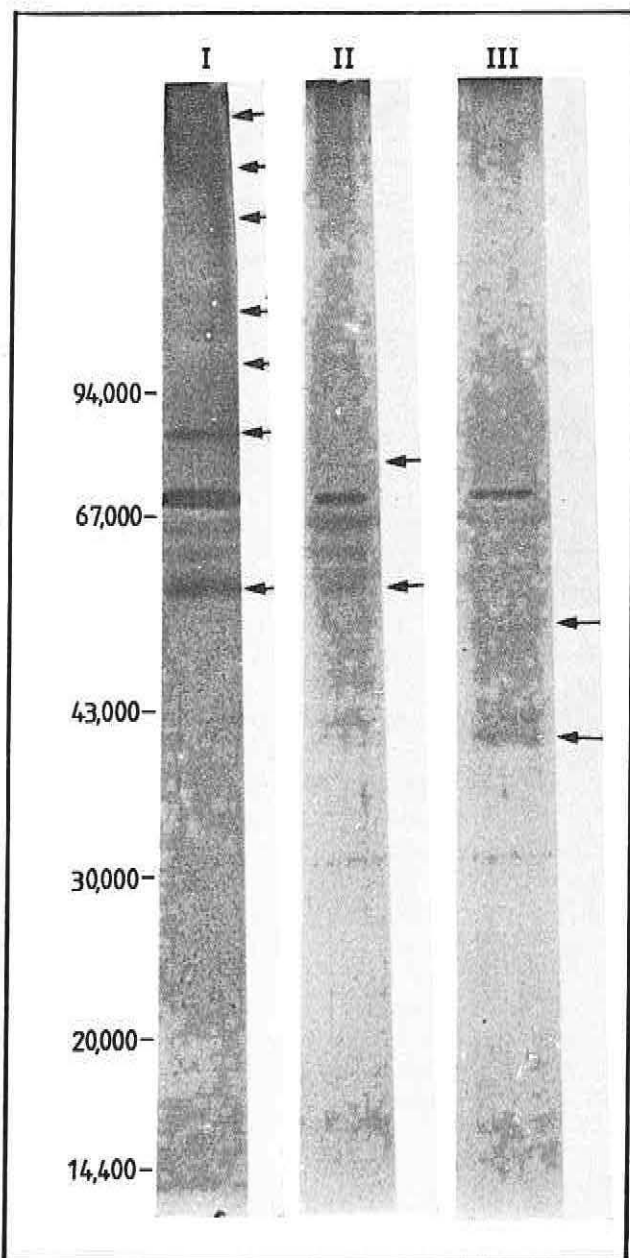


Figure 2.3 5%-20% gradient SDS-PAGE analysis of protein subunits, dissociated from antigen-antibody complexes from agarose gels. On the left are the molecular weight markers. The lanes marked I, II and III correspond to the peaks demonstrated in Figure 2.2.

Table 2.1 Comparison of the protein subunits obtained from three peaks of immunoprecipitate dissociates resulting from immunoreaction between naive rabbit sera to *R. appendiculatus* soluble whole-antigen extract obtained from Sephacryl S-200 fractionation, the proteins resolved on a 5%-20% gradient SDS-PAGE and silver stained

Estimated molecular weight (daltons)	Peaks		
	I	II	III
170000	++++	—	—
155000	++++	++	+
134000	++	—	—
110000	+	—	—
90000-92000	±	±	±
82000	++++	—	—
78000	—	++	—
70000	+++	+++	+++
68000	+	+	+
62000	+	+	+
60000	+++	++	++
58000	+	+	++
56000	+	—	+
54000	—	+	+
43000	+	+	++
40000	+	+	+
32000	+	+	+
30000	+	+	+
22000	+	+	+

— Protein band absent.

± Protein band not clearly distinguished.

+ Protein band present but faint.

++ Protein band prominent.

+++ Protein band very prominent.

++++ Protein band very prominent but smearing.

Table 2.2 *R. appendiculatus* tick midgut antigens recognized by purified IgGs from rabbits immune to tick infestations and those from soluble whole-tick antigen extract recognized by purified naive rabbit IgGs to tick infestations

Molecular weight (daltons)	Antigens recognized by	
	Immune serum IgGs (mol wt daltons)	Naive serum IgGs (mol wt daltons)
160000	—	—
155000	—	155000
—	—	130000
115000	—	120000
—	—	105000
90000-92000	—	90000-92000
79000	—	78000
67000	—	70000
62000	—	—
60000	—	60000
—	—	56000
54000	—	54000
—	—	52000
48000	—	—
43000	—	43000
40000	—	39000
—	—	30000
25000	—	—
—	—	17000
—	—	16000
12000	—	—

— = absent.

2.2 IMMUNIZATION OF RABBITS BY TICK INFESTATION AND SALIVARY GLAND ANTIGENS: LACK OF BOOSTER EFFECTS

M. Nyindo, R. Chesang and P. Muteria

In the *ICIPE 1986 Annual Report* we noted the usefulness of salivary gland antigens (SGA) as a vaccine antigen that reduced the engorged weights of adult ticks, nymphs and larvae. Immunized rabbits reduced the engorged weights of adult ticks by 35% compared to the controls. In 1987 we investigated the effects of SGA on animals that have had a previous exposure to ticks by infestation. We also investigated the effects of infestation on animals that had been vaccinated with SGA. Our interest was to discover whether SGA would induce a booster effect on rabbits previously sensitized by infestation and vice versa.

Twenty-four rabbits were used in the experiment. They were divided into two equal groups. *Group 1*. Animals in this group were divided into a further three groups of four rabbits each: Group I animals were infested once with 30 male and 30 female adult *Rhipicephalus appendiculatus*; Group II animals were inoculated with 5 mg soluble protein of SGA in incomplete Freund's adjuvant and given a booster injection three weeks later; Group III animals were treated similarly to the second group, but three weeks after the booster injection the rabbits were infested once with 30 male and 30 female *R. appendiculatus*. Three weeks after this sensitization all 12 animals were challenged with 30 male and 30 female ticks, 100 nymphs and 100 larvae. The engorged weights of adult ticks, nymphs and larvae were determined. As shown in Figure 2.4, the control weights of engorged nymphs were 8.0 mg. At challenge the Group I rabbits had developed resistance and nymphal engorged weights were reduced to 2.4 mg. Animals that had received two injections of SGA (Group II) developed immunity to larval infestation and reduced engorged weights to 5.0 mg. Those rabbits that had two vaccinations with SGA followed by one infestation (Group III) also mounted a protective response and reduced the nymphal engorgement weights from 8.0 mg to 3.0 mg.

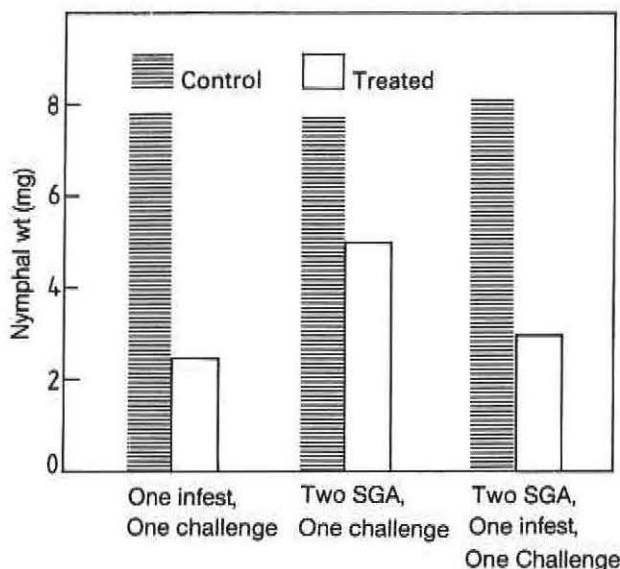


Figure 2.4 The effects of three immunization regimens on the engorged weight of *R. appendiculatus* nymphs.

Group 2. In Figure 2.5 are shown the effects on nymphal engorgement weights after one infestation (Group I), three inoculations with SGA (Group II) and one infestation followed by three inoculations with SGA (Group III). One infestation induced an immune response that reduced the engorged weights of nymphs from 8.0 mg to 3.8 mg. Three injections with SGA produced similar results. In the third category, one infestation followed by three injections with SGA did not augment the immune response above that induced by one infestation.

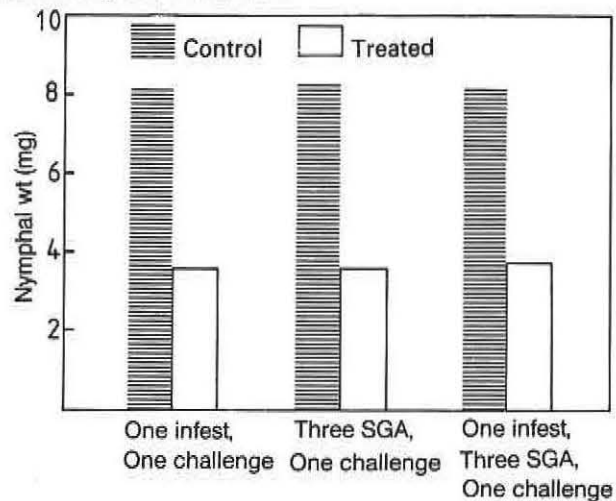


Figure 2.5 The effects of three immunization regimens on the engorged weight of *R. appendiculatus* nymphs.

The following data can be interpreted from the data.

- SGA is a potent immunogen that induces a 50% reduction in engorged weights of nymphs.
- One infestation with adult *R. appendiculatus* produces a marked effect on challenge with nymphs (reduction in engorged weights from 8.0 mg to 2.4 mg).
- A prior exposure of rabbits to adult *R. appendiculatus* followed by three injections with SGA has no booster effect on the already established immune response. Similarly, a previous experience of rabbits to SGA followed by infestation does not improve the immune response above that imposed by one infestation.
- The data show that SGA and infestation produce comparable but independent immune responses in rabbits. The mechanisms mediating one response are probably different from the mechanisms responsible for the other. It is also possible that each antigenic exposure induces a threshold response that cannot be altered by another (perhaps related) antigen. The significance of this data in relation to possible vaccination of livestock with SGA and the interaction of this immunogen with natural infestation should be investigated further.

2.3 THE INDUCTION OF ARTIFICIAL IMMUNITY IN SHEEP TO *RHIPICEPHALUS APPENDICULATUS* ANTIGENS

B. E. L. Wishitemi, P. B. Capstick and A. O. Mongi

Sheep were immunized with antigens extracted from the midgut and female reproductive organs of partially and fully

engorged female ticks, respectively. Ticks subsequently fed on these hosts showed significantly reduced feeding and reproductive performances, and in some cases high mortality was recorded, especially in animals immunized with solubilized midgut-membrane antigens.

Serological tests were performed on both groups of antigens, using sera obtained from the immunized animals. Double immunodiffusion studies revealed circulating antibodies in the sera from the immunized animals. Enzyme-linked immunosorbent assay (ELISA) also detected the presence of antibodies in the sera obtained from animals immunized with solubilized midgut-membrane antigens. The characterization of the solubilized midgut-membrane antigens by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblots identified 27 protein subunits with molecular weights ranging from 21 000 to 105 000 daltons.

Compared to control and other experimental animals, the sheep immunized with solubilized midgut-membrane antigens significantly controlled tick populations in paddocks seeded with *R. appendiculatus* nymphs. Adult blanket drag counts showed that the mean number of ticks from the paddocks containing animals immunized with solubilized midgut-membrane antigens was significantly lower than that of the controls. Similar results were observed during larval blanket drags and adult whole body counts. These experimental results confirm the possibility of controlling *R. appendiculatus* ticks by appropriate immunization of their hosts.

2.4 CORRELATION OF HOST RESISTANCE WITH INTRADERMAL RESPONSE

P. B. Capstick, J. J. de Castro, S. Nokoe and M. N. Nyindo

One hundred bulls were subjected to monthly tick collections and subsequently ranked in order of susceptibility to ticks (see 'Selection of Boran Cattle for Tick Resistance', the last paper in this chapter). Twenty-three bulls with rankings ranging from 1 to 7 were selected and subjected to intradermal testing with 0.1 ml of antigen derived from cell cultures of embryonating *Rhipicephalus appendiculatus* eggs (see the *ICIPE 1986 Annual Report*).

After one hour the resultant lesion was measured with calipers and the increase in skin thickness and the area of the lesion recorded. The volume of the inflammatory reaction was calculated by multiplying the increase in skin thickness by the area of the reaction.

The relationship between resistance ranking and the increase in skin thickness was calculated and is shown in Figure 2.6. There was a significant correlation between resistance ranking and increase in skin thickness. The greater increase in skin thickness was associated with the highest resistance ranking.

The relationship between reaction volume and resistance ranking was also positively correlated. There was no difference demonstrated between the correlations for reaction volume and skin thickness. The use of the reaction volume for judging resistance in this test gave no positive

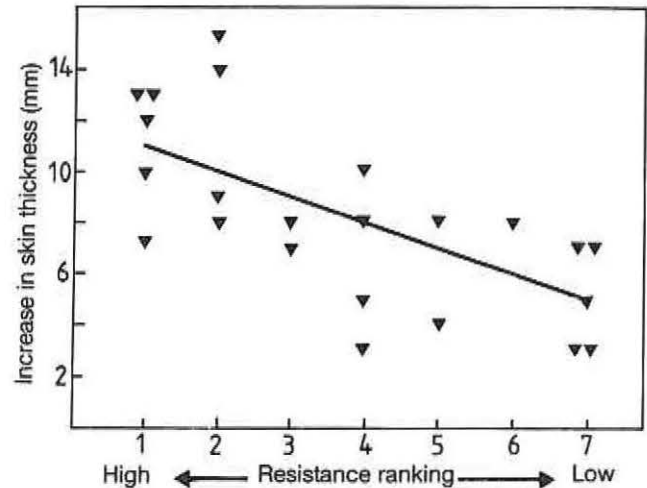


Figure 2.6 The relationship of bovine tick resistance ranking to the increase in skin thickness following intradermal testing with *Rhipicephalus appendiculatus* derived antigen.

advantage over the increase in skin thickness and takes considerably more time to obtain. This parameter is thus not recommended for use in the test.

2.5 TICK POPULATION MODELLING

S. Nokoe and D. Punyua

Population and climatic data on the tick species *Rhipicephalus appendiculatus*, *R. evertsi*, *Amblyomma variegatum* and *Boophilus decoloratus* collected over several years were studied, and in some cases re-analysed as preliminary steps in the production of tick population models. The locations covered include Rusinga Island, Mutara Ranch and Intona.

Experiments were also initiated to provide information on tick development and survival rates under natural conditions (R. M. Newson and F. Gigon) and the pick-up/drop off rates (A. Latif and D. K. Punyua). Data collection is in progress, and data will be analysed as soon as replicated information has been obtained.

Major significant results of data from Rusinga Island, where no tick control strategies are being carried out, indicate an equilibrium state of tick species with host and climate. Correlation among species is highest between adults of *R. appendiculatus* and nymphs of *A. variegatum*. Rainfall was found to be positively and significantly correlated with the adults of *R. evertsi* but negatively correlated with larval counts of *A. variegatum*. Analysis of climatic and tick population data from Intona indicated that the most important climatic variables were the cumulative rainfall, relative humidity and average temperature seven days prior to tick collections. These results are quite useful in the selection of parameters or variables in deterministic models.

Discriminant analyses were also carried out on data from a study of artificial infestation of cattle with *R. appendiculatus* (carried out by A. Latif, D. K. Punyua and P. B. Capstick). It appears possible to develop an index to separate *R.*

appendiculatus resistant from *R. appendiculatus* susceptible cattle. The most important variables in the discriminant model were the number of engorged females, the mean engorged weight of the female and the mean engorged nymphal weight. The implications are that these three variables, when considered jointly, are adequate for the assessment of resistance. Data from a new study using more animals than those used in this study are being collected for analyses.

2.6 SURVIVAL OF *RHIPICEPHALUS APPENDICULATUS* ON THE GROUND

R. M. Newson and J. Mugane

In this study we calculated the survival rates of batches of unfed *Rhipicephalus appendiculatus* nymphs and adults placed on the ground at two sites: the Kenya Agricultural Research Institute, at Muguga, and Intona Ranch, in Narok District. The ticks were contained in nylon gauze bags held in small protective cages that were placed in good grass cover at soil level and were sampled approximately once a month.

Results have now been obtained from the first three of four series placed on the ground at these two sites. These results have been fitted to the model $y = a + bx + c/x$, where y = the number alive after x days and a , b and c are constants. Data collected for the first three periods, which have necessitated our modifying the results given in the *ICIPE 1986 Annual Report*, are shown in Table 2.3. The Intona site has a higher and more uniformly distributed rainfall than Muguga and this is reflected in longer tick survival, though both sites vary considerably from one period to the next, and the fate of batches of ticks placed one metre apart in apparently uniform habitat also varied widely.

Table 2.3 Estimated 50% mortality in days of unfed *R. appendiculatus* adults and nymphs at Muguga and Intona

Site	Month of exposure			
	May 1985	August 1985	December 1985	June 1986
<i>Muguga</i>				
Adult	225	323	178	257
Nymph	158	132	75	167
<i>Intona</i>				
Adult	245	359	185	254
Nymph	214	182	146	197

We are continuing to analyse the data so as to incorporate them into a tick population model.

2.7 ECOLOGICAL STUDIES ON *AMBLYOMMA VARIEGATUM*

F. Gigon

Ecological studies on *Amblyomma variegatum*, which began in 1986, are designed to provide a better understanding of this species' ecology and to collect the data necessary for

producing mathematical models simulating tick population dynamics and their fluctuations under different possible control regimens and strategies. These studies fall under the following four topics.

- A study on the survival of the various stages of *A. variegatum* under field conditions conducted in contrasting biotopes selected from the range of *A. variegatum* potential distribution.
- A survey of the meteorological and micrometeorological field conditions conducted in conjunction with the survival experiments.
- The collection of data on the life-cycle of *A. variegatum* for the purpose of testing suitable models.
- A study of host-seeking behaviour to increase understanding of the crucial transition between the off-host and on-host phases.

First results

(1) Survival experiments on *A. variegatum* have been implemented in the following three contrasting biotopes in Kenya.

(a) Nairobi area (eco-zone III-5) is temperate with two relatively well defined rainy seasons.

(b) Trans-Mara Division (eco-zone II-4) is also temperate but lacks real dry seasons, having a more uniform rain pattern.

(c) The shore of Lake Victoria (Rusinga Island, eco-zone IV-3) has a hot climate with two rainy seasons and its humidity and wind are influenced by the lake, the consequences of which have not yet been determined.

After five months exposure, nearly 100% adult *A. variegatum* of both sexes are still alive, but the nymphs started dying abruptly. Technical problems arose with the exposure system for the larvae, which were released in batches of 50 in nylon tubes (300 × 20 mm) to allow a good equilibrium between inner and outer climates: they nevertheless died off completely within three months. This did not match visual observations under natural conditions in the same biotope, where *Amblyomma* larvae were seen to survive for at least five months. However, these larvae were clustered at the top of grass stems in much bigger numbers than our sample size of 50. We therefore believe that clustering in large numbers (probably the whole offspring of one female) provides mutual protection against dehydration for all larvae except the most peripheral ones.

(2) During early climate measurements it became evident that constant recordings should be made at different levels above the ground surface. Data loggers are now recording temperature and humidity at three levels, and these data will be complemented with rainfall data. The equipment being used is unsatisfactory but is giving interesting information that will allow us to build a standard 'ecological meteorological station' that will in turn enable us to make comparisons between classically assessed climates and related micro-climates, with particular reference to tick life.

(3) Six months ago in the Trans-Mara we began monthly collections of ticks, including immatures, on a typical Maasai cattle sample. Commonly found species were *Rhipicephalus appendiculatus*, *Boophilus decoloratus*, *A. variegatum*, *R. evertsi* and *A. cohaerens* (sorted by magnitude). Interestingly, all of them so far display a remarkable lack of

seasonality, though the same species have been demonstrated elsewhere to show some seasonality, which is supposedly related to different rainfall patterns. That the area of our study has no contrasting rainfall patterns is a possible explanation for the lack of seasonality in tick numbers there.

2.8 POPULATION DYNAMICS OF LIVESTOCK TICKS ON RUSINGA ISLAND

D. K. Punyua, A. A. Latif, P. B. Capstick and S. Nokoe

Studies were begun in January 1986 and continued in 1987 to observe the repeatability, or its absence, in the activity pattern of the common ixodid tick species on the local cattle of Rusinga Island. The animals sampled in 1986 were used again in 1987. We sampled monthly 10 head of cattle each on four farms and 5 head of cattle on one farm, making a total of 45 animals per month. We compare below the results of 1986 with those of 1987.

Rhipicephalus appendiculatus remained the dominant tick species on the island cattle. A single adult peak of activity occurred in March 1986, after which the activity suddenly dropped to a low level (in June) and remained relatively low for the rest of the year (Figure 2.7). The observed increase in December 1986 remained at that level in 1987 until February, when there was a marked drop in activity, which remained low until August. In September a slight increase was

observed, and if the results of 1986 are to be repeated in 1987 there should be an increase that will continue until December/January. It appears that tick activity generally starts to increase steadily from September and reaches a peak in February/March, when it declines to reach a low level in June/August.

The adult infestation level of *R. evertsi evertsi* was low this year, as it was in 1986 (Figure 2.8). The slight increase, two-fold, observed between March and May of 1986 appeared in January/February of 1987.

The activity peaks in January, June and September/December for adults of *A. variegatum* in 1986 occurred again this year with almost the same magnitude of activity (Figure 2.9). Like *R. appendiculatus*, *Amblyomma variegatum* appears to have a prolonged high level of activity between September and January, the activity dropping in February and rising again briefly in June before dropping again and remaining low until August.

In 1986 adult *Boophilus decoloratus* had three small activity peaks, one each in March, June and December (Figure 2.10). Although the population numbers remained almost at the same level in 1987, only a single peak, in February, was observed.

It appears that the pattern of tick activity for at least three of the tick species (*R. appendiculatus*, *A. variegatum* and *R. evertsi evertsi*) is a steady increase from September to February/March, when activity starts to drop. A brief peak may also be observed in June (*A. variegatum* and *B.*

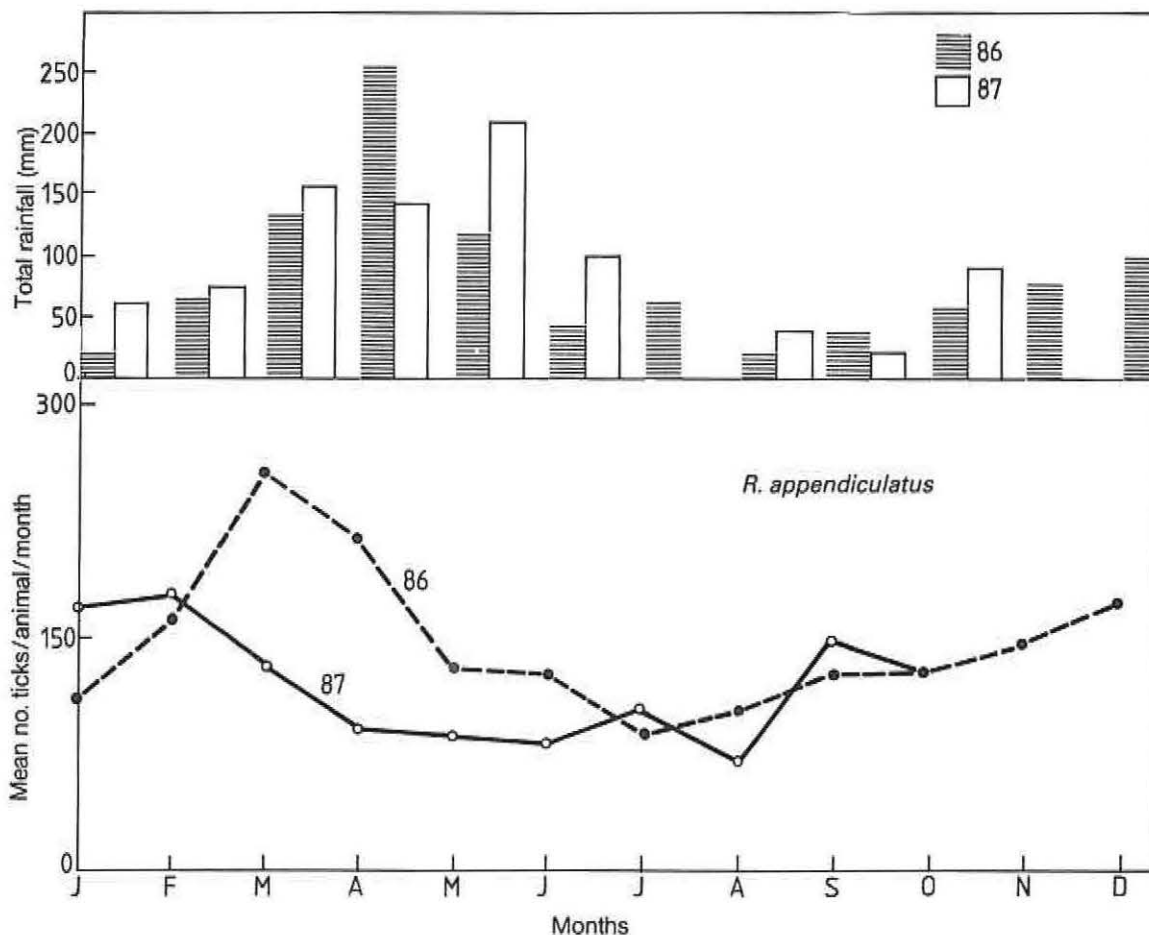


Figure 2.7 Numbers of *Rhipicephalus appendiculatus* on cattle on Rusinga Island during 1986 and 1987.

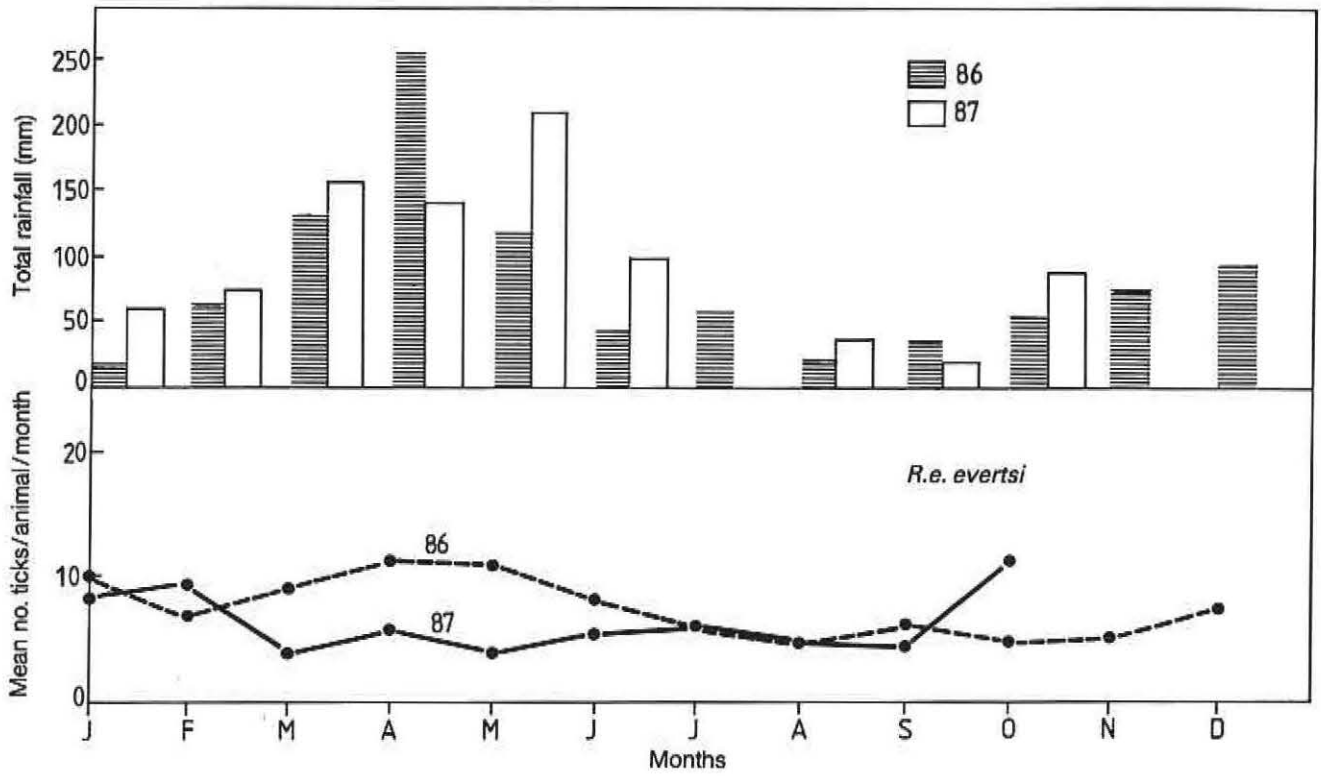


Figure 2.8 Numbers of *Rhipicephalus evertsi* on cattle on Rusinga Island during 1986 and 1987.

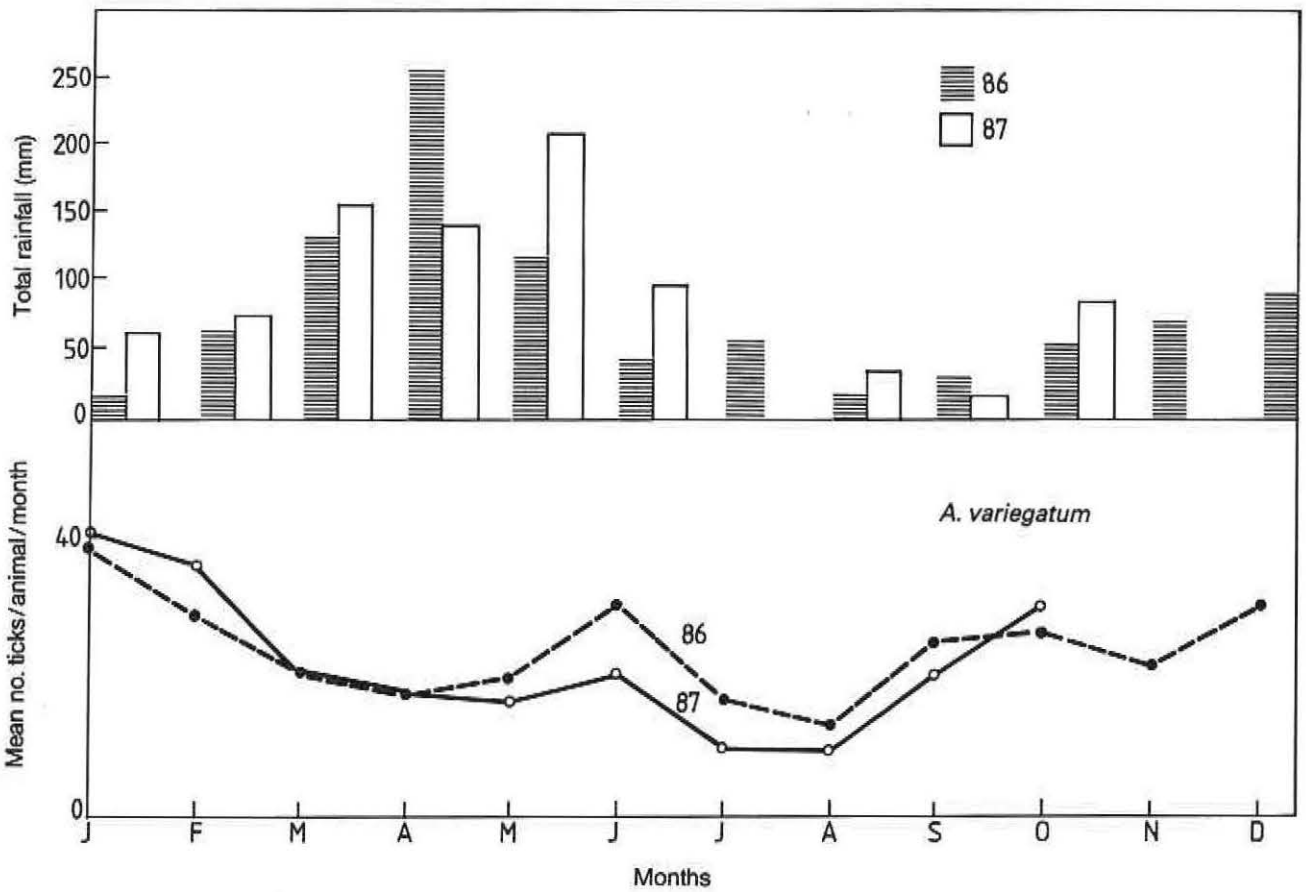


Figure 2.9 Numbers of *Amblyomma variegatum* on cattle on Rusinga Island during 1986 and 1987.

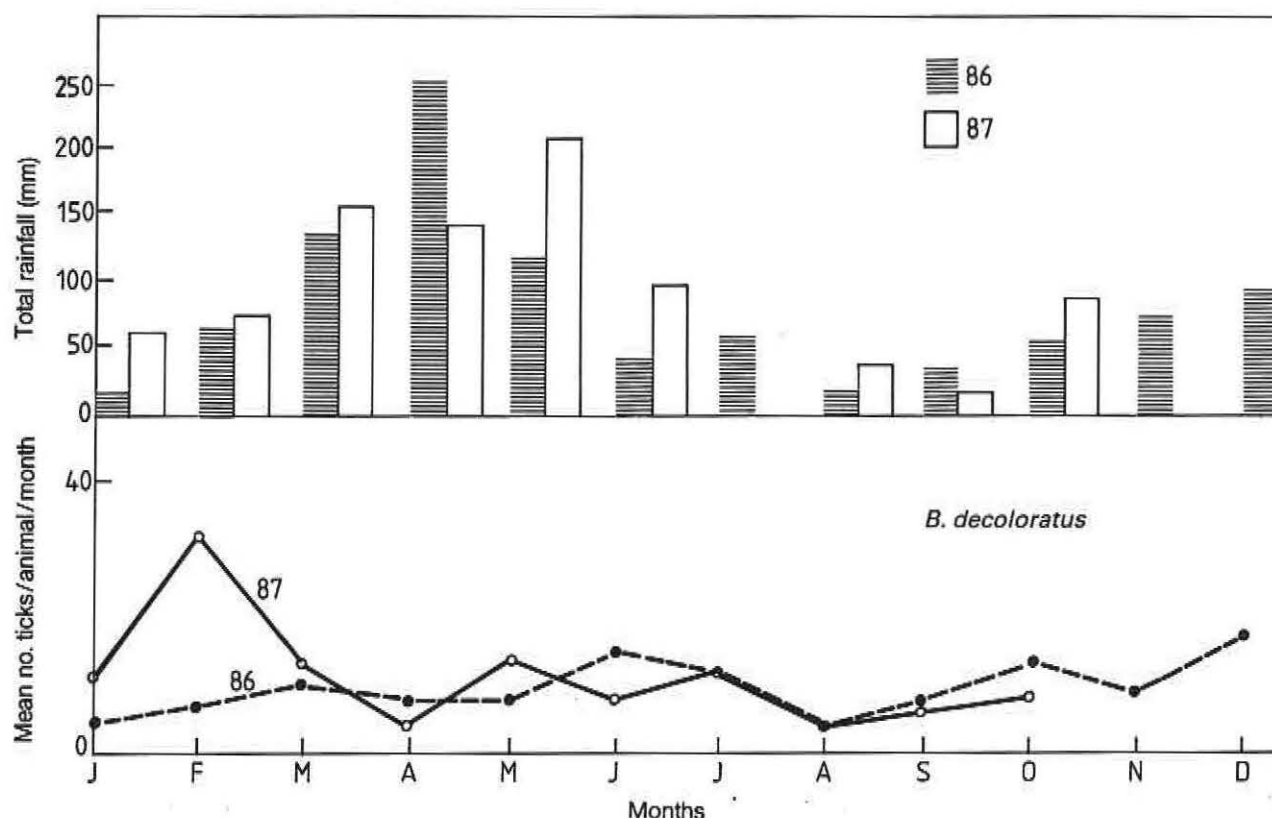


Figure 2.10 Numbers of *Boophilus decoloratus* on cattle on Rusinga Island during 1986 and 1987.

decoloratus), before activity suddenly drops again to a low level in August.

We attributed the conspicuous sudden drop in tick activity in February/March of 1986, at the height of the rainy season, to agricultural and husbandry practices. That the same drop in activity occurred in 1987 appears to confirm this interpretation of the results.

2.9 TSETSE AND TRYPANOSOMIASIS SURVEY ON RUSINGA ISLAND

D. K. Punyua, L. H. Otieno and P. B. Capstick

In 1985, 200 cattle from Rusinga Island were sampled for haemoparasites, the sampling including buffy coat examinations for trypanosomes. While none of the animals was found to be infected, we looked for the presence or absence of tsetse flies on Rusinga Island before assuming that trypanosomiasis was absent on the island. As reported in the *ICIPE 1986 Annual Report*, to do this a few traps were put out and over 600 tsetse flies were captured from one site. All the flies were identified as *Glossina fuscipes*. We then conducted a major search for more flies, possibly of different species, as well as a search for infection.

In 1987, 25 traps were put out in four transects. The first transect, in Utajo (the eastern part of the island), covered an area from the shore of Lake Victoria over a hill to the lake shore on the other side of the island. This narrow strip of the island had only three traps. The second transect, in Kakrigu (the southern part of the island), stretched from the lake shore (1230 m) towards the Lugongo Ridge (peak 1500 m)

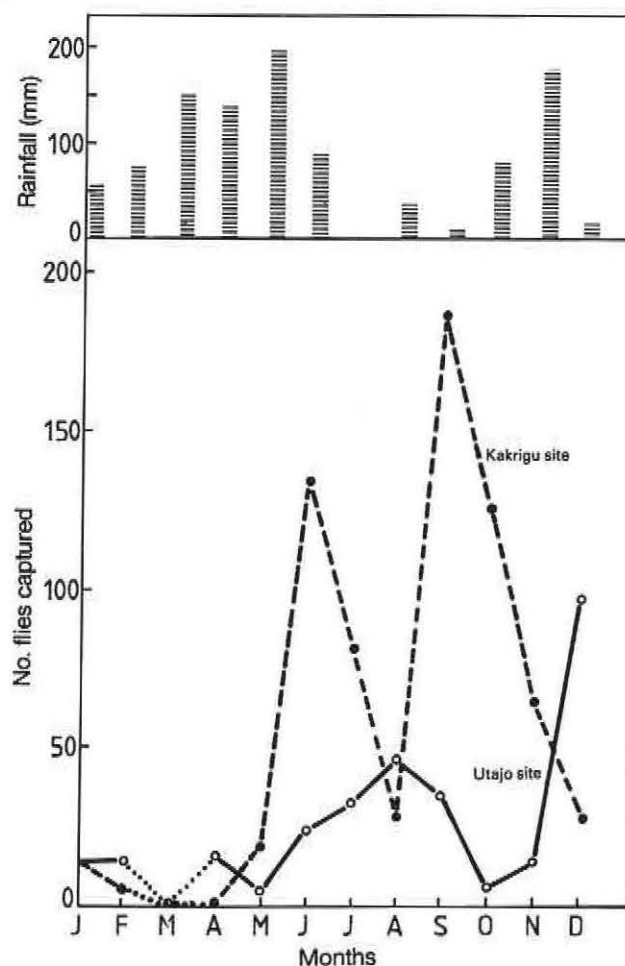


Figure 2.11 Population changes of *Glossina fuscipes* at two trap sites on Rusinga Island in 1987 in relation to rainfall.

through thicket up to the 1300-metre contour. This transect had eight traps. The third transect crossed the lowland on the western side of the island from the lake shore in the south to the lake shore in the north. This transect had six traps. The fourth and longest of the transects stretched from the northern lake shore through settlement areas and thicket toward the Lugongo Ridge up to the 1300-metre contour. This transect had eight traps. Once a month the traps were positioned, left for 24 hours and then were collected. The captured flies were then identified, sexed, dissected and examined for parasites.

After ten months of trapping, only the Kakrigu lake shore and the western lake shore at Utajo yielded flies. A total of 1344 flies, all of them *Glossina fuscipes*, were captured and examined for parasites. Only one parasite was found. This was found in the gut and identified as a reptilian species. The fly population from the two sites was plotted; Figure 2.11 shows that the major fly activity is highest during the dry months of June to September.

2.10 THE POPULATION OF *RHIPICEPHALUS APPENDICULATUS* AND ASSOCIATED SPECIES ON CATTLE IN WESTERN KENYA

D. K. Punyua, A. A. Latif, S. K. Nokoe and
P. B. Capstick

Throughout 1987 we monthly inspected forty-five head of cattle from five randomly selected farms for tick species and populations on the cattle. The common tick species observed whose populations were monitored include *Rhipicephalus appendiculatus*, *R. evertsi*, *Amblyomma variegatum* and *Boophilus decoloratus*. The dominant species is *R. appendiculatus*.

Preliminary results indicate a strong association between the adults of *R. appendiculatus* and the nymphs of *A. variegatum*. Rainfall is positively and significantly correlated with the adult population of *R. evertsi* ($r = 0.503$) and negatively correlated with larval counts of *A. variegatum* ($r = -0.41$) and nymphs of *R. appendiculatus* ($r = -0.27$). A time series analysis confirmed the effect of seasonality.

A further attempt is being made to describe the population changes for the various instars using mainly climatic data.

2.11 DEVELOPMENT AND SURVIVAL OF *RHIPICEPHALUS APPENDICULATUS* COMPARING LABORATORY AND LOCAL FIELD STRAINS IN THE FIELD

R. M. Newson, F. Gigon, D. K. Punyua and
J. G. Mugane

Field-derived and laboratory strains of *Rhipicephalus appendiculatus* were used in this study because of the differences noted by J. W. Chiera (see previous ICIPE annual reports) in the tick-host relationship between laboratory and field strains of *R. appendiculatus*.

Three sites with convenient facilities were chosen for the study: Mbita Point Field Station (MPFS); Lolgorien Research Station (Ministry of Livestock Development), near

LTRP's Intona Ranch site; and a new site near Nairobi. Ticks from field-collected engorged females from Rusinga Island and from the study herd at Lolgorien are reared for use. A laboratory-reared strain from Kikuyu, in Kiambu District, has had to be established because of the difficulty of obtaining repeated field material in Central Province, where there is a long history of intensive dipping. For the same reason, no herd studies are being done to complement the Nairobi study. In many cases the field strains were studied in parallel with the Muguga laboratory strain of *R. appendiculatus*.

Electronic data loggers are in use at each study site (see report by F. Gigon in this chapter) to record temperature and relative humidity in the micro-habitat, with the vegetation kept trimmed to approximately 10 cm high to simulate heavy grazing. These studies will be expanded to include observations of vegetation 30 cm high to simulate tick refugia after the short rains in November.

At the Lolgorien site every month since March 1987 adults of *R. appendiculatus*, *Amblyomma variegatum*, *Amblyomma cohaerens* and *Boophilus decoloratus* have been collected from ten adult cattle and ten calves from an undipped herd of Maasai cattle. The immature stages of these species are sampled by a skin-scraping technique on the neck. In addition, ten samples are taken of ticks from the vegetation at three sites used by the cattle. This area has a high and evenly distributed rainfall and the numbers of *R. appendiculatus* have been notable for their high but relatively uniform level throughout the period. Data on *B. decoloratus* should give a useful comparison with the Mutara Ranch studies, and numbers have been very low. Two studies on the rate of development of all stages of *R. appendiculatus* and the survival during development have been completed on a site at the Ministry of Livestock Development Lolgorien Research Station, and long-term survival studies of unfed stages of the tick are under way.

The results for the parasitic stages on Rusinga Island (see reports by A. A. Latif and D. K. Punyua in this chapter) will be correlated with development and survival studies being made on the site at MPFS, where the third development study and second study of the unfed stages are now in progress.

Two development studies have been made at the Nairobi site, the last site to be used. An interesting feature was the delay in the onset of moulting development in engorged larvae and nymphs, and egg-laying in engorged females, under hot, dry ground conditions (a feature noticed occasionally in earlier studies). The first survival study was also begun.

The following are the most notable points in the study to date: (1) a wide variation continues to be observed during both the development and the survival of the host-awaiting stages that are seen in samples placed less than 1 m apart in habitats selected to be as uniform as possible, (2) in those cases where the development of field and lab strains have been compared, the lab strain has developed appreciably faster than the field strain.

2.12 TICK-HOST RELATIONSHIP ON RUSINGA ISLAND

A. A. Latif, D. K. Punyua and P. B. Capstick

2.12.1 Assessment of tick challenge on the pasture

Four susceptible head of Friesian cattle were used as bait animals to assess the level of adult *Rhipicephalus appendiculatus* challenge on the pasture. At the same time, assuming that the initial rates of pick-up were similar, the numbers of ticks engorging on the individual cattle could be compared.

Twenty cattle on two study farms were ranked for total tick counts for five months in succession and four animals with consistently low counts (judged to be highly resistant to ticks) and four animals with consistently high counts (low resistance) were selected on each farm. The method of ranking was described in the *ICIPE 1986 Annual Report*.

On day 0 the four selected animals on each farm were deticked and two bait cattle were released to graze with them, in the company of the rest of the farm herd. The Friesians received extra nutrition and were monitored closely (see report on disease survey). On day 8 the experimental and bait cattle were brought to Mbita Point Field Station, where cloth bags were put over their ears to collect all the female ticks that engorged and detached themselves from the cattle. On one farm an average of 20 ticks dropped from the highly resistant cattle, 40 ticks dropped from the low resistant cattle and 140 ticks dropped from the bait cattle. On the other farm the numbers of ticks were 2, 27 and 38. Thus there was not only a clear difference in the level of tick populations on the farms, but in each case the challenge experienced by the resistant animals was 7–19 times higher than the number of ticks actually engorging on them. The local tick populations are obviously heavily dependent on animals of low resistance for maintaining their numbers.

This experiment is now being repeated with 30 cattle on three farms, plus six test animals.

2.12.2 Rating cattle for resistance by artificial tick infestation

Each of the eight cattle from the two farms and the four Friesian steers (see above) then received an artificial tick challenge of 25 males plus 25 females, 100 nymphs and 100 larvae of *Rhipicephalus appendiculatus*. The feeding performance of the females is summarized in Table 2.4. There are again clear differences among the groups of animals—those with high resistance, those with low resistance and the susceptible Friesians. The survival of the female ticks on the susceptible cattle was about 5.5 times that of the resistant group.

Table 2.4 Feeding performance of 25 female *R. appendiculatus* on two groups of cattle of different levels of resistance to tick infestation from Rusinga Island compared with susceptible controls (means \pm standard deviation)

Resistance level	Number engorged	Engorged weight (mg)
High	4.0 \pm 0.7	207.5 \pm 28.2
Low	8.3 \pm 5.7	242.2 \pm 22.5
Susceptible	19.3 \pm 6.3	301.8 \pm 35.6

The mean weights of the nymphs and larvae that fed on the Rusinga Island cattle did not differ significantly among the groups and taken together were 4.4 mg \pm 0.5 standard deviation (S.D.) and 0.38 mg \pm 0.06 S.D., respectively. These weights were, however, significantly lower than those from the Friesian steers (7.0 mg \pm 0.03 S.D. and 0.47 mg \pm 0.02, respectively).

The percentages of engorged larvae and nymphs that successfully develop to the next instar is being examined in another experiment.

2.13 RESISTANCE INDUCED BY THE LABORATORY AND FIELD-DERIVED STRAINS OF *RHIPICEPHALUS APPENDICULATUS*

J. W. Chiera

A preliminary investigation was carried out to find out if the differences that have been observed between the laboratory and field-derived strains of *R. appendiculatus* in relation to host resistance (reported in previous annual reports) could be explained in immunological terms. Rabbits were made resistant to the laboratory strain and to a field strain, after which their sera were used to find out what proteins they recognize. Using immuno-blotted proteins of the whole-tick homogenate, some differences were observed in terms of the proteins recognized. However, more work needs to be done to show that these differences are not artifacts and that they are related to the differences observed by feeding ticks.

2.14 CATTLE PRODUCTIVITY ON RUSINGA ISLAND

A. A. Latif, D. K. Punyua and P. B. Capstick

Preliminary data on productivity of livestock from Rusinga Island, obtained through a questionnaire, were reported earlier (*ICIPE 1985 Annual Report*). A systematic collection of data from ten collaborating farms started in 1987. There seems to be a natural synchronization of calving on the island, the cause of which has not yet been investigated. Figure 2.12 shows the frequency of calves born each month during 1986 and 1987. In both years, most calves were born in June/July (64.8% and 58.5%, respectively). Out of the 37 cows that calved between March and October 1986, only 6 had calved a second time by November 1987. The calving interval for the majority of cows is in excess of two years.

The calves born last year that survived an East Coast fever natural challenge are weighed each month. Table 2.5 shows the first weight of calves at birth and their subsequent weight gain/loss each month. There was a steady gain in weight during the first and second month (average of 25%) which dropped to 4% by the third month. This drop was due to the incidence of East Coast fever at this age. After the age of 4 months the calves became highly infested with endoparasites which, together with tick-borne diseases and poor nutrition during the dry season, contributed to weight loss and emaciation. These calves will continue to be observed closely until the age of first calving.

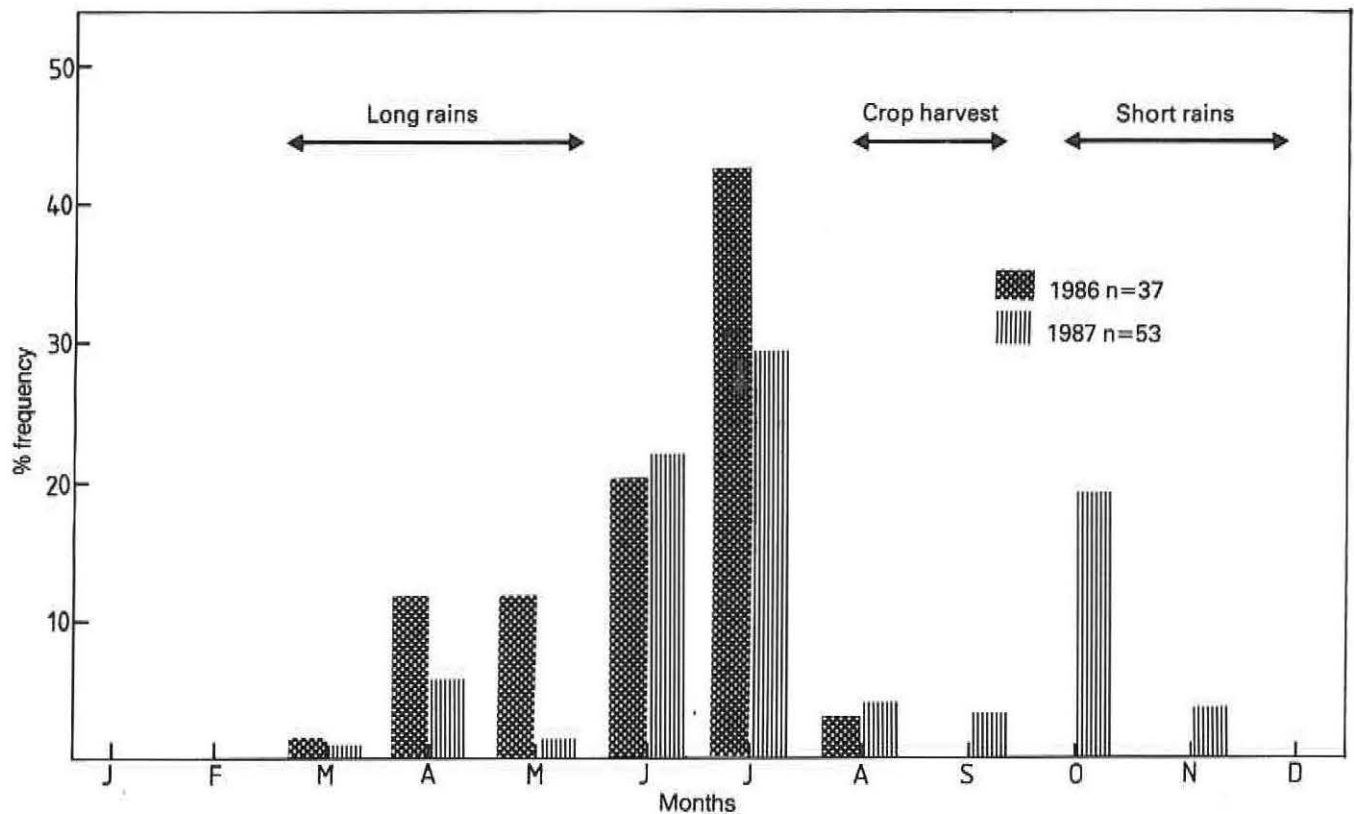


Figure 2.12 The percentage frequency distribution of calves born on ten farms during 1986 and 1987 on Rusinga Island.

Table 2.5 Liveweight of calves at birth and subsequent gain/loss each month on Rusinga Island, 1987

	1 May	2 June	3 July	4 Aug.	5* Sept	6* Oct
Mean weight (kg)	21.5	28.1	29.3	34.6	33.0	29.5
± 1 standard deviation	3.9	5.3	5.9	8.4	6.0	1.8
% gain/loss (+/-)	+27.4	+23.5	+4.1	+15.4	- 4.8	-11.9
n	35	28	30	24	5	4

* Few calves reached the age of 5-6 months.

2.15 CATTLE DISEASE SURVEY ON RUSINGA ISLAND

A. A. Latif, D. K. Punyua and P. B. Capstick

2.15.1 Exposure of Friesian cattle to natural tick-parasite challenge

Susceptible Friesian steers were bought from Manera Farm, Naivasha, where regular tick control is practised from the steers birth, and brought to Rusinga Island in an experiment to assess the natural tick challenge on the pasture. The Friesians were maintained tick-free by spraying twice weekly with Bacdip (Bayer). However, on the island, theileriosis, babesiosis and anaplasmosis were all reported in the local cattle (*ICIPE 1986 Annual Report*), while heartwater was yet to be confirmed. Vaccines against East Coast fever (ECF) of the Rusinga strain are not yet available. Therefore, each animal received 20 ml of Tetroxyl LA (Wellcome), on the day the animal was exposed to the natural field challenge; the dose was then repeated twice at 5-day intervals. This

simulates the well-documented artificial infection-treatment method as a means of protection.

In September 1987 six steers were released onto three farms for a period of six days and then brought back to Mbita Point Field Station. The animals were monitored closely clinically and parasitologically. Table 2.6 shows the performance of the cattle on the three farms. Farm 6 seemed to have the highest ECF challenge: both animals reacted severely. They had two episodes of schizont parasitaemia, the second episode being long. Though the two animals received one treatment on days 32 and 34 respectively, with Clexon (Wellcome), animal No. 616 died due to acute ECF infection. Although the tick challenge on Farm 28 was the highest of the three farms, only one animal reacted severely and it then recovered. On the other hand, Farm 16 had the lowest tick/ECF challenge and only one animal showed a mild reaction. No treatment with Clexon was required for animals on these farms.

Anaplasma marginale of low parasitaemia and *Babesia bigemina* of high parasitaemia were both detected in the

Table 2.6 Performance of Friesian steers treated on day 0 with tetroxyl and exposed to natural tick/East Coast fever challenge on Rusinga Island

Farm no.	Animal no.	No. of engorged ticks	Feeding period (days)	Days to temp. (40.0° C)	Days to schizonts	Days to piroplasms	Days of treatment*	Days to death
6	616	45	7-10	15	15; 30-40	15	32-34	42
6	632	43	7-10	5	5; 30-39	5	32-34	—
28	656	151	6-12	15	NPS	19	Nil	—
28	668	207	6-11	20	21-24	12	Nil	—
16	661	16	7-11	6	6-7	7	Nil	—
16	689	34	7-12	13	NPS	16	Nil	—

* Clexon 20 mg/kg.

second and third weeks, respectively, of exposure of susceptible cattle. All animals were treated with Imizol (Wellcome) combined with terramycin and recovered.

In March/April 1987 four Friesian steers from the same farm were also brought to the island. They received the same treatment schedule as described above but they were also protected with Clexon during the ECF reactions; none of the animals died of the disease. However, one animal died of acute heartwater infection and massive numbers of *Cowdria* were demonstrated in the brain smear. This confirms the presence of the disease on the island.

Tick pick-up of *Theileria* parasites was attempted on these animals and the infected ticks will be used for the development of an ECF vaccine (in collaboration with the Kenya Agricultural Research Institute).

2.15.2 Studies on the epidemiology of ECF on Rusinga Island

Within the Rusinga Island disease survey, additional studies, such as the epidemiology of ECF, have been incorporated in collaboration with other national and international institutes. The present study is a collaboration between the Kenya Agricultural Research Institute (KARI), the International Laboratory for Research on Animal Diseases (ILRAD) and ICIPE. The information obtained will be of value in creating host/tick/disease models.

Pregnant cows in their last trimester had been identified on the collaborating farms. Pre- and post-calving sera and colostrum were regularly collected from these animals. Sera were also taken regularly from the new-born calves, weekly for the first three months and then monthly for one year. Clinical and parasitological examinations of these calves were also carried out carefully to determine the time of exposure to the natural ECF challenge after birth. All the sera are now being examined at ILRAD to identify the ECF-immune status of the calves and dams.

2.16 SELECTION OF BORAN CATTLE FOR TICK RESISTANCE

J. J. de Castro, P. B. Capstick, S. Nokoe, M. Malonza, O. Okello and H. Kiara*

Between January and June 1987 tick data were collected monthly from 100 bulls. At the same time, data on other

parameters, such as visual assessment of tick burden, skin thickness, number of skin folds and coat colour, were collected to establish any relationship between tick burdens and any of these parameters with the aim of simplifying the ranking system for future use.

By far the most common tick species found was *Boophilus decoloratus*, followed in decreasing order by *Rhipicephalus evertsi*, *Hyalomma truncatum*, *R. pulchellus*, *R. appendiculatus* and *R. hirti*.

All 100 bulls were ranked from 1 to 7 for each species (when enough numbers were present) and the total tick burden month by month. Bulls ranked consistently for *B. decoloratus* and also for total tick burdens (highly significant values were obtained with both Kendall tau-*b* and Spearman rank correlation tests). *B. decoloratus* was also highly correlated to total tick burdens for all months. The other tick species present, however, did not show a consistent trend and further work is needed to elucidate these findings.

The assessment of tick burdens by visual observation was found to be significantly correlated with the real tick burden on the animal, suggesting that visual assessment of tick burdens may be a useful method in routine evaluation of animals for selection purposes. Although its degree of concordance between observers was very high ($r = 0.9$; $P < 0.001$) when tested by Spearman correlation, its reliability should be further tested for different tick burdens. The analyses also suggested that animals with thinner skins may harbour less ticks.

Results so far indicate that selection for *B. decoloratus*, the main tick species on the ranch, is possible and this may be greatly facilitated by the ranking of the animals by visual assessments.

A ranking system has been devised to enable the ranch manager to preserve those bulls showing the desirable characteristics; this information has now been included in the ranch's records.

Other bulls and heifers on the ranch are being assessed with the aim of creating experimental herds of tick-resistant animals for future studies.

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MEDICAL VECTORS RESEARCH PROGRAMME

- 3.1 Leishmaniasis epidemiology in West Pokot District: entomological studies **56**
- 3.2 Leishmaniasis epidemiology in West Pokot District : animal reservoirs **56**
- 3.3 Leishmaniasis epidemiology in Baringo District: blood-meal analysis of phlebotomine sandflies **57**
- 3.4 ELISA and Leishmanin tests for kala-azar **58**
- 3.5 Experimental infection of *Aedes aegypti* **58**
- 3.6 Sandfly distribution in the Marigat area **58**
- 3.7 *Leishmania donovani* in hamsters **58**
- 3.8 Interaction of *Leishmania*, bacteria and fungi **59**
- 3.9 Leishmanial parasites: identification and characterization **59**
- 3.10 Parasitology of malaria: glucose-6-phosphate dehydrogenase deficiency and its effect on susceptibility of man to malaria **60**
- 3.11 Ecology of mosquito vectors of malaria in Baringo District **60**



3

Medical Vectors Research Programme

Leishmaniasis and malaria, on which the Medical Vectors Research Programme (MVRP) focuses its activities, are major drawbacks to development. MVRP research in 1987 was focused on the following research areas.

Entomological work. Studies were undertaken in West Pokot District to provide information on species composition, habitat, feeding preference, seasonal abundance and natural Leishmania infection. The abundance of sandfly species in animal burrows and termite mounds was seasonal, and peak infection rates of Leishmania in sandflies occurred in February. An analysis of the blood-meals of phlebotomine sandflies showed that most of those found in tree holes and animal burrows feed on lizards, rodents, canids and birds, as well as man. Studies on the factors affecting distribution of sandflies at Marigat, in Baringo District, revealed that temperatures in the micro-habitats of sandflies in animal burrows and termite mounds were fairly constant and that the vertical distribution of sandflies was greater in wooded areas than in open fields.

Reservoir hosts and laboratory animals. A search for Leishmania infection in ruminants revealed for the first time the occurrence of Leishmania-like parasites, which have yet to be characterized, in goats. Through studies on laboratory animals, a medical technique of splenic puncture was successfully adapted to the detection of Leishmania donovani in live experimental animals.

Distribution of leishmaniasis. Collaborative work with the Kenya Ministry of Health using enzyme-linked immunosorbent assay (ELISA) and Leishmanin tests showed that in West Pokot District the disease may be more abundant in low than in high lands. The work also showed that malaria was more common than leishmaniasis.

Interaction of Leishmania and non-leishmanial micro-organisms. Wild sandflies sometimes carry bacteria and fungi. In vitro studies showed that the toxicity of bacteria and fungi to Leishmania is not universal.

Parasite characterization. Characterization of newly isolated leishmanial parasites using isoenzyme electrophoresis and molecular biology techniques was continued in 1987.

Studies on mosquitoes and malaria. The abundance of mosquitoes in leishmaniasis endemic areas prompted the undertaking of pilot work on the infectivity of Leishmania to mosquitoes. In the study Leishmania donovani in the mosquito Aedes aegypti survived for 20 days. Studies on the ecology of Anopheles gambiae at Marigat demonstrated that most of the larval mortality in the swampy study area was caused by predation.

Deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) in mammals is a protective phenomenon against infection with malaria parasites. Studies carried out in this area demonstrated that human-derived malaria parasites, Plasmodium falciparum, were able to produce G6PD if grown in G6PD-deficient blood cells. This possibly explains why hemizygous G6PD-deficient people lack protection from malaria.

3.1 LEISHMANIASIS EPIDEMIOLOGY IN WEST POKOT DISTRICT: ENTOMOLOGICAL STUDIES

M. J. Mutinga, C. M. Mutero, A. Ngindu, F. Amimo, F. M. Kyai, P. Munguti and D. M. Mativo

Studies on the sandfly fauna of West Pokot District were initiated in December 1986. The investigations were carried out mainly in Kacheliba, where a high concentration of kala-azar cases was observed during a previous clinical survey. The study area comprised three settlements: Sangakai, Kongelai and Pole. The main objective of the one-year field study on sandflies was to provide baseline information on species composition, habitat preference, seasonal abundance and natural *Leishmania* infection rates.

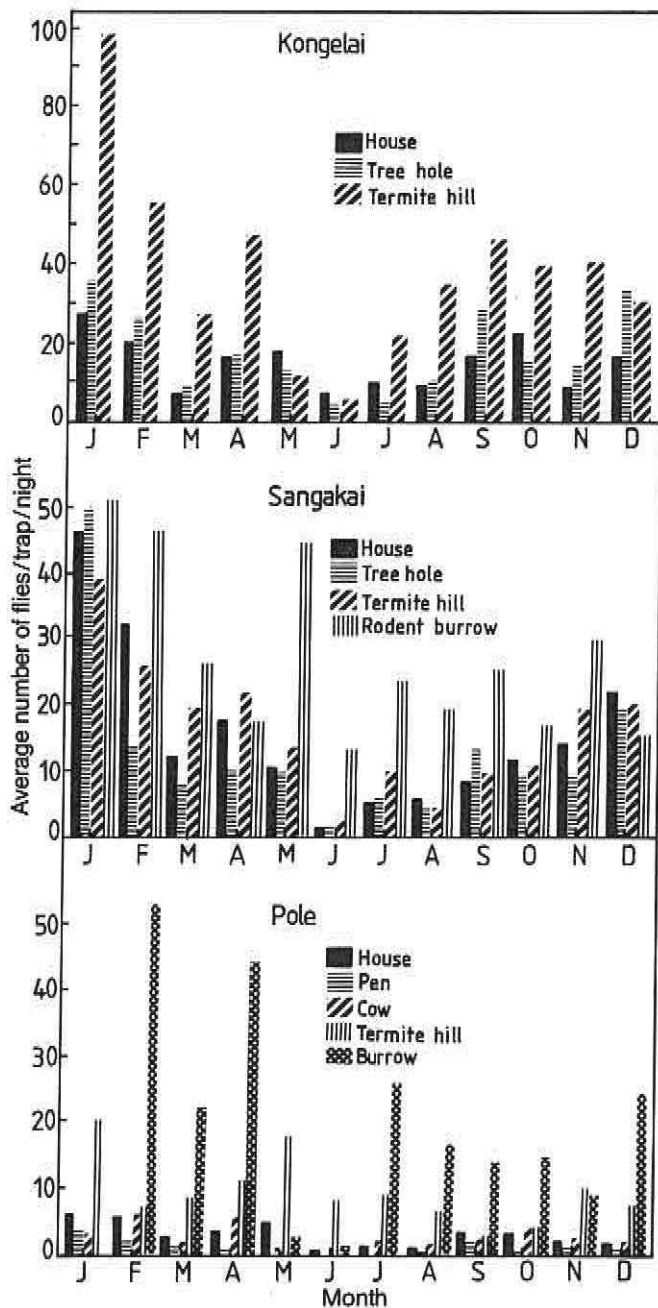


Figure 3.1 The prevalence of phlebotomine sandflies in the visceral leishmaniasis foci of Kongelai, Sangakai and Pole.

Sandflies were sampled once weekly from each of the three settlements. Sticky traps measuring 1 x 1 m were placed in various habitats previously associated with the breeding and resting of sandflies. Flies thus collected were identified at the species level and counted and the parous ones were dissected for detection of parasites.

The species recorded from the study area were *Sergentomyia antennatus*, *S. bedfordi*, *S. ingrami*, *S. clydei*, *S. adleri*, *S. africanus*, *S. affinis*, *S. schwetzi*, *S. rodhaini*, *S. squamipleuris* and *Phlebotomus martini*. *S. antennatus*, *S. bedfordi* and *S. schwetzi* were the most abundant species during the study.

The seasonal variation and the relative density of sandflies in the three settlements is provided in Figure 3.1. Animal burrows yielded the highest number of flies in Sangakai. Two major peaks in fly abundance were observed, one in January and one in May. Similar burrows also formed the main source of flies in Pole, with peak numbers being recorded in January and April. In Kongelai, most flies were collected from termite hills. The population showed density peaks in January and September.

Natural infection of sandflies with *Leishmania* promastigotes occurred during most of the year. The highest monthly infection rate was 1%, observed in February 1987.

3.2 LEISHMANIASIS EPIDEMIOLOGY IN WEST POKOT DISTRICT: ANIMAL RESERVOIRS

M. J. Mutinga, C. M. Mutero, A. Ngindu, F. Amimo, F. Kyai, D. M. Omogo, P. Munguti and D. M. Mativo

Animal reservoirs for the three forms of leishmaniasis in Kenya have been documented and add to the complexity of

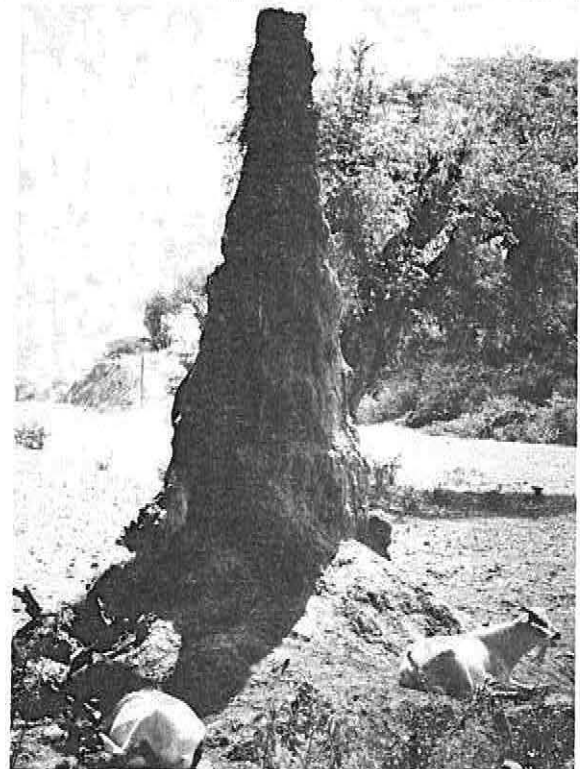


Figure 3.2 Goats resting at the foot of a termite hill, a resting and breeding site of sandflies.

the epidemiology of the disease. An intensive search in East Africa for animal reservoirs of leishmaniasis, including domestic animals, has continued.

Goats and sheep from slaughterhouses were inspected and spleen and liver smears were made and stained for detection of amastigotes. Cultures of the same organs were made in an NNN diphasic medium. Of 457 goats, leishmanial amastigotes were found in 6 and culture isolations made in 4. This is the first time goats have been found to harbour leishmanial organisms. The implications of this may be far-reaching because goats are closely linked with man in all leishmaniasis-endemic areas. Goats also roam those habitats favoured as breeding and resting sites of sandflies, such as termite hills (Figure 3.2), caves and wooded areas at both low and high altitudes. Investigations are under way to study how extensively the goats are exposed to infection and their susceptibility in the laboratory.

3.3 LEISHMANIASIS EPIDEMIOLOGY IN BARINGO: BLOOD-MEAL ANALYSIS OF PHLEBOTOMINE SANDFLIES

M. J. Mutinga, C. M. Muteru, C. C. Kamau, M. Basimike, F. M. Kyai, D. M. Omogo, P. Munguti, D. M. Mativo and S. Mutua

Sandflies are haematophagous insects and the females require a blood-meal to develop eggs. Both males and females also require sugar daily, obtained from plant juices, for energy. In the process of feeding on vertebrates for a

blood-meal, the females transmit human and animal leishmaniasis. Because wild and domestic animals are known to be reservoirs of leishmaniasis, an evaluation was made of the potential vector species of visceral leishmaniasis and cutaneous leishmaniasis in the endemic focus of Marigat, in Baringo District.

Sandflies were collected from resting and breeding sites, such as termite hills, animal burrows, tree holes, houses, thickets, open areas and rock crevices. The flies were then washed in detergent saline to remove castor oil and the blood-fed ones were sorted out. The abdomens of the fed flies were severed and squashed onto Whatman filter paper no. 1. The head of each specimen was then mounted on a glass microscope slide for species identification.

The filter papers were then sent for analysis to Dr. G. Staak, of the Robert von Ostertag Institute, in West Berlin.

The results of these investigations are summarized in Figure 3.3. Blood-engorged flies were found in houses, termite hills, animal burrows, rock crevices, tree holes, tree bases, tree canopies and chicken coops. The majority of blood-fed flies were encountered in tree holes and animal burrows. The preferred hosts of those found in tree holes were monitor lizards, followed by ruminants, rodents, man, hippos, canids and birds. The preferred hosts of those in animal burrows were rodents, followed by ruminants, canids, man and birds. These results indicate that tree holes and animal burrows may be only the resting sites of fed flies. The study area is arid and hot and thus tree holes and animal burrows would provide good shelter, especially for delicate fed flies.

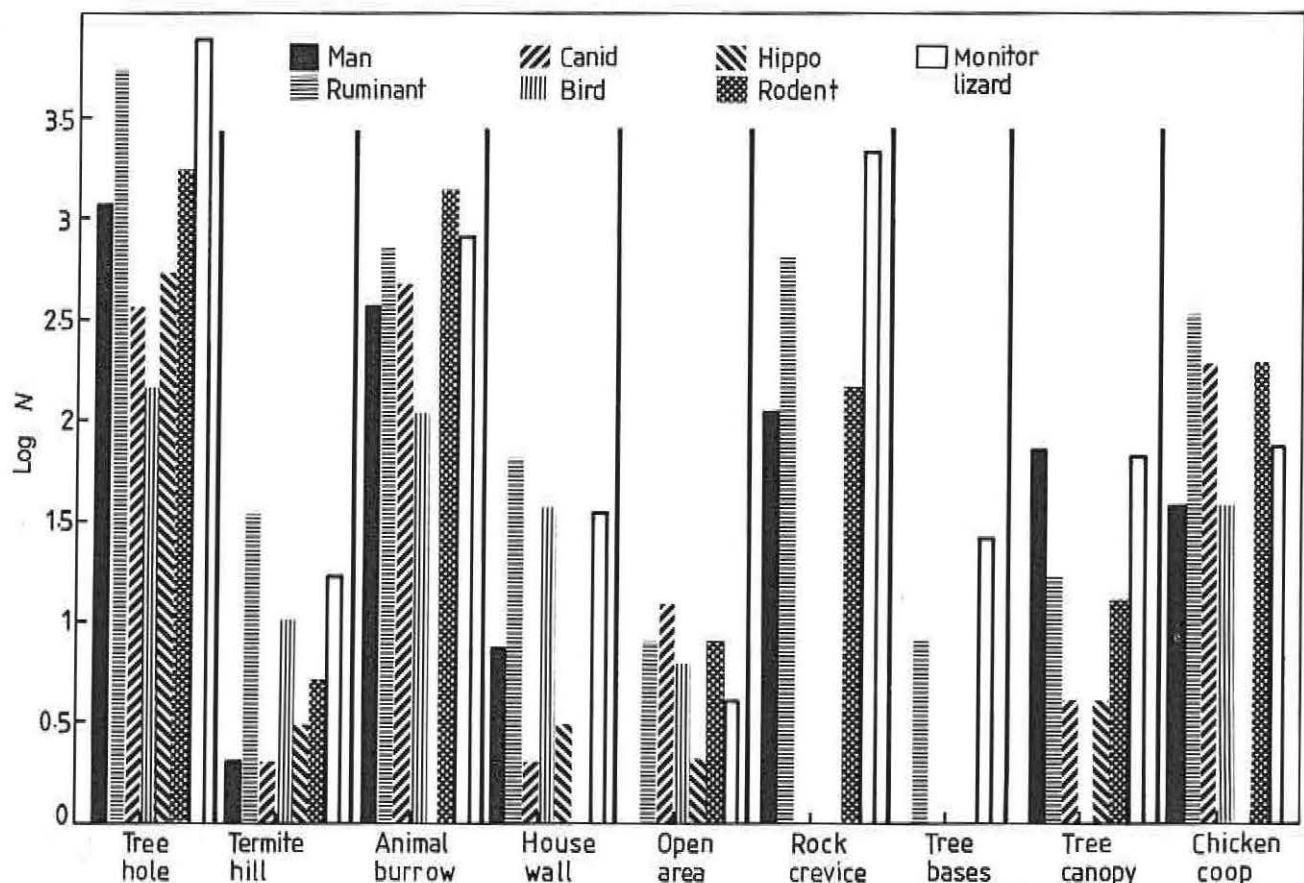


Figure 3.3 Host preferences of sandflies at Marigat by identified blood meals of flies captured in different situations.

3.4 ELISA AND LEISHMANIN TESTS FOR KALA-AZAR

C. M. Mutero, A. Ngindu, M. J. Mutinga,
T. K. Arap Siongok, P. Kenya, F. Amimo, F. Kyai
and D. Omogo

Prior to September 1986 a clinical survey was carried out in West Pokot District to determine the prevalence of kala-azar in the human population. Diagnostic methods included the Leishmanin skin test, splenic aspiration and the enzyme-linked immunosorbent assay (ELISA). Results of the first two methods were immediately available in the field, but blood samples were transported to ICIPE headquarters, Nairobi, for ELISA analysis. Blood slides for examination of malarial parasites were also sent to Nairobi.

From September 1986 blood samples from more than 2000 people were analysed by ELISA. In this method, filter-paper specimens containing dried blood were soaked in distilled water at room temperature for two hours. The samples were then wrung out with forceps and the eluates provided the required serum dilutions. The latter were reacted against *Leishmania* antigen. Reactions between positive sera and the antigen were indicated by a clearly visible reddish-yellow colour. No such colour change was observed for negative sera.

The ELISA results showed that kala-azar in West Pokot had an uneven distribution. The disease was most frequent in the low-lying areas around Kacheliba and Sigor. It was absent in the high-altitude areas around Kaibichbich. Kacheliba had the highest incidence of the disease and was accordingly chosen as an appropriate place for further studies on kala-azar vectors and reservoirs.

An examination of blood slides from the clinical survey showed that malaria was more common than kala-azar. Malaria, which had been observed in the human population during the earlier clinical survey, could therefore be responsible for a large proportion of the enlarged spleens occurring in the population.

3.5 EXPERIMENTAL INFECTION OF *Aedes Aegypti* WITH *Leishmania donovani*

C. M. Mutero, M. J. Mutinga, R. Musyoki and
D. Omogo

A laboratory experiment was begun in 1987 to determine whether *Leishmania* parasites are capable of developing in a mosquito host. We investigated the development of *Leishmania donovani* promastigotes in *Aedes aegypti*. *Leishmania* parasites were grown in a RPMI culture medium and fed to *A. aegypti* females from the mosquito colony at ICIPE. More than 50% of the mosquitoes acquired hind gut infections four days after taking the infective feed. Massive multiplication of promastigotes occurred between 6 and 17 days post infection. The highest concentration of promastigotes was initially in the pyloric and later in the rectal region. A maximum life span of 20 days was observed for *L. donovani* promastigotes in the mosquito. Morphologically intact promastigotes infected the *A. aegypti* faeces.

Further studies in this area will be undertaken in the laboratory and field to better understand the implication of the reported host-parasite interaction.

3.6 SANDFLY DISTRIBUTION IN THE MARIGAT AREA

M. Basimike

Studies on factors affecting sandfly distribution and abundance, begun in 1985, continued in 1987, when observations were undertaken on relationships between sandflies and the vegetation cover, especially sandfly vertical distribution and the microclimates in termite hills and animal burrows.

Resting sandflies on standing vegetation were collected using sticky traps pinned high up, and low down, on tree trunks. Two species of the *Phlebotomus* group (*P. martini* and *P. rodhaini*) and nine species of the *Sergentomyia* group (*S. bedfordi*, *S. antennatus*, *S. ingrami*, *S. africanus*, *S. affinis*, *S. schwetzi*, *S. adleri*, *S. clydei* and *S. squamipleuris*) were collected from large trees, open woodland and bushy sites in both dry and wet seasons. *S. bedfordi* was collected in large numbers around tree bases in woods and open woodlands. A high prevalence of sandflies was observed around large trees, probably due to the high humidity caused by the canopy cover. A high sandfly density per trap per habitat was monitored high up in large trees during both seasons.

The average nightly catches showed that large numbers of female sandflies were collected from heights of 2 to 11 m in woods and 4 to 9 m in open fields. The numbers of females were almost the double of males at each height. Between 0 and 2 m, male sandflies slightly outnumbered females. Much variation occurred in the vertical distribution of different sandfly species in the Marigat forested area. Thus, *S. bedfordi* and *S. antennatus* were the only species to reach a height of 11 m (corresponding to the canopy level). Furthermore, both species were the commonest sandflies at all heights, with their greatest density between 1 and 2 m in woods and 0 and 1 m in open fields. The high flight heights of both species suggest that their preferred sources of blood-meals are mammals, reptiles or birds of arboreal habits. Species such as *S. ingrami*, *S. affinis* and *S. adleri* were collected mainly at ground level and were designated lower-zone species; *S. africanus* and *S. clydei* appeared to search for food above the ground, preferring the middle zone (2 to 5 m).

Temperature and humidity in burrows and termite hills were monitored from November 1986 to August 1987 and these data were correlated with the numbers of sandflies collected from both sites. These investigations revealed that humidity and temperature have an important influence on sandfly population densities.

3.7 *Leishmania donovani* IN HAMSTERS

J. B. Kaddu and R. M. Musyoki

In experimental infection and transmission of *Leishmania donovani*, which involve feeding sandflies on infected

hamsters, the conventional way of detecting parasitaemia is to examine smears and/or cultures of the splenic tissues of sacrificed hamsters. To identify and maintain parasitaemic animals, however, it is necessary to demonstrate parasitaemia without killing the animals. Through splenic aspiration we have, for the first time, demonstrated viable amastigotes of *Leishmania donovani* in live hamsters. This work has shown that it is possible to detect parasitaemia in live hamsters. The technique has various advantages: (1) it allows animals to be maintained after detection of parasitaemia, (2) it increases accuracy during experimental infection because sandflies are fed only on those animals confirmed to be parasitaemic, (3) it minimizes the cost of maintaining unwanted refractory animals and (4) it provides a way of initiating *L. donovani* cultures from hamsters without sacrificing the hamsters.

3.8 INTERACTION OF LEISHMANIA, BACTERIA AND FUNGI

J. B. Kaddu, M. P. Nyamori and R. M. Musyoki

Bacterial infections and mycoses are suspected to be among the factors influencing the susceptibility of sandflies to *Leishmania*. Because of this, the effect of a 'bacterium MVRP-1' and a 'fungus MVRP-1' on the growth of *Leishmania donovani*, *L. major* and an uncharacterized reptilian *Leishmania* was investigated *in vitro*. Both the bacterium and the fungus were previously isolated from laboratory-reared Kenyan sandflies: *Sergentomyia schwetzi*, *S. antennatus* and *S. bedfordi*. The bacterium was tested against all the *Leishmania* isolates while the fungus was tested against *L. donovani* alone. The cultures were incubated at 24–30°C and their growths monitored daily with a haemocytometer for 5 to 21 days. The flasks containing bacteria lost the *Leishmania* parasitaemia in 5 days post-inoculation. The flasks containing fungi, similar to the control, maintained high parasitaemias throughout the study. The study shows that the bacterium MVRP-1 is toxic to *L. donovani*, *L. major* and the reptilian *Leishmania* but that *L. donovani* can coexist with the fungus MVRP-1.

3.9 LEISHMANIAL PARASITES: IDENTIFICATION AND CHARACTERIZATION

N. N. Massamba, B. N. Odera and R. K. Rotich

To provide diagnostic, epidemiological and taxonomic information, crucial both in gaining better understanding and for controlling diseases, parasites of medical and economic importance need to be identified and characterized.

Classical characterization methods rely largely upon morphology, but many parasites that are morphologically indistinguishable show different behavioural characteristics. Biochemical and molecular biological methods are valuable in such cases and should provide greater powers of resolution. MVRP studies continued in 1987 include adapting leishmanial field isolates *in vitro* culture, cloning wild isolates, mass cultivation of cloned and uncloned leishmanial populations and isolating and purifying DNA to

identify repetitive and single-copy DNA sequences that may be used as probes to distinguish leishmania species and subspecies. The objective is to establish whether genomic differences detectable with DNA sequence probes can provide information about the phylogenetic interrelationships between and within leishmanial parasite species. A genomic expression library constructed from cloned leishmania DNA IC-140 has been screened with radiolabelled total DNA from cloned leishmania DNA stock IC-140 and with total DNA from leishmania marker strain IC-235 (WHO marker strain LRC-L37).

Twenty-five clones showed strong positive hybridization signals and five of them have been selected for further characterization.

Total protein electrophoresis and enzyme electrophoresis have both been useful for interspecific differentiation of protozoan parasites such as leishmania, but total protein patterns are complex and tedious to analyse. Measuring differences in the electrophoretic mobility of enzymes helps to determine the genetic diversity of organisms, but different species often have similar electrophoretic patterns. Thus we plan to supplement enzyme electrophoresis with the highly discriminatory method of isoelectric focusing to identify leishmanial parasites. Thirty leishmanial isolates and six WHO marker strains have been prepared to date for isoenzyme analysis. The protein cell extracts in the form of beads were resolved on cellulose acetate electrophoresis and studied for 15 enzyme systems (Table 3.1). Five enzyme systems have been selected for constructing the enzyme profiles (zymograms) since they have been consistently well resolved, well stained and reproducible. These include malic acid (malate) dehydrogenase EC 1.1.1.37 (MDH), alanine amino transferase EC 2.6.1.2 (ALAT), lactic acid dehydrogenase EC 1.1.1.12 (LDH), mannose phosphate isomerase EC 5.3.1.8 (MPI) and superoxide dismutase EC 1.15.1.1 (SOD). They are now being used to identify and characterize the isolates from the field.

Table 3.1 Enzymes examined by cellulose acetate electrophoresis

Enzyme	EC number	Abbreviation
Alanine amino transferase	2.6.1.2	ALAT
Aldolase	4.1.2.13	ALD
Aspartic amino transferase	2.6.1.1	ASAT
Fructose kinase	2.7.1.1	FK
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PD
Glucose phosphate isomerase	5.3.1.9	GPI
Lactic acid dehydrogenase	1.1.1.12	LDH
Malic acid dehydrogenase	1.1.1.37	MDH
Malic enzyme	1.1.1.40	ME
Mannose phosphate isomerase	5.3.1.8	MPI
Nucleoside hydrolase	3.2.2.2	NH
6-phosphogluconate dehydrogenase	1.1.1.44	6PGD
Phosphoglucomutase	2.7.5.1	PGM
Proline dipeptidase	3.4.3.4	PEPD
Superoxide dismutase	1.15.1.1	SOD

3.10 PARASITOLOGY OF MALARIA: GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND ITS EFFECT ON SUSCEPTIBILITY OF MAN TO MALARIA

C. C. Kamau

A genetic factor that limits the growth of malarial parasites in the human host is glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) deficiency. The protection phenomenon is manifested in heterozygote females who are genetic mosaics, but surprisingly not in homozygous-deficient females or hemizygous-deficient males. Recently it was shown that this lack of protection may be due to the production by the parasite growing in such deficient cells of its own G6PD. But those experiments employed laboratory strains of the parasite grown continuously *in vitro*. The purpose of the work reported here was to investigate if the production of parasite-specific G6PD does occur *in vivo*.

Immature (ring stage) *Plasmodium falciparum* obtained from patients were cultured through one life cycle only, in a RPMI-1640 medium, by the petridish-candle jar method. The mature (trophozoite and schizont) parasites were harvested and fractionated by density centrifugation. The mature parasite fraction and the red blood cell (uninfected) fraction (Figure 3.4) were sonicated and then subjected to G6PD electrophoresis in cellulose acetate gel (cellogel).

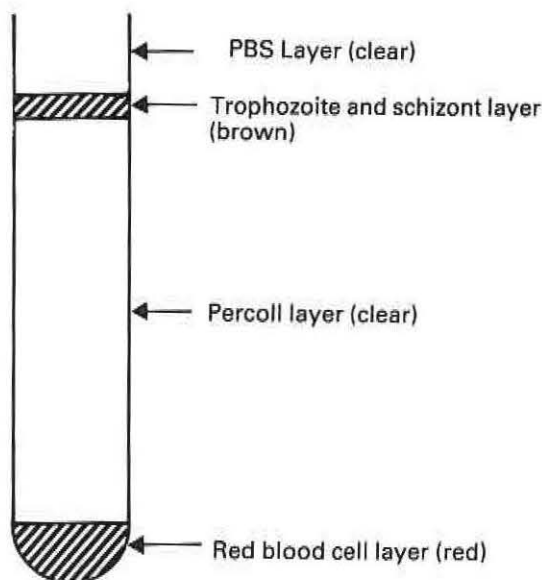


Figure 3.4 The harvesting and fractionation of malarial parasites by density centrifugation.

Electrophoresis results (Figure 3.5) suggest that *P. falciparum* does produce its own G6PD when growing in enzyme-deficient red cells *in vivo*. This would explain why hemizygous-deficient males are not protected from malaria while the heterozygous-deficient females are protected.

In G6PD hemizygous- or homozygous-deficient individuals, the parasite is able to grow significantly following its adaptation by producing its own G6PD in the host cells (which are one type). But growth in heterozygote individuals

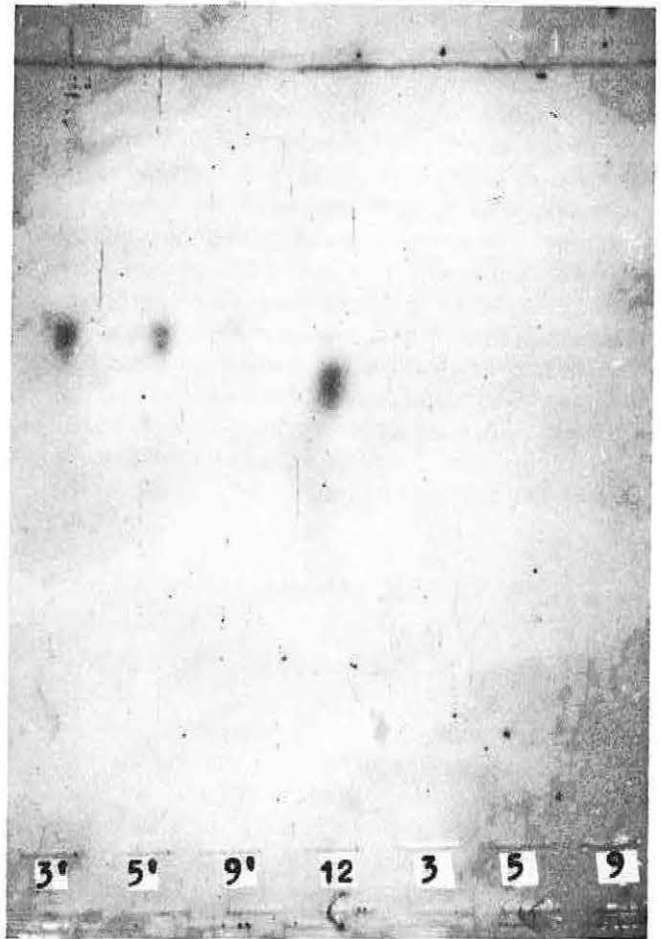


Figure 3.5 The detection by electrophoresis of glucose-6-phosphate dehydrogenase (G6PD) in *Plasmodium falciparum* cultivated in G6PD-deficient erythrocytes.

is poor because the parasite must pass from G6PD-normal to G6PD-deficient cells within the same individual.

3.11 ECOLOGY OF MOSQUITO VECTORS OF MALARIA IN BARINGO DISTRICT

I. Aniedu

A major area of investigation during 1986 and 1987 was the ecology of the immature stages of mosquitoes. Accurate estimations of immature developmental rates and survivorship are essential in the study of the population dynamics of mosquitoes in an area because larval population estimates are required to compare population sizes before and after control programmes.

The Lobo Swamp in Marigat, Baringo District, supports mosquito breeding all year round. In seasonal abundance studies, gonoactive females and first-instar larvae were collected throughout the year, indicating overlapping generations. The vertical life-table method was thus considered suitable for estimating the survivorship of immature *Anopheles gambiae sensulato* in this habitat. Two experiments were conducted, one in a rainy season (June 1986) and the other in a dry season (February 1987). In each case the method of Service (1973) was used. Two hundred dips were made daily from pre-determined areas of the

swamp for ten consecutive days, using a standard aluminium dipper. The pre-imagines taken in the 200 dips were pooled and returned to the laboratory where they were counted, identified and scored to instars.

First, the age-specific distribution curves were constructed, using the sampling data. Since these experiments were conducted under conditions that assumed a stable larval population throughout the sampling period, the age-specific distribution curve is assumed to simulate the time-specific survivorship curve (Service 1973, 1977). The stage-specific survival rates, shown in Table 3.2, were then generated from these curves.

Survivorship from first instar to adult was 0.085 during the rains (June) but dropped to zero during the dry season (February). Mortalities of immature stages were high in the second, fourth and pupal stages during the dry season, accounting for the big difference in overall survivorship between the two periods. The proportion dying daily in each instar was also consistently higher in the dry season than in the rainy season; in agreement with this, K (the generation mortality or sum of all the intermediate mortality factors) was also higher.

There is evidence that most of the larval mortality at the swamp was due to predation, but the data on this aspect of the investigation are not yet fully analysed.

Table 3.2 Stage-specific survivorship of immature *A. gambiae* in a larval habitat in Baringo District

Habitat	Instar	N	S1	S	D1	P1	k
Loboi (June 1986)	I	474	590	1.000	220	0.373	0.203
	II	150	370	0.627	235	0.635	0.438
	III	124	135	0.229	35	0.259	0.130
	IV	64	100	0.169	45	0.450	0.230
	P	54	55	0.093	5	0.091	0.041
	A			50	0.085*		
Loboi (Feb. 1987)	I	875	1095	1.000	435	0.397	0.220
	II	226	660	0.603	460	0.697	0.519
	III	183	200	0.183	70	0.350	0.187
	IV	57	130	0.119	105	0.808	0.716
	P	6	25	0.023	25	1.000	—
	A			0	0.000*		

N: Number collected/stage duration.

S1: Number entering stage (estimated from survivorship curve).

S: Cumulative survivorship from one stage to another: * = overall first instar to adult survivorship.

D1: Number dying during each stage, (S1₁ - S1₂, etc.).

P1: Proportion dying in each instar, D1/S1.

k: 'Killing power' of the mortality factors acting on each stage, (log S1₁ - log S1₂, etc.).

** Total generation mortality (K).

TSETSE RESEARCH PROGRAMME

- 4.1 Population manipulation of *Glossina pallidipes* 65
- 4.2 Parasitoids and predators of *Glossina pallidipes* at Nguruman 68
- 4.3 Pupal ecology 68
- 4.4 Trapping studies on *Glossina longipennis* at Nguruman 68
- 4.5 Trypanosome/vector interactions and disease epizootiology 69
- 4.6 Tsetse genetics 69
- 4.7 Enzyme polymorphism in *Glossina longipennis* 70
- 4.8 Reproductive physiology of *Glossina pallidipes* mating behaviour, feeding and insemination in a laboratory strain 70
- 4.9 Hormonal regulation of the onset of female receptivity 71
- 4.10 Effect of a juvenile hormone analogue on tsetse development 71
- 4.11 Protein synthetic activity of the milk glands during larval development 72
- 4.12 Epidemiology of animal trypanosomiasis in Lambwe Valley 72
- 4.13 Further isoenzyme analysis of cattle stocks from Lambwe Valley 73
- 4.14 Sensitivity of *T. congolense* stocks to samorin and berenil 74
- 4.15 Antibacterial spectrum of insect immune haemolymph factors 75
- 4.16 Pathogenicities of entomopathogenic bacteria for adult tsetse *Glossina morsitans morsitans* 75



4

Tsetse Research Programme

Tsetse-transmitted African trypanosomiasis has hindered the full exploitation of livestock in some 38 countries in Africa, an area estimated to be 10 million sq km. Restrictions on keeping domestic livestock limit also the development of farming systems dependent on animal traction. Besides the economic losses, trypanosomiasis has a direct effect on human health. Tsetse control has therefore been one approach to contain the scourge of trypanosomiasis. Tsetse control measures have included habitat destruction, application of insecticides and genetic manipulation. These methods have not controlled tsetse on a long-term basis and the measures directed against trypanosomes have not produced the major breakthroughs that Africa requires.

The failure to achieve long-lasting success in tsetse control suggests that new strategies are required. The ICIPE Tsetse Research Programme (TRP) has consequently put much effort into collecting baseline field data to elucidate—and to help make predictions regarding—vector-disease dynamics. In this way we may avoid relying on inappropriate control technologies.

The points enumerated below highlight the TRP activities carried out in 1987.

- *Suppression of the tsetse population in Nguruman, southwestern Kenya, with the help of members of a local group ranch*
- *Monitoring the decline of cattle trypanosomiasis as one indicator of the effectiveness of tsetse suppression*
- *Observations on natural enemies of tsetse pupal stages*
- *Development of effective traps for *G. longipennis**
- *Studies on tsetse population genetics*
- *Studies on the reproductive biology of *G. pallidipes**
- *Studies on the spread of *G. pallidipes* outside its natural habitat in Lambwe Valley and the consequent increase in cattle trypanosomiasis*
- *Characterization of trypanosome stocks from tsetse flies and cattle from Lambwe Valley, and their relationship to human isolates*
- *Studies on the immune mechanism in tsetse flies*

4.1 POPULATION MANIPULATION OF *GLOSSINA PALLIDIPES*

R. D. Dransfield, R. Brightwell, C. Kyorku and B. Williams

Too often in the past, tsetse control has benefited the foreign expert more than the local people, with the emphasis on high technology coupled with little understanding of either vector or disease dynamics. The Nguruman project is an attempt to redress this imbalance. From May 1983 to December 1986, our principle aim was to obtain in-depth knowledge of what factors determine and regulate tsetse population size and distribution. This culminated in the development of a

population simulation model. At the same time, research was carried out on devising novel cost-effective trapping systems, which led to the creation of the NGU trap, baited with cow urine and acetone. Our aim in 1987 was to suppress the tsetse population in the study area using the new traps both to test the population model and to assess the feasibility of involving the local, Maasai, community in tsetse control.

Trap manufacture by members of the Olkeramatian Group Ranch started in January. We held a general meeting at the ICIPE field station with Maasai community leaders and interested local people, followed by training sessions in the local homestead ('manyatta'). The response was enthusiastic and by the end of the month nearly 100 traps were ready for deployment. People who made the traps put

their cattle marks on them so they could be identified in the field. In response to a questionnaire, very few said they had any difficulties in making the traps, and most felt they could make more without further assistance from ICIPE. They expected that the traps would greatly reduce the number of tsetse, but estimates on how long this would take varied from a few months to ten years! The operation demonstrated

clearly that the NGU traps could be made by the local people with a minimum of training and equipment.

The traps were deployed early in February 1987 over approximately 100 sq km of grassland and woodland (Figure 4.1, Box [1]), with the traps concentrated in the woodland at a density of 2-3 per sq km. Higher densities of traps were deployed in a narrow strip of woodland joining the study

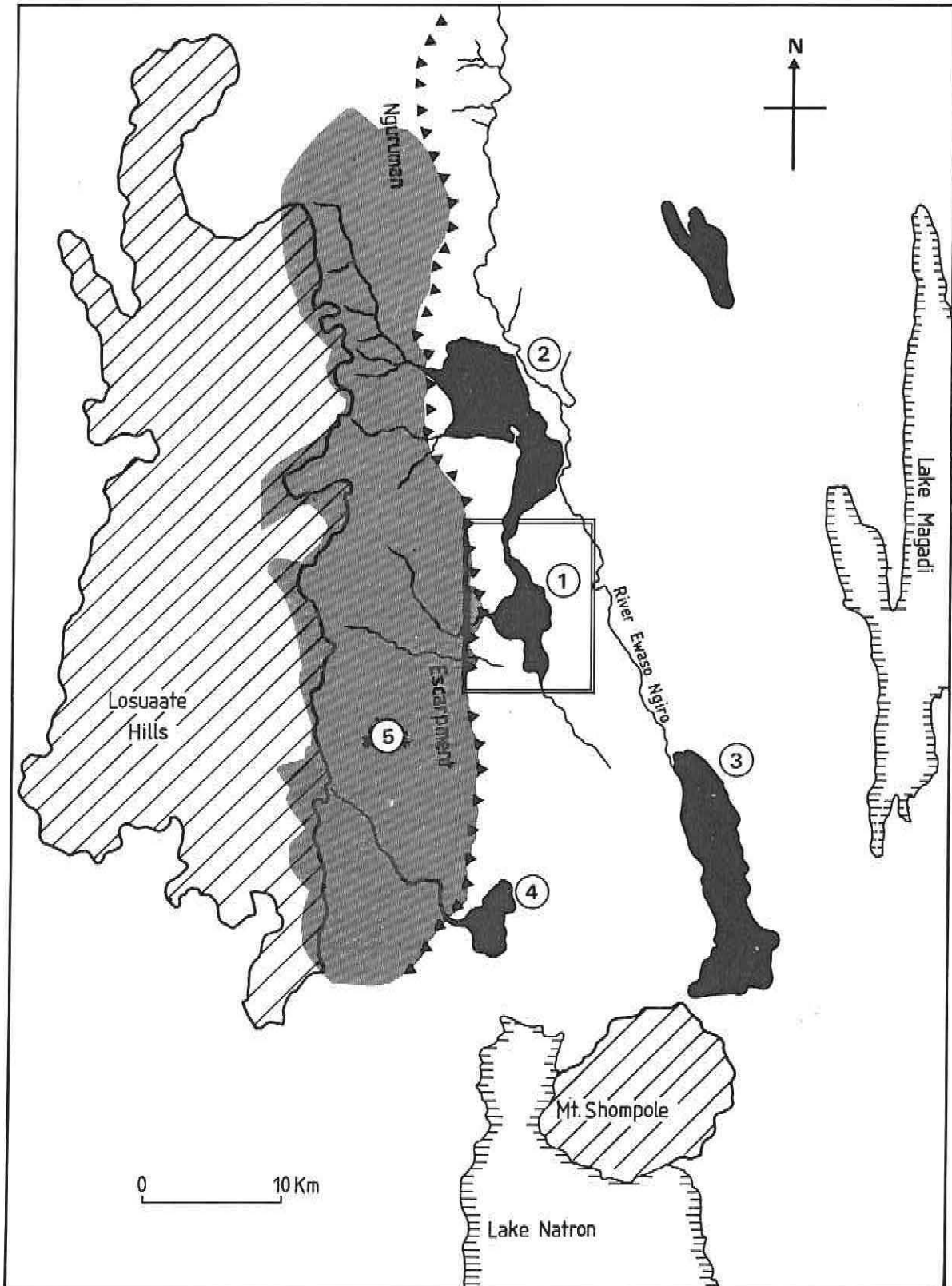


Figure 4.1 Map of Nguruman area, Kajiado District, Kenya. Black areas represent *G. pallidipes* subpopulations below the escarpment; the shaded area represents the more diffuse escarpment population. Area (1) is the suppression zone.

area to the northern area (2) and in the Sampu Valley, coming down from the escarpment (5) as barriers to reduce invasion pressure.

Wooden poles to support the traps were cut locally, and the bases treated with termiticide. We used sisal string initially to tie the trap to the poles, but this was soon replaced with thin wire. All traps were baited with cow urine, collected by the local people, and dispensed from 1-kg discarded cooking fat tins. The top of each tin was covered with polythene, with a hole cut at the top edge to give a release rate of about 1 ml/hour during the day. Acetone was dispensed from 200-ml bottles, with a hole in the lid giving a release rate of about 150 mg/hour. The flies collect in the polythene bag cages, where they die rapidly from heat stress; the cages are usually cleared of dead flies by ants. We found it necessary to smear the base and top of the cage with 'geneterrent'—a mixture of car grease and chilli powder that deters genets (and most other animals) from eating the flies and damaging the cage in the process.

The effects of the trapping were monitored in two main ways. Sampling for one week each month with baited biconical traps was continued both inside and outside the suppression zone. In addition, 20 representative NGU traps were designated monitoring traps, greased to keep ants out and emptied 2–3 times each week for counting the flies. Tsetse were also marked each month both inside and outside the suppression zone to estimate population sizes and assess the amount of movement between different areas.

Initial catches by the traps were very high and from the catches in the monitoring NGUs, we estimated that in the first three weeks over 200 000 female *G. pallidipes* were removed from the population, thus increasing the natural mortality rate by 3% per day. During the dry season the barriers were effective, with catches in the biconical traps declining rapidly within the suppression zone (Figure 4.2). With the early onset of the rains in March, catches increased again slightly and there was evidence that flies were invading the area despite the barriers. After June, however, numbers declined sharply to give a 99% reduction by October, as predicted by the model. In the short rains in November there

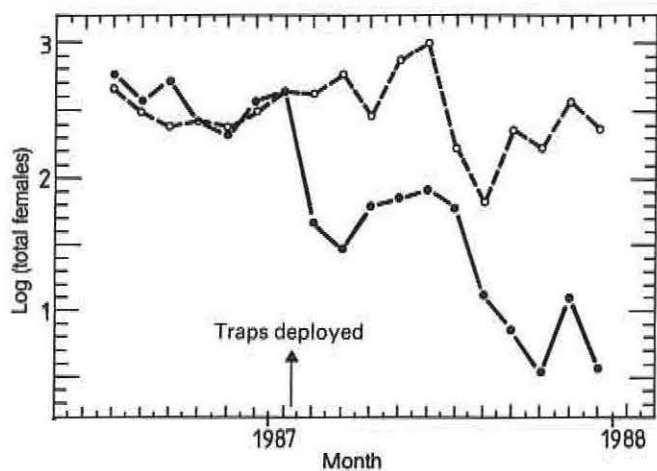


Figure 4.2 Catches of female *G. pallidipes* in odour-baited biconical traps before and after trap deployment within suppression zone (solid line) and 5 km to north outside suppression zone (dotted line).

was again evidence of a small reinvasion, but numbers dropped again in December.

Catches in the monitoring NGUs along the riverine thickets (Oloibototo River) of both males and females are shown in Figure 4.3. These catches show similar trends to catches in the biconical traps, although the initial drop in February is not apparent. The most interesting point is that the short-lived November invasion is much more evident for the females than for the males. The females appear to disperse much more rapidly than the males, with mass movements occurring on cool days (with maxima below 30° C). Many of these females come from the escarpment, and it is now clear that the cooler escarpment acts as a reservoir for tsetse, with escarpment flies restocking the lowland areas after long dry periods.

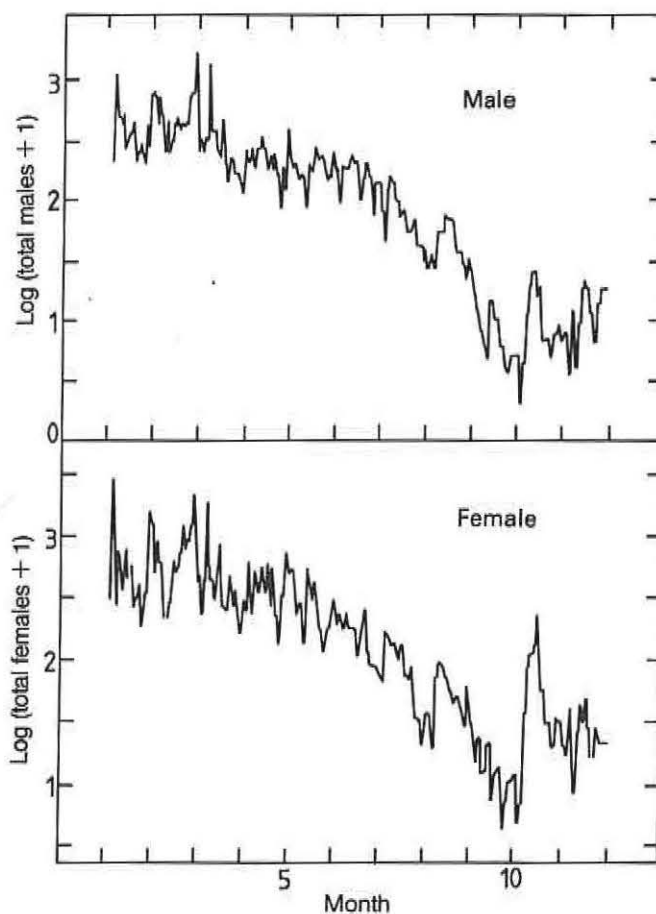


Figure 4.3 Catches of male and female *G. pallidipes* in monitoring odour-baited NGU traps within the suppression zone.

The evidence of fly movement through our barriers has led us to increase trap density both in the area to the north and along the base of the escarpment. Between October and December a further 80 traps were deployed at 10 traps per kilometre. Although it would probably be more efficient to distribute the traps over a larger area, especially on top of the escarpment, this is neither logistically nor politically feasible, since the escarpment area is outside the Group Ranch. Future research will therefore concentrate on improving the efficiency of the barrier and on identifying the factors responsible for large-scale movements of flies between

ecological zones. This will enable spatial heterogeneity and fly movement to be incorporated realistically into the model, allowing the most efficient use of traps and the most cost-effective means of breaking disease transmission.

4.2 PARASITOIDS AND PREDATORS OF *GLOSSINA PALLIDIPES* AUSTEN AT NGURUMAN

D. A. Adabie, R. D. Dransfield, T. S. Dhadialla and S. Essuman

Studies were continued on the natural enemies of tsetse at Nguruman. There were seasonal fluctuations in the rate of parasitization of the pupae by two dipteran species of the family Bombyliidae, *Exhyalanthrax lugens* and *E. beckerianus*. Together these species caused about 12% mortality. These parasitoids were not tsetse specific, nor was the mortality density-dependent, so parasitism was not a factor in the regulation of tsetse populations.

Potential tsetse predators at Nguruman were sampled by several methods. Pitfall traps and constant-time searches of the soil were effective for capturing pupal predators. Hand-nets and biconical traps proved suitable for sampling potential adult predators. There was no apparent relationship between numbers of tsetse and abundance of potential predators, indicating that none of the predators considered was specific to tsetse.

Serological analysis of predator gut contents to identify natural predators of tsetse was carried out using the agar gel double immunodiffusion technique. The gel precipitin test proved specific to *Glossina*, and the length of time a tsetse remained detectable in guts of predators varied from a minimum of 9 hours for the cricket *Liogryllus bimaculatus* to 48 hours for another cricket, *Phaeophilacris* sp.

The relative proportion of positive results in field-collected predators varied, but of the adult predators Asiliidae (25.0%), Hymenoptera (15.8%) and Odonata (15.2%) consistently had higher proportions of positive results and were abundant in the field. This suggests that they were the most important natural predators of adult *G. pallidipes* at Nguruman. Of the pupal predators, 12.9% of crickets tested gave positive results.

4.3 PUPAL ECOLOGY

P. Muange and R. D. Dransfield

Studies have been continued at Nguruman on the pupal ecology of *Glossina pallidipes*. The objectives of the study were to improve the sampling technique for pupae and to carry out more detailed studies on mortality factors affecting puparia in larviposition sites.

Two techniques were used to sample pupae in larviposition sites, the time-constant hand-searching method (two man-hours) and the quadrat method. The time-constant hand-searching method gives only a relative estimate of numbers of pupae in larviposition sites. Moreover, sampling efficiency may vary seasonally. To quantify absolute numbers in larviposition sites, an

alternative method of quadrat sampling was tested. Simple larviposition traps were made of 100 cm × 50 cm × 8 cm metal trays with a wire-mesh bottom and soil from natural larviposition sites. These traps were left in recognized larviposition sites. The soil was then sieved each month and all pupae in it collected. This technique proved ineffective: it yielded only 3 live puparia compared to 193 puparia obtained using time-constant hand-searching.

Efforts were then made to quantify the factors affecting the efficiency of hand-searching. In the first experiment, the size of the larviposition site was varied. Three sites of different sizes were used. Thirty empty pupal cases marked inside were buried randomly at a depth of 3 cm in each category of site. The sites were hand-searched for two man-hours. Three replicates were made in each site category. The recovery rate was then related to the size of the sites.

Studies on mortality factors affecting pupae in larviposition sites are being continued. Field-collected pupae have been weighed to differentiate live from dead pupae. Live pupae were kept in vials to wait for the emergence of adults, or to detect mortality due to parasitism or other factors. Adults of two dipteran species of the family Bombyliidae—*Exhyalanthrax lugens* and *E. beckerianus*—have emerged from some of these pupae.

Further investigations are under way on the nature and extent of puparial mortality. These studies involve the burial and later exhumation of laboratory-bred puparia in typical larviposition sites and at varying densities. It is hoped that puparia can soon be obtained from the *G. pallidipes* colony being established at Nguruman for these field studies.

4.4 TRAPPING STUDIES ON *GLOSSINA LONGIPENNIS* AT NGURUMAN

C. Kyorku, R. Brightwell and R. D. Dransfield

Studies were undertaken to develop an effective trap for sampling *Glossina longipennis* and for subsequent studies on the dynamics of the resident population of *G. longipennis* at Nguruman. Several known tsetse trap types plus new trap designs and various odour baits were tested in a series of Latin square design experiments.

A new trap designed at Nguruman, the NG2B trap, and the Zimbabwe F3 trap were found to be the most effective traps for *G. longipennis*. When baited with cow urine or buffalo urine (1000 mg/hr) and acetone (500 mg/hr), they caught on average five times more than a similarly baited biconical trap. Furthermore, these traps caught twice as many females as the biconical trap. Since the NG2B trap is far cheaper and easier to construct and handle than the F3 trap, the former is recommended as a sampling tool for *G. longipennis*.

Subsequently, regular monthly sampling with the NG2B trap was introduced in June 1986 to study some aspects of the population dynamics. Generally, catches of both sexes declined in the dry season from July to October, increased again till January and declined again from February to April. The low catches in the dry season probably reflect the adverse effects of low relative humidity and high temperature.

The spatial distribution of *G. longipennis* was also investigated by siting traps in the different vegetation types.

In most months *G. longipennis* concentrate (60%–80%) in the open woodland bordering the riverine thickets. Flies spread out into the open plains (10%) in cool months, especially during the rains, but only move into the riverine thickets (30%) in hot dry months.

The activity pattern of *G. longipennis* was determined more precisely (it is common knowledge that *G. longipennis* is a crepuscular species). From 15-minute interval collections made from an odour-baited electrocuting screen, peak catches were recorded at 1830–1845 hr in the evening (sunset 1800 hr), with a smaller peak at 0600–0615 hr in the morning (sunrise 0630 hr) (Figure 4.4). It was also observed that males regularly flew earlier than females but both peaked and both stopped flying at the same times.

Light intensity was apparently the major factor influencing activity: catches rose with decreasing light intensity from 1745 hr, peaked at 90 lux units and decreased steeply thereafter when light intensity also drops sharply.

4.5 TRYPANOSOME/VECTOR INTERACTIONS AND DISEASE EPIZOOTIOLOGY

S. R. Tarimo and T. K. Golder

To determine whether tsetse manipulation at Nguruman, described in the preceding papers, had any effect on the

incidence of trypanosomiasis in cattle, trypanosome infection in both tsetse and cattle were monitored before and during the manipulation period. Figure 4.5 shows a record of the decline of trypanosome infection in cattle. *Trypanosoma congolense* was the most abundant trypanosome species observed in cattle. In tsetse flies, however, *T. vivax* was the most abundant. The decline in the prevalence of trypanosome infections during the manipulation period shows clearly that there was low tsetse challenge.

4.6 GENETIC VARIATION IN TWO FIELD POPULATIONS AND IN LABORATORY-REARED *G. PALLIDIPE* AUST.

S. R. Tarimo

Although knowledge of tsetse genetics may be important in determining the best type of control approach, there is little information available. It is thus impossible to postulate ways of making use of genetics to control tsetse and the disease it transmits.

Field collected *G. pallidipes* from Nguruman and Lambwe were compared with laboratory-reared samples using polyacrylamide gel electrophoresis. The percentage of polymorphic loci was 35.7 for Lambwe and 14.3 for Nguruman. The respective mean heterozygosity was 15.7%

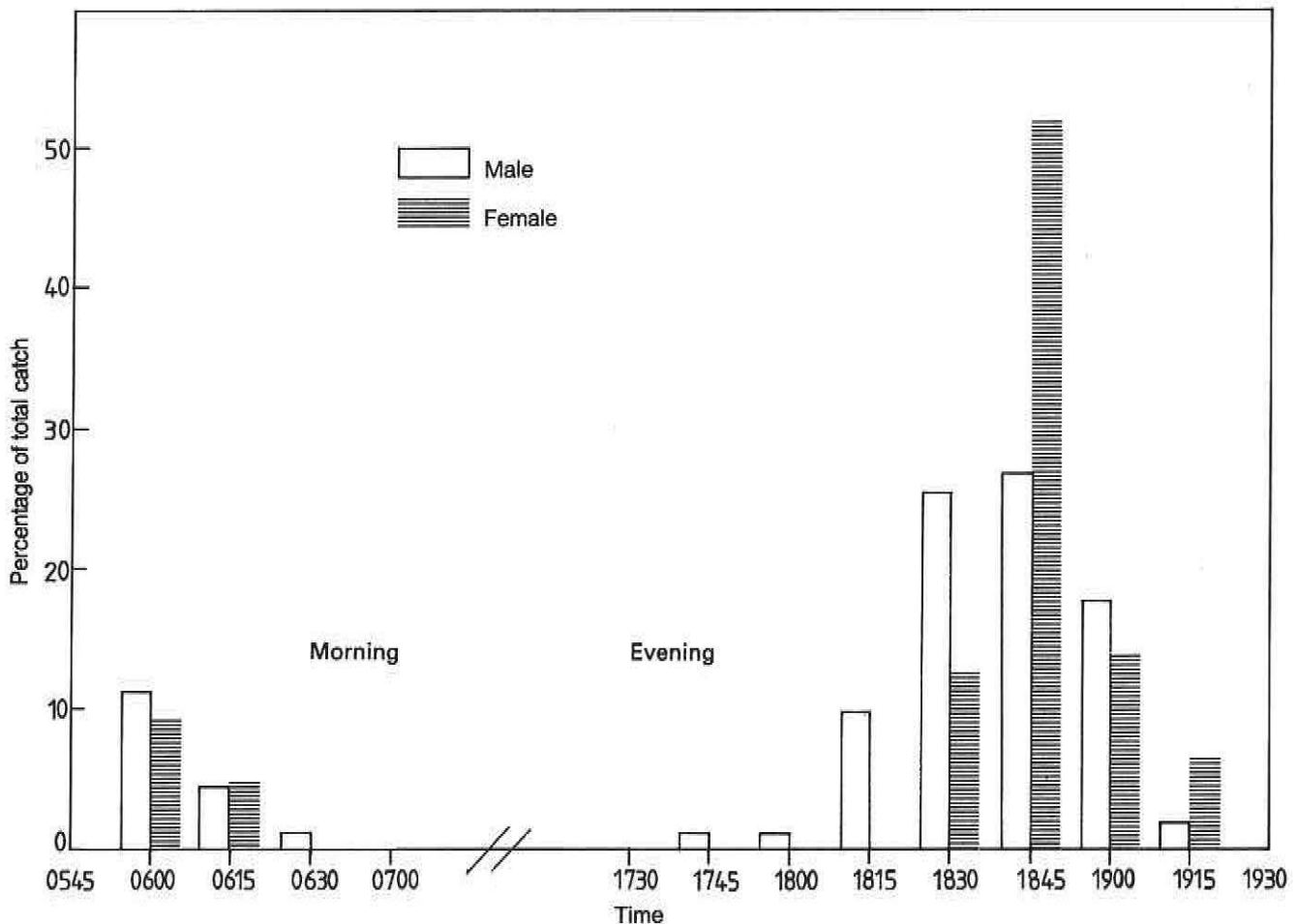


Figure 4.4 Diurnal activity of *G. longipennis* in Nguruman. Notice the higher catches in the evening compared to early morning catches.

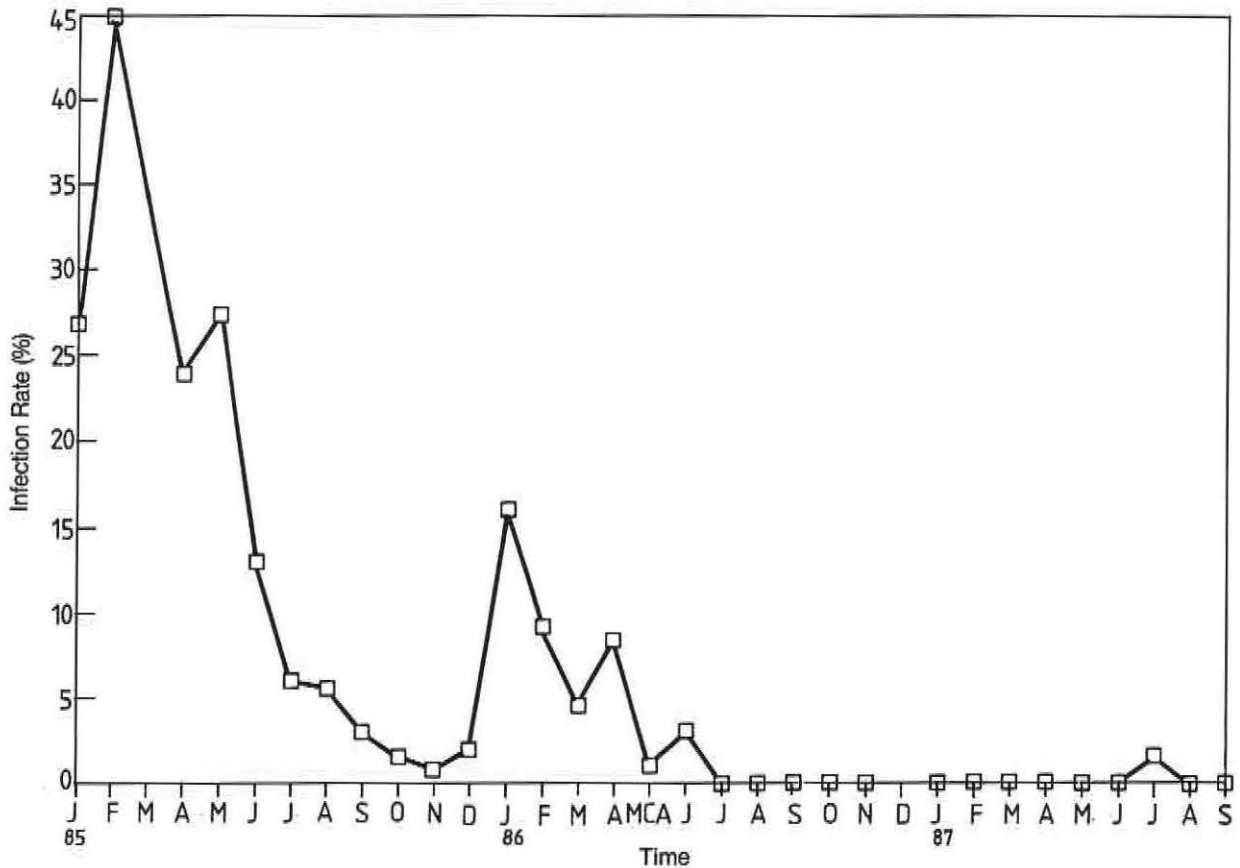


Figure 4.5 Decline in cattle trypanosomiasis in Nguruman as a result of suppression of *G. pallidipes* using traps.

and 9.9%, respectively. The mean allele frequency for esterase-1 and octanol dehydrogenase was 0.379 and 0.088 for Lambwe and 0.454 and 0.020 for Nguruman. There were no significant differences between Lambwe flies collected from the field and those reared in the laboratory. Phosphoglucumutase, phosphoglucoseisomerase, hexokinase and adenylate kinase are reported for the first time in *G. pallidipes*.

4.7 ENZYME POLYMORPHISM IN *GLOSSINA LONGIPENNIS*

S. R. Tarimo

Much work has been done in recent years on *Glossina morsitans* genetics and in the *palpalis* groups. However, little similar work has been done on the *fuscus* group. The only available information on this group is a report on the chromosome number of two of its members: *G. fuscus congolensis* and *G. brevipalpis*.

The electrophoretic mobilities of enzymes, allele frequencies and heterozygosities at six loci in field-collected *G. longipennis* were observed using polyacrylamide gel electrophoresis. The mean average heterozygosity was observed to be 4.5%. Octanol dehydrogenase and a thoracic esterase were polymorphic. The mean allelic frequency for octanol dehydrogenase was 0.176; that of esterase-1 was 0.095. This is the first report on enzyme polymorphism in *G. longipennis*.

4.8 REPRODUCTIVE PHYSIOLOGY OF *GLOSSINA PALLIDIPES*: MATING BEHAVIOUR, FEEDING AND INSEMINATION IN A LABORATORY STRAIN

M. F. B. Chaudhury

G. pallidipes originating from Lambwe Valley is being reared in the ICIPE laboratory successfully. This has encouraged us to study physiological aspects of *G. pallidipes* males and females not studied in detail in the past due to lack of a laboratory colony of the species. To establish baseline information on the performance of the new colony, as well as to compare the data with information from *G. pallidipes* strains from elsewhere, we have investigated the mating, insemination, larval development and progeny production of *G. pallidipes* in this colony.

Although most males of all known *Glossina* species become sexually mature between day 4 and 6, most *G. pallidipes* males do not show any interest in mating at that age. About 50% of the males become sexually mature and appetitive when they are at least 9–10 days old. More than 90% of the two-week-old males are capable of mating and inseminating sexually mature females. A few (less than 20%) males of 6 to 8 days old show normal copulatory behaviour but these males can neither inseminate the females adequately nor produce a complete spermatophore. These results do not agree with those obtained from *G. pallidipes* of other strains.

A detailed study on the development of the male reproductive organs of *G. pallidipes* from our colony indicates that although males 5 days old possess their full complement of spermatozoa and other testicular materials, the development of male accessory glands is incomplete at that time and they do not have adequate accessory reproductive gland (ARG) secretion. The inability of males to successfully inseminate during these days of adult life can be attributed primarily to the insufficiency of ARG secretions. This is exemplified by the occurrence of undefined and incomplete spermatophores in uteri of some females that have copulated with 6- to 8-day-old males. Where females were inseminated by 6- to 9-day-old males, the mean spermathecal value (MSV) observed in these females was extremely low. A clear correlation is observed between the increase in diameter of the ARG and the amount of sperm transferred by males from day 6 to day 10.

From experiments designed to establish if feeding and production of ARG secretions are coupled to the ability of males to inseminate, it is clear that the number of blood-meals has a direct influence on the quantity of sperm transferred. A detailed study on the developmental biology and the synthetic activity of the ARG is in progress.

From results of experiments to determine if sexual behaviour and capability to inseminate are under hormonal control, it appears that injection of brain extracts from sexually mature males into 5- to 7-day-old males increases copulatory behaviour and in some cases results in incomplete insemination. The brain extracts used for these experiments were obviously of very low activity. If neurosecretions affect sexual behaviour and maturation, it is reasonable to expect a high rate of induction of insemination in injected flies. However, the relatively low activity of the extracts can be attributed to several factors, one of which could be that the neurosecretory granules in extracts of neurosecretory cell parykaria may be hormone precursors and these granules may mature as they pass down the axon to the neurohaemal area, which is the dorsal aorta in the case of *Glossina*.

Since newly emerged males have a small amount of secretion in their ARG it seems likely that the process of synthesis of secretion commences before adult emergence. The full capacity for synthesis is attained only after a blood-meal. The preliminary results indicate that feeding and neurosecretions are coupled to the process of sexual maturation, which is attained at a slower rate in *G. pallidipes* than in other species of *Glossina* studied.

4.9 HORMONAL REGULATION OF THE ONSET OF FEMALE RECEPTIVITY

L. M. Riddiford* and M. F. B. Chaudhury

Unlike most other *Glossina* species, female *Glossina pallidipes* normally do not mate until 6–9 days after eclosion, just as the first egg is matured and ready to ovulate. To determine the physiological basis of this delay in mating, virgin female *G. pallidipes* from an Mbita Point colony were tested once daily for mating from day 3 to day 12 with males between 14 and 25 days of age. Testing was done either individually or in groups of 5 to 10 for a period of 30 minutes

to 1 hour and was usually done 2 hours before the lights were turned off, although mating was also observed between 2 and 3 hours after the lights were turned on if the flies had not been fed. As shown in previous studies, the peak mating occurred on day 8, with some females mating as early as day 6. Irrespective of the age of the female, males would attempt copulation, but the younger females successfully rejected them.

Injection of 1 μ l water on day 2 advanced the peak mating to day 7 with 3 of the 29 mating either on day 3 or 4. When 1 ng of 20-hydroxyecdysone (20HE) was injected on day 2, females mated earlier, with peak mating on days 5 and 6. A higher dose of 2 ng 20HE appeared to be somewhat less effective, and 10 ng 20HE proved toxic. Due to a lack of flies, lower doses were not tested. Females that emerged from field-collected puparia (from Nguruman) showed a similar response.

Haemolymph was collected from virgin females from day 1 to day 8 and from mated females at various stages in the larval cycle and processed for ecdysteroid measurement. The ecdysteroid titres were determined by radioimmunoassay by Dr. Thomas Kelly, of the United States Department of Agriculture, in Beltsville, Maryland. The preliminary results based on two pooled samples each day during the first 8 days indicate that there is a small but significant peak (55 ng/ml 20HE equivalents) on day 5, then by day 7 it has dropped to basal levels (about 5 ng/ml). The titre then begins to rise rapidly after the larva hatches, showing a peak (1.0–1.5 ng/ml) in the early third instar, then dropping sharply after parturition. These titres are similar to those in *Glossina fuscipes*.

These data together suggest that ecdysteroids, presumably from the developing ovaries, may act on the nervous system of the female *G. pallidipes* to turn on her receptive behaviour.

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4.10 EFFECT OF A JUVENILE HORMONE ANALOGUE ON TSETSE DEVELOPMENT

L. M. Riddiford*

A potent juvenile hormone analogue—2-(1-methyl-2-[4-phenoxyphenoxy]ethoxy) pyridine (S31183), obtained from Sumitomo Chemical Co.—was applied to mated female *G. pallidipes* collected at Nguruman. The females were then caged either separately or in groups of ten, and the puparia collected daily and kept for 35 days until emergence or dissection. Application of 1- μ l acetone had little effect on development of subsequent offspring. By contrast, as little as 0.002- μ g S31183 was sufficient to prevent eclosion of puparia produced up to about 17 days after application. When 2- μ g S31183 was applied, puparia deposited over the next two months never eclosed. One female that had received this treatment deposited a puparium 95 days after treatment which was able to develop normally to the adult but was unable to eclose. Puparia from treated females typically developed to the eye pigmentation stage, but at the higher doses showed severe deficits in thoracic and abdominal bristle development.

Although a total of 60 females was treated with each dose (0.002, 0.02, 0.2, and 2- μ g S31183; 1- μ l acetone), the number of puparia recovered was not commensurate with the number treated. The primary reason for this was the finding that over half the females collected and brought into the laboratory never larviposited due to abnormalities in oogenesis and ovulation. All had full spermathecae, but females were observed often to have two eggs developing at one time or to have one egg half ovulated with the next egg developing. Since these flies were not dissected until after they had been in the laboratory for 1 to 3 weeks it is not known whether this is a characteristic of the flies trapped at that time or due to some factor involved in their maintenance in captivity. Furthermore, although we tried to select the healthiest-looking flies for treatment, in some groups some flies died within two weeks of the treatment. These deaths were unrelated to dose of hormone.

This pilot study extends the findings of Langley on *G. morsitans* (personal communication) to *G. pallidipes*. Langley's study used only 2- μ g S31183 and showed that puparia deposited over 45 days after treatment were unable to eclose. The studies with *G. pallidipes* show that S31183 is a potent and long-lasting JH analogue that could have potential field application to reduce tsetse populations.

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4.11 PROTEIN SYNTHETIC ACTIVITY OF THE MILK GLANDS DURING LARVAL DEVELOPMENT

L. M. Riddiford*

Although total protein synthesis by both the milk glands and the fat body of tsetse flies has been measured during larval development, no one had studied the pattern of synthesis to determine how it might change over this cycle. Therefore, with the help of Dr. Dhadialla, I pulse-labelled milk glands and fat body from individual female *G. pallidipes* (primarily from field trapping) at various stages of the pregnancy cycle, then electrophoresed proteins of both the tissue and the incubation medium, and subjected them to fluorography.

Preliminary experiments giving 35 S-methionine to females for a period of 5 hours showed that *in vivo* the milk gland was not synthetically active until the larva had hatched. Synthesis of several proteins, including prominent 70, 55, 19 and 16 kD polypeptides, then began and continued until the larva was at least a mid-third instar. The third-instar larval gut contained large amounts of newly synthesized protein, primarily composed of the 16 and 19 kD polypeptides and lower molecular weight polypeptides, although some 55 and 70 kD bands were also present. The major protein in the larval gut was a 19 kD protein.

The *in vitro* pulse-chase experiments confirmed that the milk gland synthesized these major proteins and secreted into the medium, primarily the 70 and 16 kD polypeptides. Interestingly, a major 13-kD polypeptide appeared in the medium after incubation of glands from females with first- and second-instar larvae. Glands from those with third-instar larvae made and secreted very little of this latter protein. At

larviposition, synthesis of most proteins ceased except for that of the 16-kD protein.

These studies then showed that the milk glands do not begin synthesizing their secretory products until the first-instar larva hatches. The major secretory products then remain constant, except for a low molecular weight protein that is made only for the first and second-instar larva. The primary protein found in the larval gut is synthesized by the milk gland. If this protein proves antigenic, it will be important to test if such an antibody could be detrimental to larval development.

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4.12 EPIDEMIOLOGY OF ANIMAL TRYPANOSOMIASIS IN LAMBWE VALLEY

L. H. Otieno, P. A. Onyango, E. Mpanga and N. Darji

A survey of tsetse outside the Ruma National Park, in the Lambwe Valley, has clearly shown that *G. pallidipes* is widespread in the settled areas adjoining the national park. Flies have been trapped on the slopes and sometimes on the top of the Ruri Hills—a distance of approximately 6 km from the edge of the national park. It is likely that animal trypanosomiasis diagnosed in cattle in this area is transmitted by flies found outside the park. Whether these flies are resident outside the park or come from the densely infested Ruma thicket is yet to be established. However, fluctuations in fly densities occasioned by the constant cypermethrin ground spraying operations appear to us to be affecting fly densities outside the park. This in turn influences the prevalence of the disease in cattle.

G. pallidipes populations were monitored in three study areas around the Ruma thicket at the eastern end of the national park: inside the thicket, the edge of the thicket and along the Lambwe River Valley.

Six biconical traps were placed in each study area 200 m apart and were used to monitor the fly populations for five consecutive days each month. Some 150–400 randomly selected cattle were examined for animal trypanosomiasis at the same time. These animals grazed mostly on the slopes of the Ruri Hills, and frequented the Lambwe River for watering.

The national park has a long history of insecticide spraying campaigns. The last series of insecticide ground spraying operations started in October/November 1984 and continued on an irregular basis up to August 1987. The effect of spraying is clearly reflected in the number of *G. pallidipes* caught throughout 1987. Low fly densities were recorded from January to May, but thereafter there was a gradual increase in fly density. A sharp increase was observed in October 1987, reaching a peak in December of the same year. The number of flies trapped inside the thicket was consistently higher than those recorded on the edge of the thicket (Figure 4.6). This situation was reversed beginning in October, when a marked increase was observed in the number of flies caught on the edge. A similar increase in the

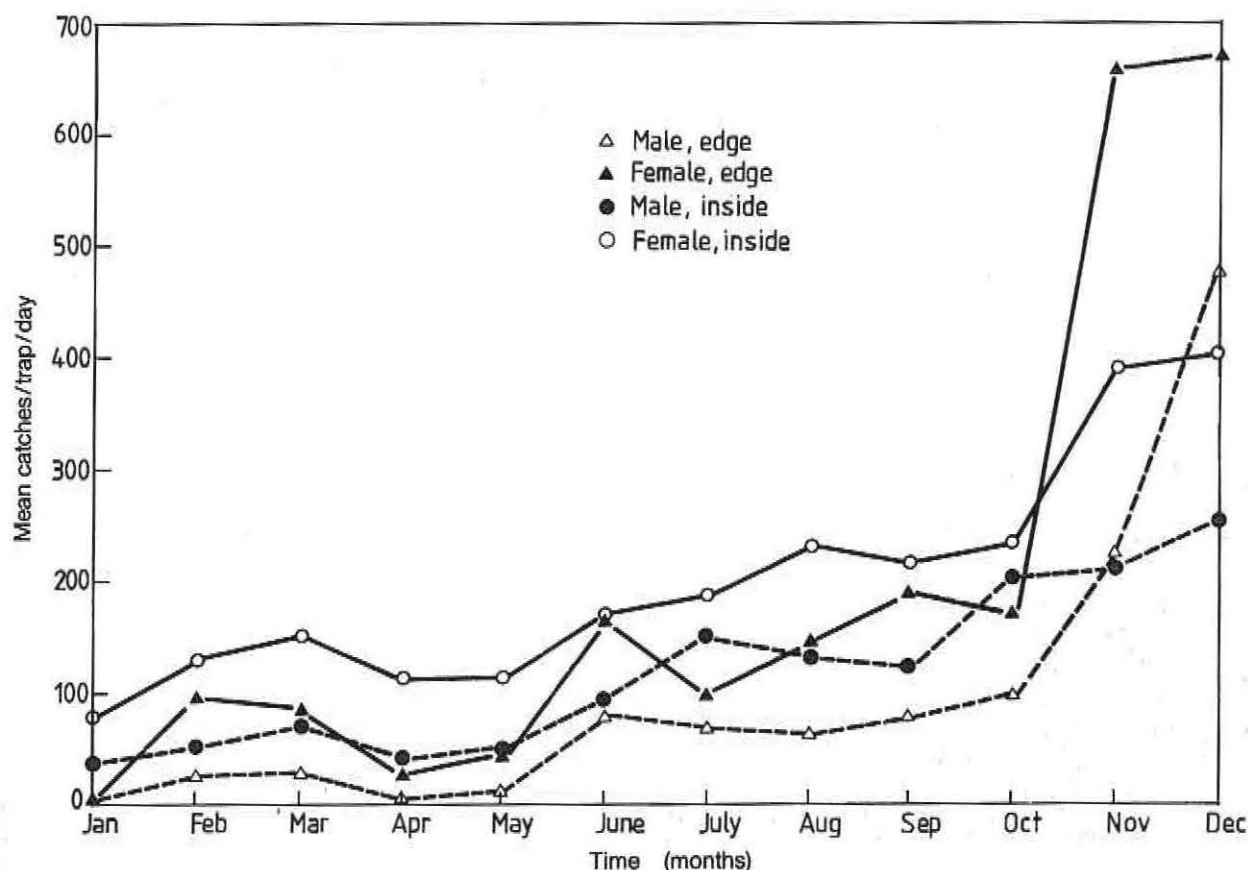


Figure 4.6 Comparison of *G. pallidipes* catches in Lambwe. The flies were sampled inside the Ruma thicket, on the edge of the thicket and along the river valley in the settled area.

number of flies caught outside the park (in the river valley) was observed also in the month of October.

The number of cattle infected with trypanosomes was low (up to 3%) for the first seven months of the year (Figure 4.7). Thereafter the infections increased to 8%, but this was quickly reduced, probably due to the application of insecticide in August. However, by December 1987 the number of infected cattle had increased threefold (21%). The very sharp increase in the number of infected cattle

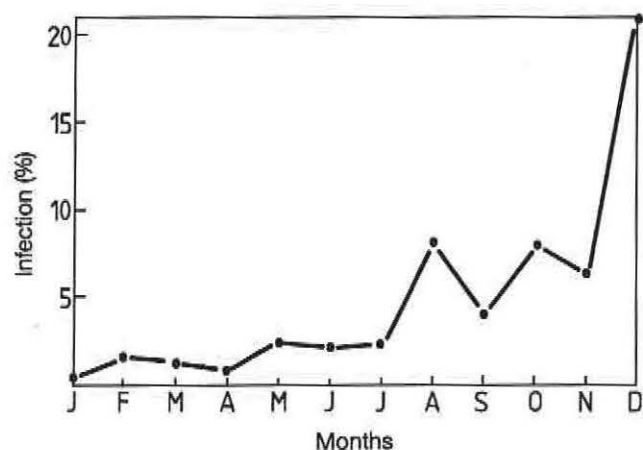


Figure 4.7 Prevalence of cattle trypanosomiasis around God Jope/Ruri Hills area of Lambwe. Notice the sharp increase in the disease incidence which coincides with the increase in fly population at the end of the year.

coincided with the period the highest densities of flies were recorded in the three study areas. It would appear from these observations that when flies reach a certain density level (saturation point) inside the thicket, they start spreading out into 'temporary' habitats where cattle/man/fly contacts become intimate, resulting in an outbreak of trypanosomiasis. If the present situation persists, it will be interesting to see how long it takes before cases of sleeping sickness are reported. For the last one year no cases of human trypanosomiasis have been reported in Lambwe Valley.

4.13 FURTHER ISOENZYME ANALYSIS OF CATTLE STOCKS OF *T. BRUCEI* FROM THE LAMBWE VALLEY

N. Darji and L. H. Otieno

In 1986 we reported the isoenzyme characterization of ten cattle stocks of *T. brucei* from areas bordering the northern part of the Ruma National Park, in the Lambwe Valley, South Nyanza. The cattle came mainly from God Jope and the Ruri Hills. This year we report the results of nine further cattle stocks isolated from the same general areas, including the Kamato area.

Isoenzyme studies have revealed that of 19 cattle stocks, 8 zymodemes were demonstrated (Table 4.1). Three zymodemes—ICZ 13, ICZ 14 and ICZ 20—have been described previously (Gibson and Welde, 1985)* as Z 78, Z

Table 4.1 Isoenzyme patterns for cattle stocks of *T. brucei* in the Lambwe Valley

Area	Cattle stocks (no.)	ALAT*	ASAT†	PGM‡	ICD§	ME	PEP ₁ ★	ICIPE Zymodeme	Zymodeme
God Jope (2) Ruri Hills (6)	C ₂ , C ₃ , C ₄ C ₅ , C ₇ , C ₈ , C ₉ , C ₁₀	2	1	1	3	1	6	ICZ ₁₃	Z78# Man infecting
God Jope (2)	C ₁₁ C ₁	1	7	1	3	1	7	ICZ ₁₄	Z85#
God Jope (1) Ruri Hills (2)	C ₁₂ , C ₁₃ , C ₁₄	1	1	1	3	1	7	ICZ ₁₆	
Otuok (1)	C ₁₅	2	7	1	3	10	7	ICZ ₁₇	
Kamato (1)	C ₁₆	2	7	1	2	23	7	ICZ ₁₈	
Kamato (1)	C ₁₇	2	7	3	2	1	6	ICZ ₁₉	
Kamato (1)	C ₁₈	2	1	1	3	1	2	ICZ ₂₀	Z76# Man infecting
God Jope (1)	C ₁₉	2	1	1	3	23	2	ICZ ₂₁	Man infecting

Patterns for glucose phosphate isomerase (GPI), nucleoside hydrolase (NH), malate dehydrogenase (MDH), threonine dehydrogenase (TDH) and peptidase₂ (PEP₂) were invariant.

* ALAT: alanine aminotransferase.

† ASAT: aspartate aminotransferase.

‡ PGM: phosphoglucomutase.

§ ICD: isolitic dehydrogenase.

|| ME: malic enzyme.

★ PEP₁: peptidase.

Described by Wellde and Gibson, 1985 (see footnote to this paper for complete reference).

85 and Z 76, respectively. Z 78 had previously been isolated from 9 head of cattle and 4 tsetse, Z 85 from 1 bovine and Z 76 from 5 humans. Zymodemes ICZ 20, ICZ 21 and ICZ 13 are closely related to each other, except for their patterns in ME and PEP₁, and as reported above, Z 20 was previously described as Z 76 and isolated from man.

In the Lambwe Valley livestock is the main reservoir of the human pathogenic trypanosome, *T. b. rhodesiense*. The peridomestic tsetse species *G. pallidipes* is possibly responsible for the transmission to man in the area. High tsetse populations in early 1983, which led to spraying the valley with insecticide, may have resulted in a high incidence of trypanosome transmission, but even at low tsetse density, the disease has remained endemic. At present, the fly population in the Ruma thicket is building up due to the cessation of insecticide spraying. *T. brucei* infections in *G. pallidipes* are gradually increasing. This, together with the large reservoir of game animals in the park, creates the risk of a serious outbreak of the disease in man and livestock.

* Gibson, W. C. and Wellde, B. T. (1985). Characterization of trypanozoon stocks from South Nyanza sleeping sickness focus in western Kenya. Trans. Roy. Soc. Trop. Med. & Hyg. 79:671-676.

4.14 SENSITIVITY OF *T. CONGOLENSE* STOCKS TO SAMORIN AND BERENIL

U. Mustafa and L. H. Otieno

T. congolense isolated from Nguruman, in the Rift Valley, and the Lambwe Valley, in western Kenya, were tested for

their sensitivity to berenil (diminazene aceturate) and samorin (isometamedium chloride). Of the six stocks tested, two stocks (category A) belonged to one zymodeme. Both were from Nguruman. Another two stocks (category B) belonged to a different zymodeme. One of these was originally isolated from Lambwe Valley and the other from Nguruman. The last two stocks (category C) were similar neither to each other nor to any of the other stocks; they were included only for comparison.

The dosage levels investigated for samorin were 1 mg, 3 mg and 10 mg per kg body weight and for berenil were 3 mg, 10 mg and 30 mg per kg body weight. All six stocks were sensitive to samorin at the dose level of 1 mg per kg, with the mean days to clearance ranging from 1.5 ± 0.66 to 3.3 ± 0.98 . Berenil at a dose of 3 mg per kg body weight failed to clear the parasitaemia in all six stocks; with a dose of 10 mg per kg body weight there was a temporary clearance, but the parasites reappeared after a few days. When 30 mg per kg was used in stocks of category B, there was temporary clearance and the parasites reappeared. The other four stocks were sensitive to this dose.

Of the many trypanocidal agents used against various forms of trypanosomiasis in livestock, berenil and samorin are known to be the most potent agents against *T. congolense* and *T. vivax* (MacLennan, 1970)*. It was once believed that resistance to berenil was unlikely to develop under field conditions since it was difficult to induce resistance in laboratory animals (Bauer, 1962)*. But recent studies have shown the occurrence of field strains of *T. congolense* in cattle which are resistant to twice the recommended curative dose of 3.5 mg/kg. Our results also show berenil-resistant

strains. Interestingly the resistant stocks belonged to one zymodeme.

*Maclennan, K. J. R. (1970). *Practical application of measures for the control of tsetse-borne trypanosomiasis of livestock in African trypanosomiasis*. In *The African Trypanosomiasis* (Edited by Mulligan, H. W.) pp. 751—765. George Allen and Unwin, London.

4.15 ANTIBACTERIAL SPECTRUM OF INSECT IMMUNE HAEMOLYMPH FACTORS

G. P. Kaaya

After immunization with non-pathogenic bacteria, most insects respond by producing several antibacterial peptides, some possessing broad-spectrum antibacterial activity. Although several peptide antibiotics have practical applications in the treatment of local and even systemic bacterial infection in man and animals, little information exists regarding the exploitation of these powerful insect antibacterial factors for therapeutic purposes.

In a recent investigation we determined *in vitro* sensitivities of several species of bacteria known to be pathogenic to man, animals and poultry to immune haemolymphs from adult tsetse and *Cecropia* pupae. Several of these bacteria were found to be very sensitive to the immune haemolymphs, and the *Cecropia* haemolymph was found to possess a wider antibacterial activity than that of tsetse and was active against 10 out of 17 tested bacteria, while that of tsetse was active against only 3 of them. The bacteria sensitive to *Cecropia* immune haemolymph were *Escherichia coli*, *Enterobacter cloacae*, *Salmonella gallinarum*, *Salmonella typhimurium*, *Pasteurella multocida*, *Pasteurella haemolytica*, *Klebsiella pneumoniae*, *Corynebacterium equi*, *Corynebacterium pseudotuberculosis* and *Actinobacillus lignieresis*. Those sensitive to tsetse immune haemolymph were only *E. coli*, *E. cloacae* and *P. multocida*. In another set of experiments, mice injected with predetermined lethal doses of *E. cloacae*, *K. pneumoniae* and *C. pseudotuberculosis* and then treated with single intraperitoneal or subcutaneous injections of *Cecropia* immune haemolymph had significantly lower mortalities than untreated controls, suggesting that immune haemolymph has therapeutic potentials.

4.16 PATHOGENICITY OF ENTOMOPATHOGENIC BACTERIA FOR ADULT TSETSE, *GLOSSINA MORSITANS MORSITANS*

G. P. Kaaya and N. Darji

Although the current literature contains several reports on the use of entomopathogenic bacteria, such as *Bacillus thuringiensis* and *B. sphaericus*, for the control of larvae of

important dipteran vectors of diseases, such as mosquitoes and blackflies, little information is available on the adulticidal effects of these pathogens. We investigated the pathogenicity of different entomopathogenic bacteria for adult male tsetse using two application techniques: (1) allowing tsetse to engorge once or twice consecutively on rabbit ears smeared with bacteria and (2) allowing tsetse to engorge on defibrinated rat blood containing two different concentrations of bacteria through artificial silicone membranes.

The bacteria used in both experiments were *Pseudomonas aeruginosa* OT 97, *Serratia marcescens* Db II, *Bacillus sphaericus* 1593-4, *Bacillus cereus* Bc 11, *Bacillus thuringiensis* serotype H-14, *Bacillus thuringiensis* serotype 5 and *Bacillus thuringiensis* serotype 1. For each investigation, 3 cages containing 25 male tsetse were used. In the bacterial smear experiment, 4 ml of a 10^7 /ml bacterial suspension in saline was applied on a shaved rabbit ear and smeared evenly, while controls were smeared with 4 ml of sterile saline. Mortalities in the tsetse were recorded for 8 days.

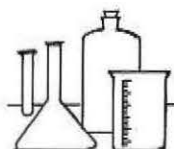
The tsetse mortalities in the smear experiments were not high, although some bacteria caused significantly higher mortalities than observed in the controls. In the single-exposure experiment, the only bacteria that caused significantly high mortalities by 8 days post-exposure were *P. aeruginosa*, *S. marcescens* and *B. thuringiensis* serotype 1. In the double-exposure experiment, significant mortalities were caused by only 4 of the 8 tested bacteria: *P. aeruginosa* (72%), *S. marcescens* (70%), *B. thuringiensis* serotype 5 (14%) and *B. thuringiensis* serotype 1 (33%), compared to a control mortality of 3%.

In the low bacterial concentration membrane feeding experiment (10^3 bacteria/ μ l of blood), *B. thuringiensis* serotype H-14 had caused a mortality of 59% by 8 days post-feeding, while all the other *B. thuringiensis* strains and *B. sphaericus* had caused very low mortalities (8%–24%). However, the other species of bacteria had caused remarkably high mortalities: *P. aeruginosa* (96%), *S. marcescens* (83%) and *B. cereus* (81%). In the high bacterial concentration (10^4 / μ l) membrane feeding experiment, all the bacteria except *B. sphaericus* had induced very high mortalities (92%–98%) by day 8 post-feeding. *B. sphaericus* had induced a mortality of 40%, while mortality in controls was 1%. Mortalities in all these experiments were observed to depend on the type of bacteria, the concentration used and the time post-exposure, up to day 8, when mortalities had stabilized. These observations clearly indicate that the most widely studied entomopathogenic bacteria, *B. thuringiensis*, including the serotypes known to be specific only for lepidopterans, and some other entomopathogens such as *P. aeruginosa* and *S. marcescens*, are pathogenic for adult tsetse provided that a high concentration of bacteria is ingested by the flies. In all of our experiments, however, *B. sphaericus* exhibited low pathogenicity for adult tsetse compared to the other species of bacteria.

Chemistry and Biochemistry Research Unit

- 5.1 A synopsis of CBRU's major accomplishments **79**
- 5.2 Analysis of the sex pheromone blend of the spotted stalk borer, *C. partellus* **80**
- 5.3 *Chilo* feeding allelochemicals **81**
- 5.4 Volatiles of cowpea **82**
- 5.5 Formation of phenolic tsetse attractants in buffalo urine: the microbes and precursors involved **84**
- 5.6 Induction of host resistance to the brown ear tick, *Rhipicephalus appendiculatus* **87**
 - A. Midgut buffer soluble antigens
 - B. Midgut membrane-bound solubilized proteins
 - C. Whole tick solubilized membrane proteins
- 5.7 Effect of pH on enzyme 2, a carboxyl proteinase from the gut of *R. appendiculatus* **89**
- 5.8 The volatile resin exudate from the stem bark of *C. rostrata*: role in plant defence **90**
- 5.9 Natural and synthetic naphthoquinones as mosquito larvicides **91**
- 5.10 A new limonoid antifeedant from *Turrea robusta* **92**

5



Chemistry and Biochemistry Research Unit

The research activities of the Chemistry and Biochemistry Research Unit (CBRU) fall into the following three major areas.

Chemical ecology of ICIPE's target pests and disease vectors. *The objective of this study is to contribute to efforts of the core programmes to develop appropriate eco-technologies for the management of the tsetse fly and the stem borer *Chilo partellus*.*

Protein biochemistry. *Until recently this work was confined to collaborating with the Livestock Ticks Research Programme on identifying tick antigens potentially useful for providing livestock with immune protection against ticks. With the return of Dr. Ellie Osir, a protein biochemist, we are now diversifying our activities to include a study of the proteins associated with diapause in *Busseola fusca*.*

Screening anti-arthropod natural products from African plants. *The aim of this work is to identify new models for anti-insect activities and to explore the possibility of exploiting the plants by developing simple processing methods suitable for cottage-type or small-scale industries. Current efforts are focussed on limonoids of *Meliaceae*, *Tephrosia spp.*, and plants that may be suitable for use in small-scale mosquito control projects or as protectants of stored grain.*

Two important items of equipment were acquired during the year—the Pharmacia Phast System for high performance electrophoretic work and an Ito centrifugal countercurrent chromatograph, a tool of growing usefulness in the separation of semi-polar and polar compounds. In addition, special funds that were available for a collaborative project between ICIPE and the University of Lund, Sweden (sponsored by the Swedish Agency for Research Cooperation with Developing Countries), enabled us to commission a linked gas chromatograph–electrophysiology recording system in the Sensory Physiology Research Unit. *We expect this system greatly to assist us in identifying active, volatile eco-chemicals.*

5.1 A SYNOPSIS OF CBRU'S MAJOR ACCOMPLISHMENTS

A. Hassanali

5.1.1 Pheromone and allelochemical studies relating to crop pests

In addition to (*Z*)-11-hexadecanal and (*Z*)-hexadecen-1-ol, identified as pheromone components of the Indian biotype of *Chilo partellus*, other minor components in the pheromone glands of the African biotype of the insect have now been positively identified. These include hexadecanal, hexadecan-1-ol, (*Z*)-7-hexadecenal and (*Z*)-9-hexadecenal. The roles of these components in the pheromone blend remain to be elucidated. An analysis of the gland contents during the first

scotophase after adult emergence has confirmed that pheromone accumulation in the gland begins from the 5th–6th hour, reaches a maximum at about the 9th hour and falls with the onset of calling behaviour (10th–11th hours).

4-hydroxybenzaldehyde and 4-hydroxybenzoic acid, the two major components of ethyl-acetate extracts of sorghum whorls, were both shown to stimulate feeding of third-instar *C. partellus* larvae, the former being much more active than the latter. Dose response studies of analogues of these compounds have shown that the phenolic aldehyde may be the most potent feeding stimulant of the various theoretical biogenetic variants. High-performance liquid chromatography (HPLC) analysis—using a refractive index detector of an active fraction of methanolic extract of the whorls of the susceptible sorghum cultivar IS 18363—has led to the

identification of sucrose, glucose, fructose and xylose. Feeding tests on these sugars both individually and combined with the phenols are currently in progress. Maximum feeding appears to be associated with a blend of the sugars and the phenols.

Last year we reported identifying volatile compounds emitted by *Sorghum bicolor* seedlings. The role of these compounds in influencing orientation, oviposition and host recognition by sorghum pests is being investigated by CBRU in collaboration with the Crop Pests Research Programme. Applying the same purging-trapping technique to cowpea (*Vigna unguiculata*) seedlings has led to our identifying a series of volatile compounds, which include pentyl formate, toluene, 2,4-dimethyl-hexan-3-ol, hexanal, *m*-xylene, *o*-xylene, 1-nonene, β -pinene, myrcene, (*Z*)-3-hexen-1-ol acetate, hexyl acetate, α -pinene, limonene, ocimene, nonanal and α -cendrene.

5.1.2 Tsetse kairomone studies

Several projects on tsetse kairomones begun in 1986 continued during 1987. A report on our (now complete) collaborative studies with the Sensory Physiology Research Unit (SPRU) on the analogues of 1-octen-3-ol using antennal movement bioassay appears in the SPRU Chapter of this Report.

Last year we reported that the two most important components of the active phenolic fraction (buffinol) of African buffalo (*Syncerus caffer*) urine were 4-cresol and 3-*n*-propylphenol. A series of analogues of these compounds have now been prepared and await both laboratory and field tests. We hope that the structure-activity relationships that emerge from these tests will help to identify a more potent attractant blend for the tsetse fly.

Progress has been made towards identifying the microbe(s) responsible for the breakdown of buffinol precursors to the free phenols. In addition, HPLC separation of the precursors followed by nuclear magnetic resonance (NMR) studies suggest that the precursors may be predominantly glucuronates, although other conjugates such as sulphates may also be present. We believe the microbe-precursor system will provide a model for the controlled release of the attractants over extended periods.

5.1.3 Tick antigen studies

Three possible sources of antigens have been investigated during the last two years for the purpose of raising antisera with potential for disrupting the midgut wall and/or the digestive system of the tick *Rhipicephalus appendiculatus*. These three sources are buffer-soluble midgut proteins, Triton X-100 solubilized midgut proteins and digestive enzymes. Results obtained with enriched or purified protein samples from these sources, however, indicate that only antisera raised from Triton X-100 solubilized midgut proteins significantly affect feeding ticks. A fraction, with a subunit molecular weight range of 60 Kd-180 Kd, obtained by successive fractionations on Sephacryl S-200 and DEAE-Sephacel was used to immunize rabbits. This caused tick feeding to be reduced by about 30% compared to ticks fed on control rabbits. Purification of the active proteins involved in these fractions is now under way.

On the other hand, in work undertaken during 1987, solubilized membrane proteins obtained from whole ticks have shown greater promise as a source of useful antigens. Ticks fed on rabbits immunized with these proteins have exhibited higher mortality, reduced engorgement weights and lower egg conversion rates than corresponding ticks fed on control rabbits. These proteins will, henceforth, be the major focus of our purification and immunological studies.

5.1.4 Anti-arthropod natural products from African plants

The volatile portion of the resin exudate from the stem bark of *Commiphora rostrata* was examined by gas chromatography and mass spectrometry and 22 components were identified. Preliminary bioassays indicate that the oil is repellent to the maize weevil and that its major components also possess antifungal properties.

We have shown that the mosquito larvicidal activity of extracts of the roots of *Plumbago zeylanica* was due mainly to the naphthoquinone, plumbagin. In 1987 bioassays using a series of naphthoquinones have shown that the activity is largely associated with the 1,4-naphthoquinone nucleus. Since naphthoquinones occur widely in tropical plants, their crude preparations may prove useful in small-scale mosquito control programmes.

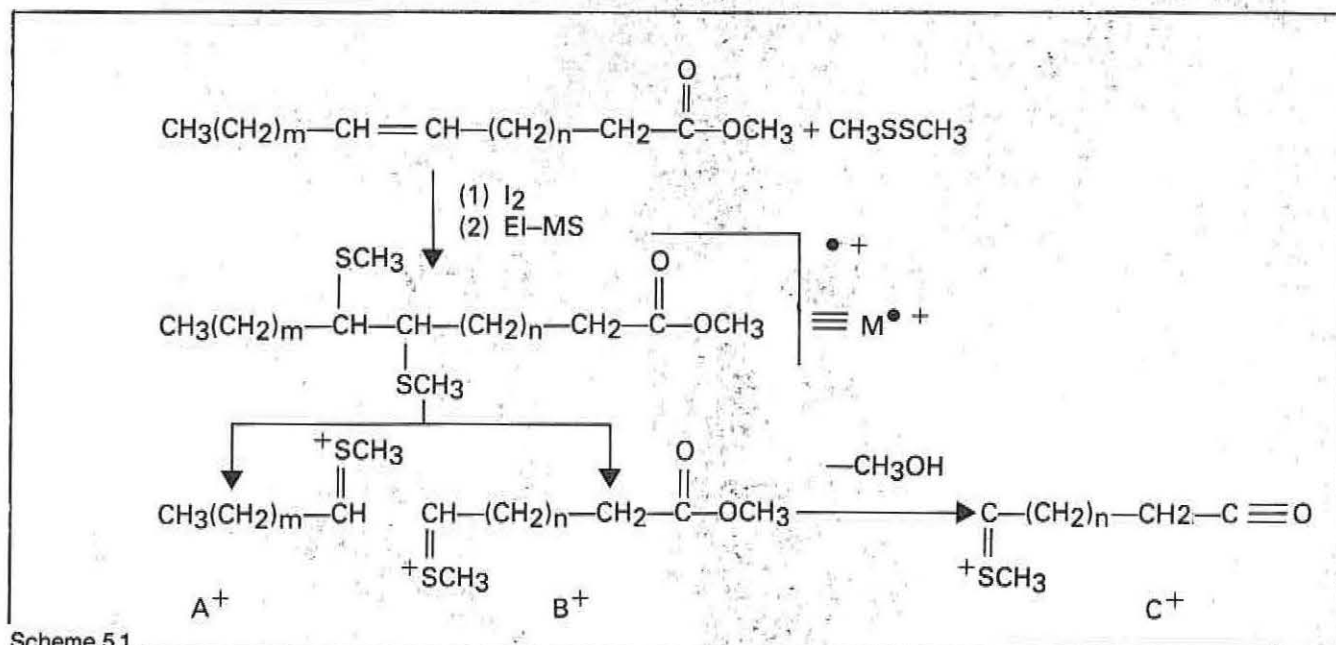
As part of our work on African species of Meliaceae, a new limonoid, mzikonone, was isolated from the root bark of *Turrea robusta* and its structure determined by 1-D and 2-D nuclear magnetic resonance (NMR) techniques. It is a useful addition to our growing number of limonoid variants for our structure-activity studies. This phase of the studies is expected to be completed next year and a full report will be included in the 1988 Annual Report.

5.2 ANALYSIS OF THE SEX PHEROMONE BLEND OF THE SPOTTED STALK BORER, *CHILO PARTELLUS*

P. G. McDowell

Last year gas chromatographic (GC) and mass spectrometric (MS) analysis of the hexane extracts of the pheromone glands of female *Chilo partellus* confirmed the presence of the known pheromone gland components, (*Z*)-11-hexadecenal (*Z*11-16:Ald) and (*Z*)-11-hexadecen-1-ol (*Z*11-16:OH). In addition, two further components were positively identified: the saturated analogues hexadecanal (16:Ald) and hexadecan-1-ol (16:OH). Two further unsaturated components were tentatively identified as (*Z*)-7- and (*Z*)-9-hexadecenal (*Z*7-16:Ald and *Z*9-16:Ald), and traces of the corresponding alcohols were also noted. The fatty acyl moieties found in the female gland were examined by extraction with chloroform:methanol (2:1) followed by evaporating the solvent and trans-methylating to the fatty acid methyl esters (FAMES) using methanolic sodium hydroxide. The FAMES were subsequently analysed by GC and GC-MS. These techniques confirmed the presence of delta-7, delta-9, and delta-11 C₁₆ FAMES.

Double-bond positions have now been confirmed by derivatizing the FAMES using dimethyl disulphide to produce bis-thiomethyl derivatives, which give highly



Scheme 5.1

characteristic mass spectral fragmentation patterns indicative of the original double-bond position (and its geometry), as shown in Scheme 5.1. GC-MS analysis of the bis-thiomethyl ethers was carried out on a 50-m CP Sil 5CB column at 250°C. Components were observed confirming the presence of Z-7-, Z-9- and Z-11-C₁₆ FAMES.

Analyses of pheromone production during the first scotophase after adult emergence have also been performed, with an extension of the analyses to the 11th hour after the start of scotophase. These analyses confirm that pheromone accumulation in the gland begins from the 5th or 6th hour onwards. Maximum production occurred at about the 9th hour, and thereafter the gland content actually falls with the onset of calling behaviour, which has been observed in the laboratory to occur in the 10th and 11th hours (Figure 5.1).

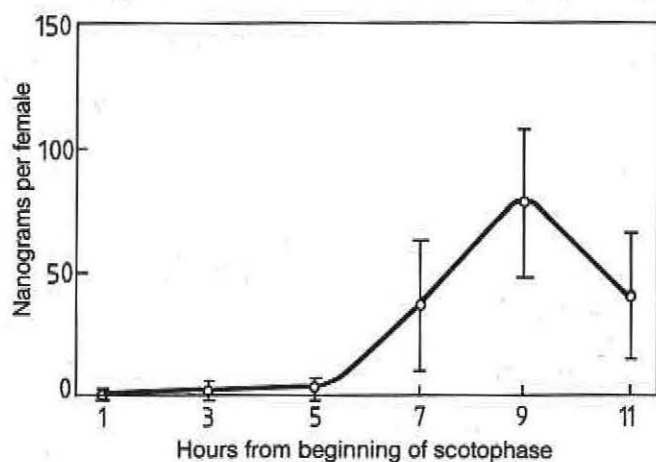


Figure 5.1 Production of (Z)-11-hexadecenal (ng/female) in *Chilo partellus* during the first scotophase following adult emergence (bars are ± 1 S.E.M.).

Biosynthetic pathways in the female gland are currently under investigation by incubating pheromone glands with deuterium-labelled fatty acid precursors. (16,16,16)-Trideuterohexadecanoic acid (2 μg) was applied to single glands held everted by micro-alligator clips, shaped to apply

sufficient pressure on the abdomen. Following this application, the insects were placed in the dark for 6–8 hours. The glands were then dissected, extracted and analysed for FAMES by GC. Chromatograms obtained in this way show the presence of small peaks due to omega-deuterated methyl hexadecanoate (D₃16:Me) and omega-deuterated Z-11-hexadecenoate (D₃Z11-16:Me). Incorporation of deuterium into other C₁₆ components is unclear since the chromatogram peaks are not well resolved. Confirmation should be forthcoming from GC-MS analyses of both the FAMES and their thiomethyl ethers.

This year we commissioned a linked gas chromatograph-electrophysiology recording system in the Sensory Physiology Research Unit. We expect this system to be of great assistance in our pheromone work and other odour-related research. Details of the system are given in the Sensory Physiology Research Unit Chapter of this Report.

5.3 CHILO FEEDING ALLELOCHEMICS

*B. Torto**

Investigations continued on the isolation and identification of *Chilo partellus* third-instar larval-feeding stimulants from extracts of sorghum whorls. Two studies were conducted: structure-activity studies on selected analogues of *p*-hydroxybenzaldehyde, the major active component of the ethyl acetate extract of sorghum, and a detailed examination of the methanol extract of the whorls of three-week-old plants of the susceptible cultivar IS 18363.

The analogues tested included *p*-hydroxybenzoic acid, *p*-hydroxybenzyl alcohol, *p*-methoxybenzaldehyde, *p*-methoxybenzoic acid and *p*-methoxybenzyl alcohol. The results show that of the hydroxy analogues tested, *p*-hydroxybenzaldehyde was most stimulatory to the third-instar larvae (Figure 5.2). For the methoxy derivatives, *p*-methoxybenzaldehyde was the least stimulatory (Figure 5.2). These results suggest that the chemoreceptors of the third-

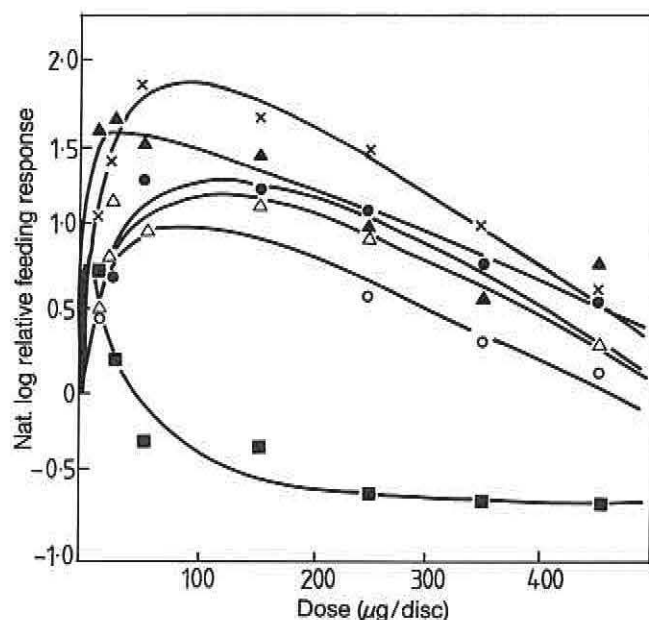


Figure 5.2 Dose response curves for the following phenolic compounds: (x) *p*-hydroxybenzaldehyde, (▲) *p*-methoxybenzyl alcohol, (●) *p*-hydroxybenzoic acid, (△) *p*-methoxybenzoic acid, (○) *p*-hydroxybenzyl alcohol and (■) *p*-methoxybenzaldehyde.

instar larvae of *C. partellus* are more tuned to *p*-hydroxybenzaldehyde than any of the tested analogues.

The methanol extract was chromatographed into four fractions (M1–M4). Fraction M4, the most polar of the four, dissolved readily in water. The fractions were tested singly and in combinations in the proportions obtained from the fractionation of the crude extract. The feeding tests on the individual fractions indicate that M4 was most stimulatory to the larvae, followed by M2, M3 and M1, in that order. The feeding response of the larvae to the mixture M1 + M2 + M3 was not significantly different from that to the control, but the addition of M4 to this mixture significantly increased the feeding response of the larvae. This mixture, however, was not as active as the crude methanol extract. With the exception of the mixture of M4 and M1, all mixtures containing M4 were more active than M4 tested singly. These results indicate that components in M2 and M3 synergize with those in M4 to enhance the feeding response.

All the fractions have been analysed by high-performance liquid chromatography (HPLC). The analyses show that M4 contained a mixture of sugars, the identities of which were determined by comparing the separation profile of the mixture to that of a standard sugar mixture. Two different columns were used: bonded phase-NH₂ (Figure 5.3a) and aminex carbohydrate HPX-87C (Figure 5.3b). The sugars identified include sucrose, glucose, fructose and xylose. Fractions M3, M2 and M1 were analysed on a reverse-phase column. From fraction M3 we identified *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid and a third component of unknown structure. Maximum activity thus appears to be associated with both the sugar and phenolic constituents of sorghum whorls. A detailed dose-response study of various combinations of these compounds,

now in progress, will help throw more light on their relative importance in stimulating feeding of *C. partellus* larvae.

*Postgraduate scholar in ICIPE's African Regional Postgraduate Programme in Insect Science.

5.4 VOLATILES OF COWPEA

W. Lwande, P. G. McDowell, P. Amoke and H. Amiani

Cowpeas (*Vigna unguiculata*) are widely grown in tropical and subtropical regions for human and animal food. In many developing tropical countries they provide a cheap source of dietary protein and energy. Cowpeas, however, are attacked by a wide range of insect pests that can cause severe crop losses.

Volatile compounds originating from the cowpea plant may play a role in orienting insect pests towards the plant and helping them recognize the plant for the purpose of feeding and oviposition. Knowledge of the volatile compounds of cowpea may therefore be useful in studies of the relationship between insect pests and cowpea plants, but we have found no report on the volatiles of cowpea. In the 1986 Annual Report we described the trapping and identification of volatiles of *Sorghum bicolor*. We report below on the identification of volatiles of cowpea.

Charcoal-filtered air was drawn over 7 four-week-old cowpea plants (VITA 1 variety) in a glass chamber and then passed through a glass tube packed with Tenax adsorbent (Tenax trap). The cowpea volatiles were trapped on the Tenax adsorbent and then released directly into a capillary gas chromatograph–mass spectrometer (GC-MS) by heating the Tenax trap. The volatiles were identified on the basis of their mass spectra and by comparing their GC retention times to those of authentic samples.

Figure 5.4 shows the GC-MS total ion current chromatogram of the trapped cowpea volatiles, Table 5.1 lists the identified cowpea volatile compounds and Figure 5.5 shows the chemical structures of the compounds in Table 5.1.

Table 5.1 Identified air-borne volatiles trapped from four-week-old cowpea (*Vigna unguiculata*) VITA 1 variety plants

Compound number	Compound
1	Pentyl formate
2	Toluene
3	2,4-Dimethylhexan-3-ol
4	Hexanal
5	<i>m</i> -Xylene
6	<i>o</i> -Xylene
7	1-Nonene
8	β-Pinene
9	Myrcene
10	(<i>Z</i>)-3-Hexen-ol acetate
11	Hexyl acetate
12	α-Pinene
13	Limonene
14	Ocimene
15	Nonanal
16	α-Cendrene

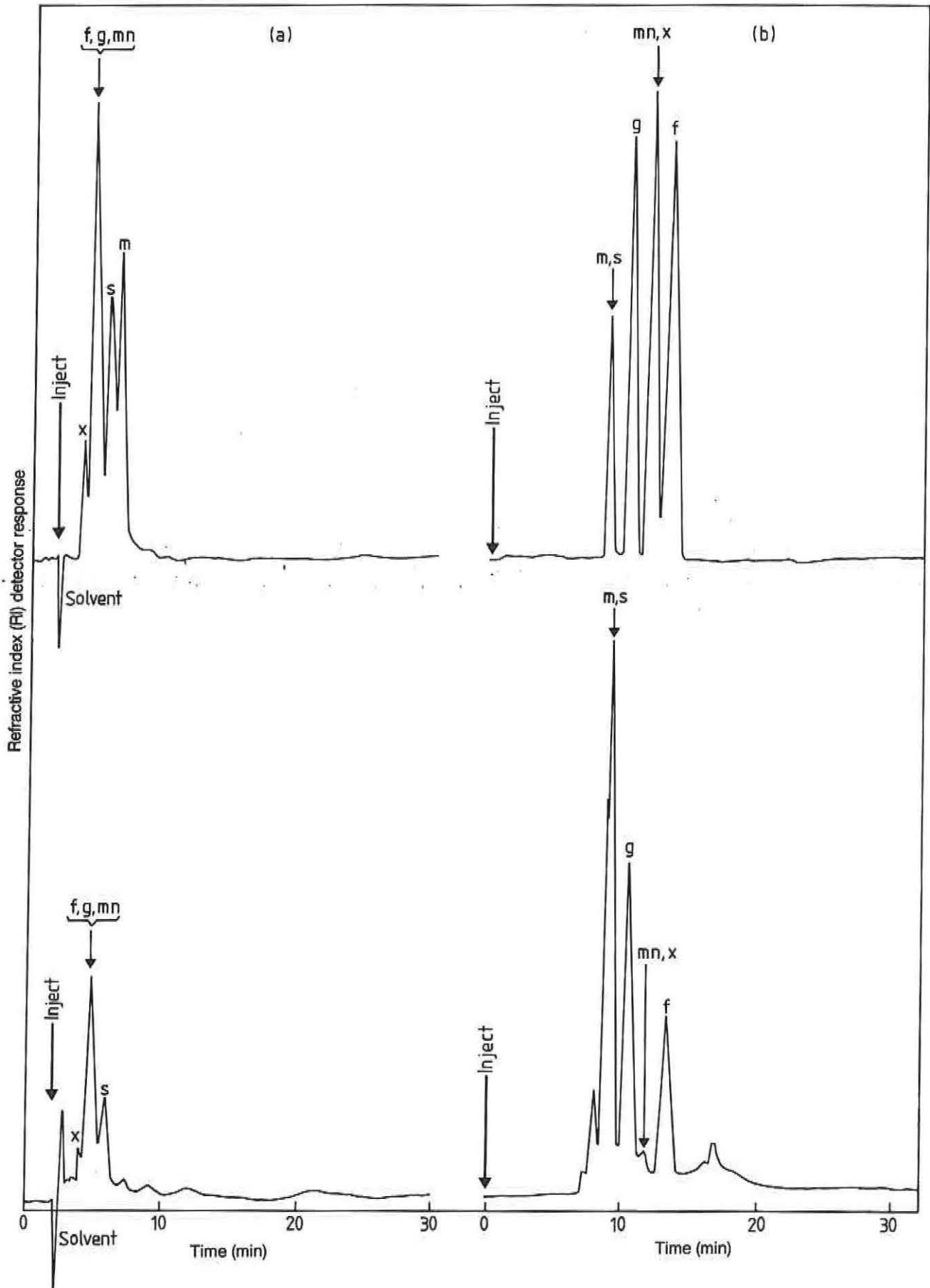


Figure 5.3 HPLC separation of the most stimulatory fraction of the methanol extract. The upper chromatogram represents a standard sugar mixture and the lower chromatogram, the active fraction (M4). (a) bonded phase-NH₂ and (b) aminex carbohydrate HPX-87C.

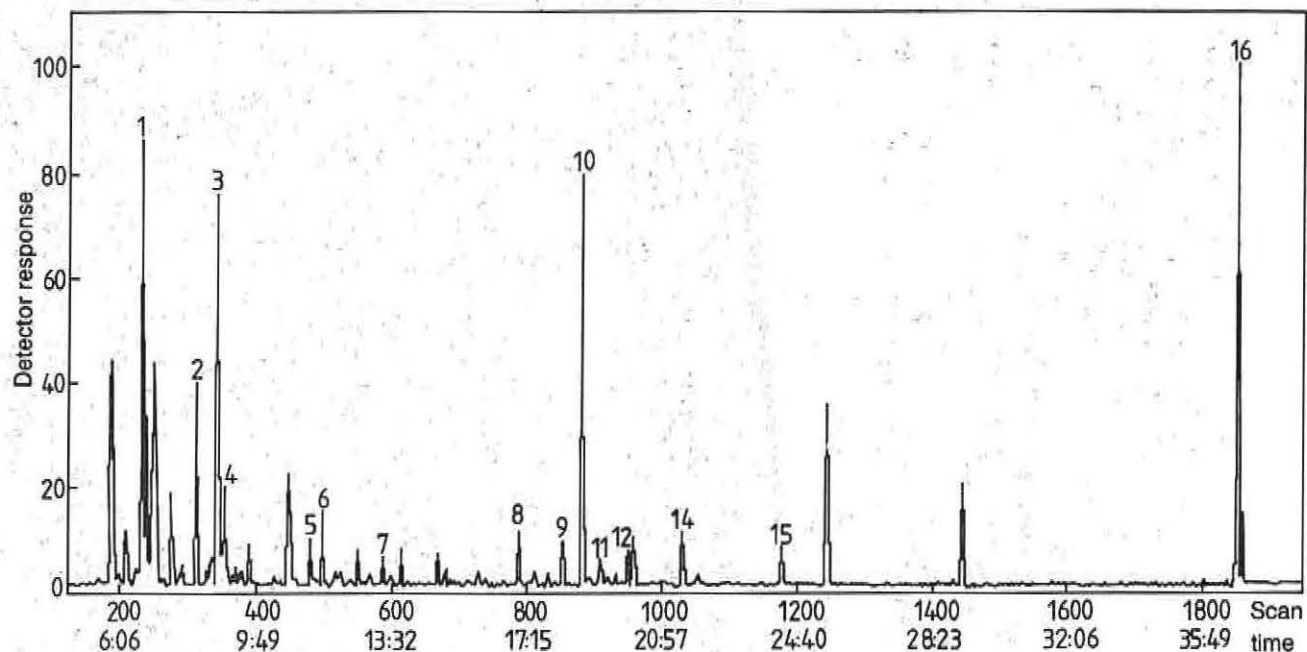


Figure 5.4 Gas chromatographic-mass spectrometric chromatogram of 16 volatiles trapped from four-week-old cowpea (VITA 1 variety) plants (listed in Table 5.1).

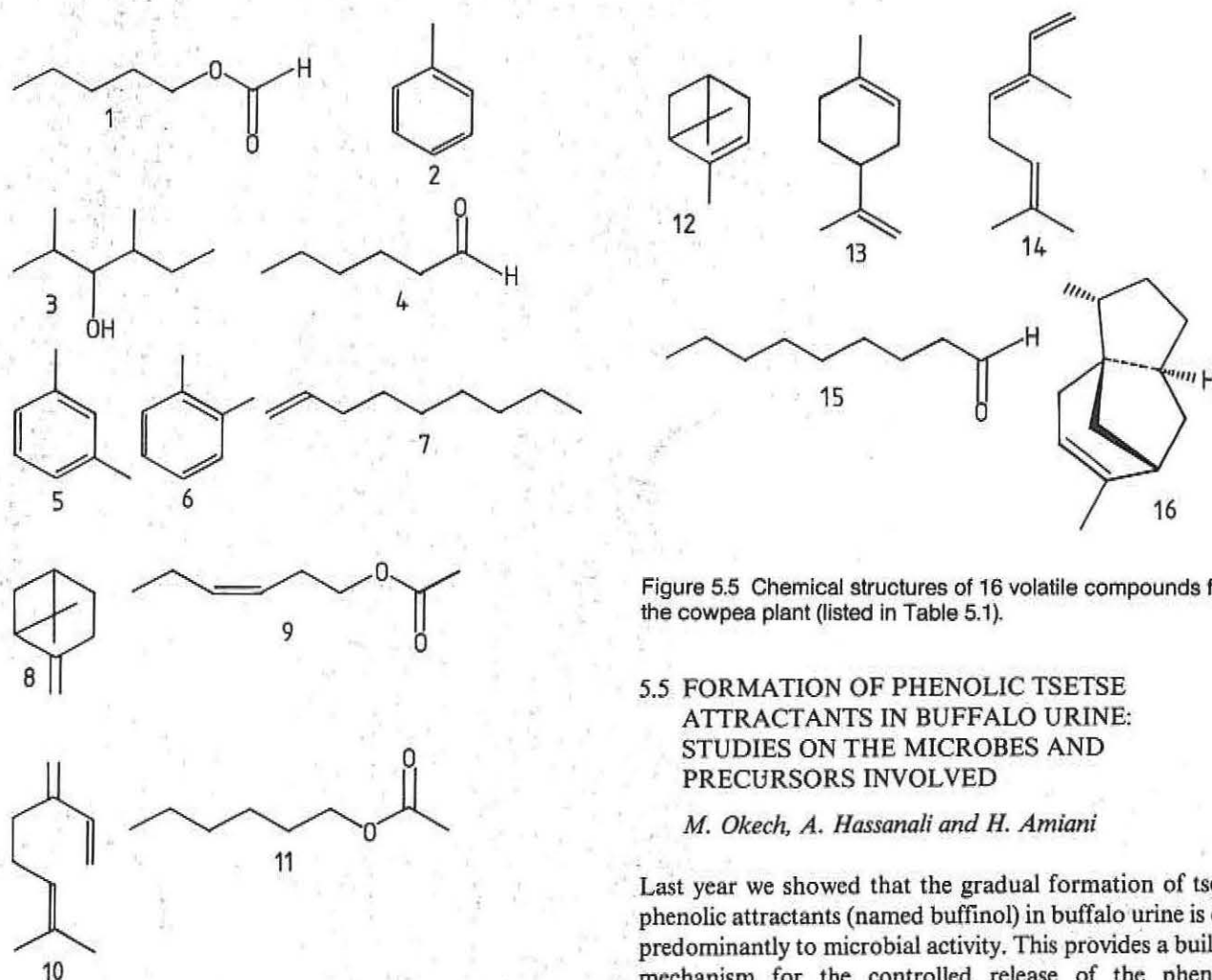


Figure 5.5 Chemical structures of 16 volatile compounds from the cowpea plant (listed in Table 5.1).

5.5 FORMATION OF PHENOLIC TSETSE ATTRACTANTS IN BUFFALO URINE: STUDIES ON THE MICROBES AND PRECURSORS INVOLVED

M. Okech, A. Hassanali and H. Amiani

Last year we showed that the gradual formation of tsetse phenolic attractants (named buffinol) in buffalo urine is due predominantly to microbial activity. This provides a built-in mechanism for the controlled release of the phenols. Although the presence of phenolic glucuronates in mammalian urines has been reported, no studies appear to

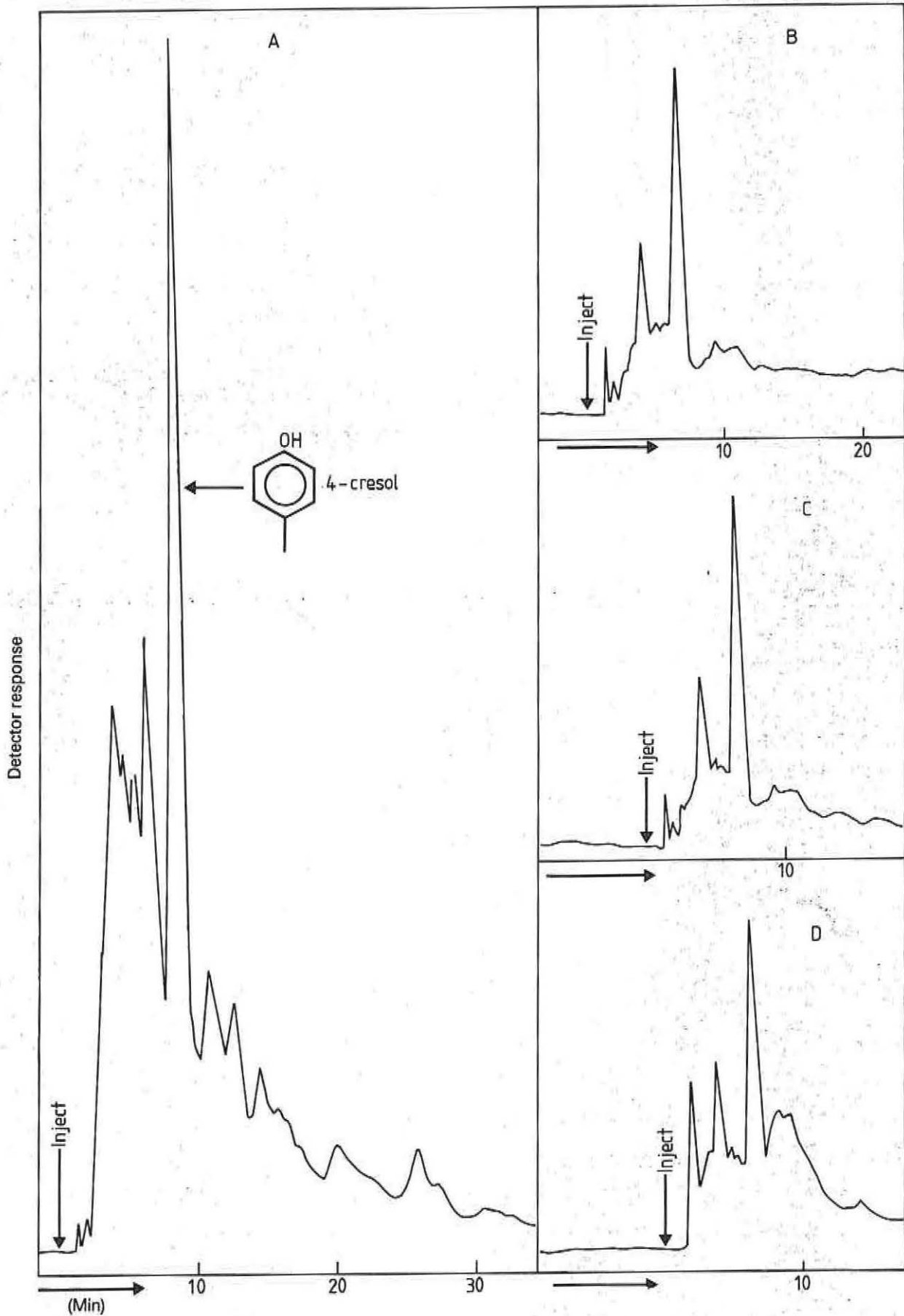


Figure 5.6 HPLC chromatograms of CH_2Cl_2 extracts of buffalo urine samples treated with different microbial isolates (A, B, C or D), showing relatively large amounts of 4-cresol and other components of buffinol in the sample inoculated with microbe A.

have been carried out on the composition of phenolic conjugates of ruminant urine. We have undertaken an in-depth study of these precursors and the microbe(s) responsible for their breakdown in the hope of using the system as a model for dispensing the attractants in a controlled fashion for sustained periods.

5.5.1 Isolating and screening microbes

Portions of buffalo urine, allowed to age under ambient conditions in the laboratory, were inoculated onto buffalo-urine agar and tryptone-soya agar plates. Colonies resulting from the growth of microorganisms were plated out onto new plates. Finally, 'pure' cultures, selected from single clones, were isolated and placed into separate tubes. Four organisms (A, B, C and D) have been isolated from the urine aged in the laboratory.

These organisms have been screened in a preliminary test for their abilities to release free phenols from the corresponding precursors. Each organism was precultured in small portions of filter-sterilized urine, which was then used to inoculate larger aliquots (100 ml) of freshly collected and sterilized (0.22 μm millipore filtration) urine. After ten days of incubation under ambient conditions, samples (50 ml) of each test urine were extracted with dichloromethane and examined by HPLC on a reverse-phase column. Results obtained (Figure 5.6) suggest that organism A is most effective in releasing phenols. These experiments are being repeated with further samples of urine for confirmation.

5.5.2 Isolating and characterizing precursors

The procedure outlined in Scheme 5.2 has been followed to isolate the precursors in crude form. Four fractions were isolated from Sephadex G-10 chromatography, as shown in Figure 5.7. Fraction 3 has shown a UV spectrum typical of

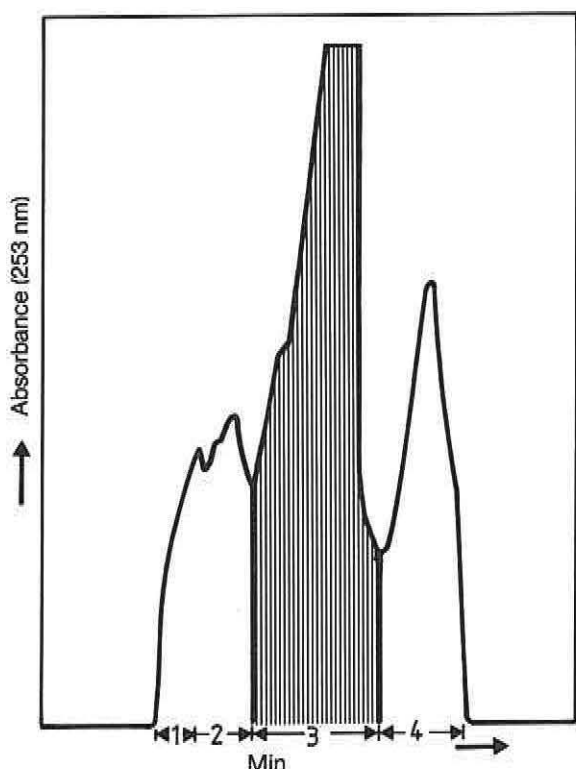


Figure 5.7 Sephadex G-10 chromatogram of freeze-dried buffalo urine.

phenolic ethers. This fraction has been further fractionated by HPLC on a semi-preparative reverse-phase column to yield two semi-pure compounds, IIa and IIIa (Figure 5.8). Preliminary spectral nuclear magnetic resonance (NMR) examination of IIa suggests that it may comprise a mixture of 4-cresol conjugates, with glucuronate as the major component. Further cycles of chromatography are currently under way in the hope of isolating pure conjugates for definitive spectral identification.

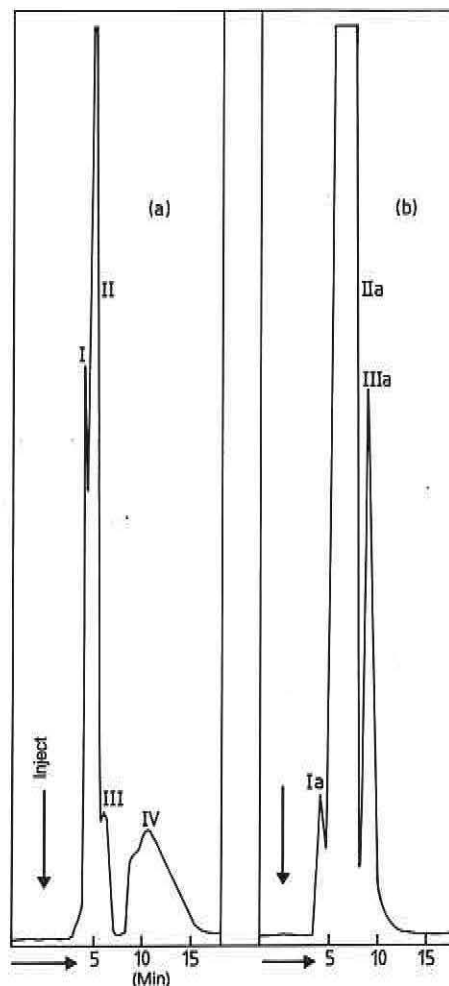
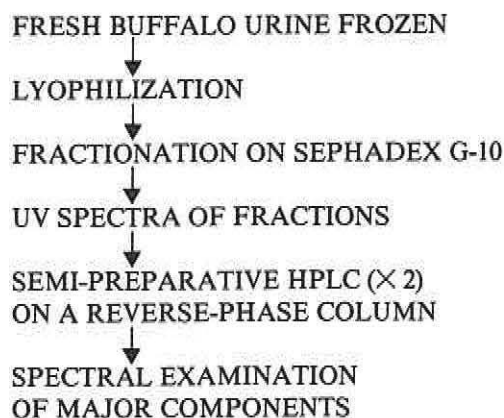


Figure 5.8 Chromatograms showing two cycles of HPLC separation of phenol precursors in buffalo urine on a semi-preparative reversed-phase column leading to the isolation of two semi-pure fractions: IIa and IIIa.



Scheme 5.2

5.6 INDUCTION OF HOST RESISTANCE TO THE BROWN EAR TICK, *RHIPICEPHALUS APPENDICULATUS*

A. MIDGUT BUFFER SOLUBLE ANTIGENS

T. S. Dhadialla and S. Essuman

In the 1986 Annual Report we described the results of a tick challenge on rabbits immunized with buffer or detergent (Triton X-100) soluble midgut antigens from the tick *Rhipicephalus appendiculatus*. Since the engorgement weights of adult female ticks fed on rabbits immunized with soluble or solubilized midgut antigens were reduced by 25% and 40%, respectively, we have used the sera from such animals to identify antigens in buffer soluble protein extracts of whole ticks. Two antigens with native molecular masses of 230 000 and 140 000 were identified with immunoblotting techniques. Protein fractionation experiments to isolate these antigens have been conducted.

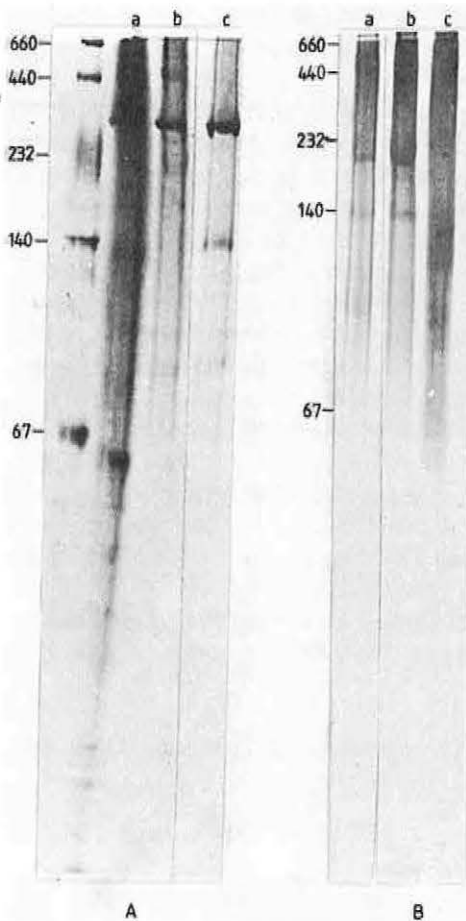


Figure 5.9 NATIVE-PAGE (7%–15% gradient) of *R. appendiculatus* protein extracts during partial purification of the 140-Kd and 230-Kd antigens. (A) Coomassie brilliant-blue stained gel: (a) high molecular weight markers, (b) 50% ammonium sulphate precipitated fraction of protein extract of whole-tick homogenate, (c) ex-Sephacryl S-200 fraction enriched for the two antigens and (d) ex-DEAS-Sephacel fraction further enriched for the two antigens. (B) Immunoblot of the same gel probed with sera from a rabbit made resistant to tick feeding by immunization with buffer soluble midgut protein extracts from partially engorged virgin females.

The two antigens were enriched by protein fractionation techniques from buffer soluble extracts of virgin female ticks that had fed for five days on rabbits. In immunoblotting experiments these antigens reacted with sera from rabbits that had been immunized with buffer soluble midgut proteins and that showed resistance to feeding by ticks (Figure 5.9).

Fractions enriched for these two antigens have been used to immunize rabbits. Four rabbits were immunized with fractions enriched for the 230-Kd and 140-Kd antigens, while four other rabbits were subjected to the same immunization schedule with a buffer-adjuvant mixture but without the proteins. Each of the rabbits was then challenged with 20 female and 30 male ticks. The mean engorgement weights of these females did not differ between ticks fed on immunized rabbits and those fed on control rabbits.

B. MIDGUT MEMBRANE-BOUND SOLUBILIZED PROTEINS

S. Essuman and T. S. Dhadialla

Immunization of rabbits with Triton-X solubilized gut membrane proteins has been shown to lead to reduced

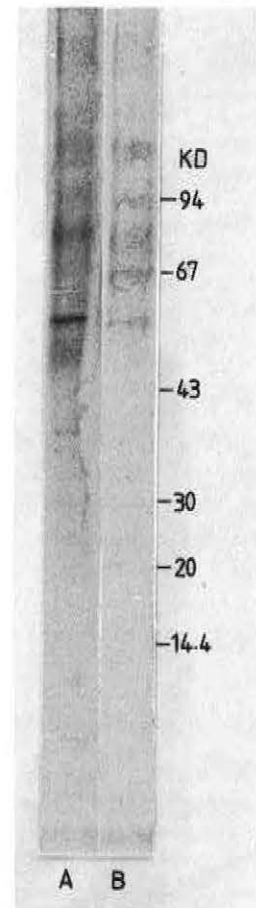


Figure 5.10 Immunoblot of an SDS-polyacrylamide gel on which protein fractions obtained from solubilized tick membrane extracts after gel permeation on Sephacryl S-200 (A) followed by anion-exchange chromatography (B) were electrophoresed. The blot was probed with serum from rabbits immunized with tick midgut solubilized membrane proteins. Protein fractions enriched for protein subunits ranging in molecular weights of 60 000–180 000 (B) were used to immunize rabbits.

feeding by ticks. We report below on our progress in isolating the antigens responsible for this effect.

The source of the membrane-bound proteins was the pellet obtained after centrifugation of partially engorged female tick homogenate. The membrane-bound proteins were extracted from the pellet using 0.5% Triton X-100 (for details of the extraction, see the 1986 Annual Report). The solubilized material was fractionated on a Sephacryl S-200 (Pharmacia) column. The fraction that was mainly enriched for proteins with a subunit molecular weight range 60 Kd-180 Kd (Figure 5.10, track A) was concentrated and chromatographed further on an anion-exchanger (DEAE-Sephacel [Pharmacia]). Serum raised specifically against midgut solubilized proteins was used to identify the protein subunits by the immunoblotting technique. The fraction obtained after anion-exchange chromatography (Figure 5.10, track B) was used to immunize the rabbits.

Seven rabbits were used for the experiment. Group one, consisting of 4 rabbits, served as the control. Group two (3 rabbits) was immunized with the tick protein sample obtained above. One immunized animal died in the course of the experiment. The immunization protocol was that described by Dhadialla and Latif in the 1985 Annual Report. Three weeks after the second booster, the rabbits were challenged with 20 female and 30 male adult ticks on one ear. The mean fed weights of the female ticks are summarized in Table 5.2. Evaluations of egg batch weight and egg hatchability are still in progress.

Table 5.2 Mean weights of female *R. appendiculatus* after engorgement on immunized and control rabbits

Group	Mean engorgement wt (mg) (\pm S.E.M.*)	% reduction in engorgement wt
Control	329 \pm 17.2	—
Immunized	229 \pm 23.2	30

* S.E.M.: standard error of the mean.

It is evident from the engorgement weights obtained in this experiment that the feeding performance of ticks fed on rabbits immunized with the membrane-bound proteins, with subunit molecular weights ranging from 60 Kd-180 Kd, was reduced by 30% compared to ticks fed on control rabbits.

C. WHOLE TICK SOLUBILIZED MEMBRANE PROTEINS

T. S. Dhadialla, B. Rutti* and M. Brossard*

We have continued trying to identify tick antigens that may be used to induce resistance in host animals to infestation by

Table 5.3 Effect on fed weights and weights of egg batches of adult female *R. appendiculatus* applied on immunized and control rabbits

Treatment	No. of ticks	% mortality	Mean wt of ticks \pm S.E.M.* (mg)	Mean wt of egg batch \pm S.E.M.* (mg)	Egg conversion factor†
Immunized	60	25.0	228.5 \pm 14.0	85.9 \pm 10.2	0.376
Control	60	3.3	365.2 \pm 11.0	195.7 \pm 7.60	0.536

* S.E.M.: standard error of the mean.

† Egg conversion factor = egg batch weight/engorged weight of female.

the tick *R. appendiculatus*. We have immunized rabbits with a protein extract from female ticks, the extract obtained by solubilizing tissues with Triton X-100. Virgin females that had fed on rabbits for 5 days were homogenized in phosphate buffered saline (PBS), pH 7.0, containing 1 mM phenylmethylsulfonylfluoride (PMSF). The homogenate was centrifuged at 12 000 g for 10 min at 4° C. While the supernatant was used to obtain protein fractions enriched for the 230 000 and 140 000 molecular weight antigens, the pellet was resuspended in PBS and allowed to settle before the supernatant was discarded. This step was repeated 15 times until the supernatant was colourless. The pellet was washed this way to make it as free as possible of contamination by host serum proteins.

The washed pellet was then homogenized in PBS containing 0.5% Triton X-100. The homogenate was centrifuged at 12 000 g for 10 min at 4° C. The supernatant (referred to as solubilized membrane proteins, SMP) was used for immunizing a group of three rabbits. Control rabbits were injected with PBS containing 0.5% Triton X-100. One week after the rabbits had been injected with the second booster injections, the rabbits were bled to collect sera. One week later, 20 female and 25 male ticks were applied on the ears of the rabbits. The ears were enclosed in cloth bags. To assess biological activity of the SMP on ticks fed on immunized rabbits, weights of the engorged ticks and their egg batches were recorded, as well as the number of ticks that died either on the host or after engorgement. The results of the tick challenge are shown in Table 5.3.

Twenty-five per cent of the ticks that fed on immunized rabbits died, compared to 3.3% of the ticks that fed on control rabbits. Engorgement weight and egg-batch weight of ticks fed on immunized rabbits were reduced by 38% and 57%, respectively, compared with the control rabbits. These results are encouraging because the decrease in egg-batch weight is not directly correlated to the amount of reduction in the engorgement weight of the ticks. There was a 30% reduction in the egg conversion factor for ticks applied on immunized rabbits, indicating that the immunization of rabbits with SMP had adversely affected one or more steps of the egg-production process.

Immunoblotting experiments are in progress to identify the antigen in the SMP extract that induced the immune response.

*Institute of Zoology, University of Neuchatel, Neuchatel, Switzerland.

5.7 THE EFFECT OF pH ON PROPERTIES OF ENZYME 2, A CARBOXYL PROTEINASE FROM THE GUT OF *RHIPICEPHALUS APPENDICULATUS*

M. R. W. Vundla and V. L. Labongo

The purification of two carboxyl proteinases (1 and 2) from the midgut of partially fed female *Rhipicephalus appendiculatus* was described in the 1986 Annual Report. In carboxyl proteinases, substrate specificity, susceptibility to inhibitors and thermal inactivation under different conditions of pH are known to vary considerably. We report below the effect of pH on some properties of enzyme 2.

The effect of pH on the activity of carboxyl proteinase 2 was determined by assaying the enzyme at maximum velocity (V_{max}) concentrations of haemoglobin (20 mg ml^{-1}) in 0.15-M buffers in the pH range 1.8–6.5, prepared from a mixture of phosphoric, formic and acetic acids and containing 0.2-M NaCl. Typically, the *R. appendiculatus* carboxyl proteinase 2 was optimally active at pH 2.6 (Figure 5.11) and is similar in this respect to carboxyl proteinases from the ticks *Argas persicus*, *Ornithodoros tholozani* and *Boophilus microplus*. In addition to the major peak, there was a distinct second peak at pH 5.0. Most cathepsin D preparations show a distinct shoulder or, as in this case, a second peak at pH 4.0–5.0. This phenomenon has been reported in ticks for *Argas persicus* and in the dipteran *Lucilia cuprina*. It has also been reported that this bimodal nature is the property of a single enzyme species and is not due to changes in the substrate. No hydrolysis of haemoglobin occurred above pH 6.0.

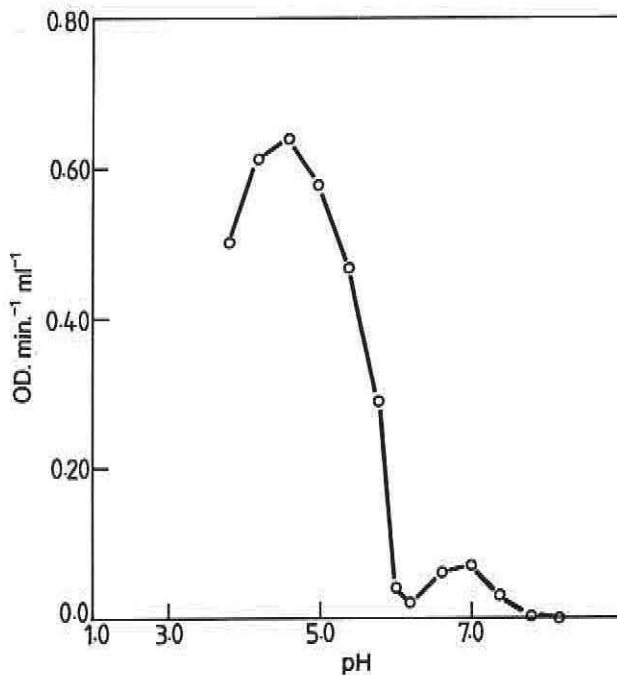


Figure 5.11 The effect of pH on the activity of *R. appendiculatus* carboxyl proteinase 2.

The inhibition of enzyme 2 by pepstatin was dependent on the assay pH. The enzyme was assayed in the presence of V_{max} concentrations of substrate and increasing concentra-

tions (1 nm–8 nm) of pepstatin at pH 1.8, 2.6 and 3.3. For each concentration of pepstatin, inhibition decreased with increasing pH. At pH 3.3, pepstatin concentrations below 3 nm had little effect on enzyme activity in contrast to the marked inhibition observed at pH 1.8 and 2.6 (Figure 5.12). These observations are in agreement with the findings of others that the binding site of cathepsin D is abolished by a conformational change as the pH rises towards 7.0.

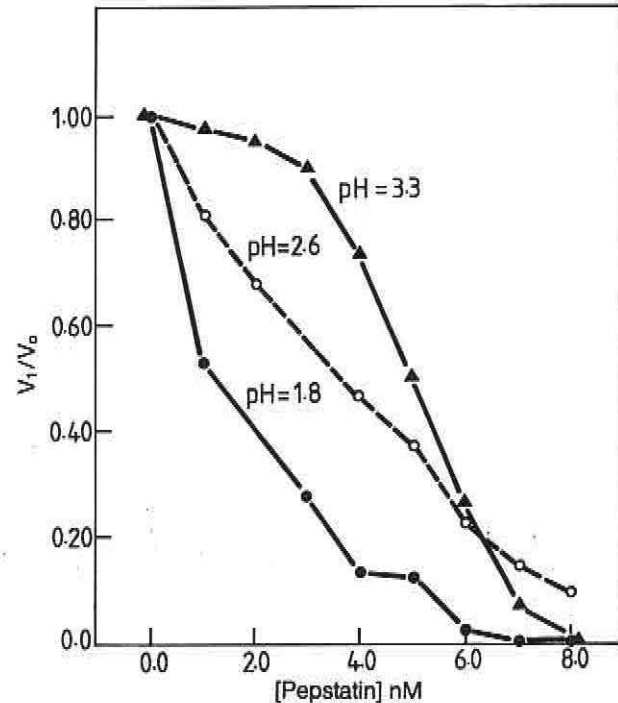


Figure 5.12 The effect of pH on the inhibition of *R. appendiculatus* carboxyl proteinase 2 by pepstatin. V_0 = no pepstatin, V_1 = inhibited reaction.

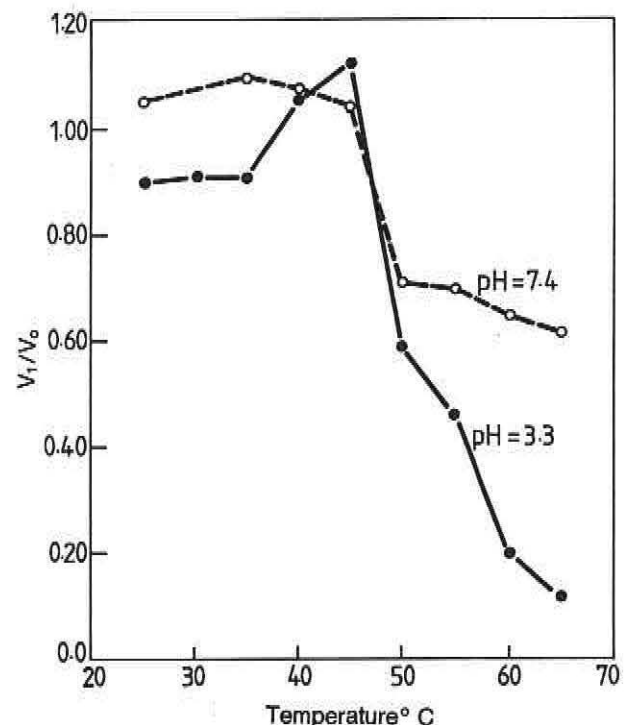


Figure 5.13 The effect of pH on the thermal stability of *R. appendiculatus* carboxyl proteinase 2. V_0 = untreated enzyme, V_1 = activity after temperature treatment.

The influence of pH on the thermal stability of enzyme 2 was also investigated. Aliquots of the enzyme were incubated at temperatures between 25° C and 65° C for 10 minutes at pH 3.3 or pH 7.4. Each sample was then brought to 4° C and assayed at 45° C and pH 3.3. The enzyme lost activity rapidly above 45° C. However, the enzyme incubated at pH 3.3 was much more sensitive to thermal inactivation so that after incubation at 65° C this enzyme had only 12% of original activity compared to 62% for the enzyme incubated at pH 7.4 (Figure 5.13). Other studies are in agreement with our observations that most preparations of cathepsin D lose activity rapidly above 50° C but that pH is an important factor. The mechanism involved in this phenomenon is not known, but other findings suggest a situation similar to that reported for pepsin, where the enzyme is stabilized by a peptide at pH values above 4.0. It may well be that the abolition of the enzyme binding site at high pH also has a part to play in the stabilization of carboxyl proteinases at high pH.

5.8 THE VOLATILE RESIN EXUDATE FROM THE STEM BARK OF *COMMIPHORA ROSTRATA*: PROBABLE ROLE IN PLANT DEFENCE

P. G. McDowell, W. Lwande and P. G. Waterman*

Commiphora rostrata (Burseraceae) is one of numerous species of this genus found in the arid areas of northern

Kenya, Somalia and southern Ethiopia. Many *Commiphora* species produce oleo-gum-resins, a number of which, notably myrrh, are of commercial significance. *C. rostrata* is a small deciduous tree rarely attaining a height of more than 3 metres. The simple leaves are edible and are reported to have a flavour of oxalic acid. The red-brown bark is smooth and contains copious amounts of a clear pungent resin, which appears to be retained under pressure. When branches or twigs are cut or bent the resin is released both as a fine spray and as a free-flowing liquid, which rapidly covers a considerable area around the point where the damage has occurred. Most *C. rostrata* trees show signs of resin flow but are conspicuous by the absence of herbivore damage or pathogen attack on their woody parts.

We studied the volatile portions of the resins from a number of Kenyan species of *Commiphora* and found them to be mainly monoterpene or sesquiterpene in composition. There do not appear to be any reports concerning the volatile resin from *C. rostrata*. We describe the results of our study on a sample of the resin obtained from plants growing in Meru National Park and discuss its probable importance in the defence of the plant.

Gas chromatography revealed the presence of at least 30 components in the oil (Figure 5.14). Table 5.4 lists 24 components from which mass spectra were obtained. Of the 22 that have been identified, 18 were characterized by direct comparison to library mass spectra and co-injection with authentic samples on two capillary columns. The major

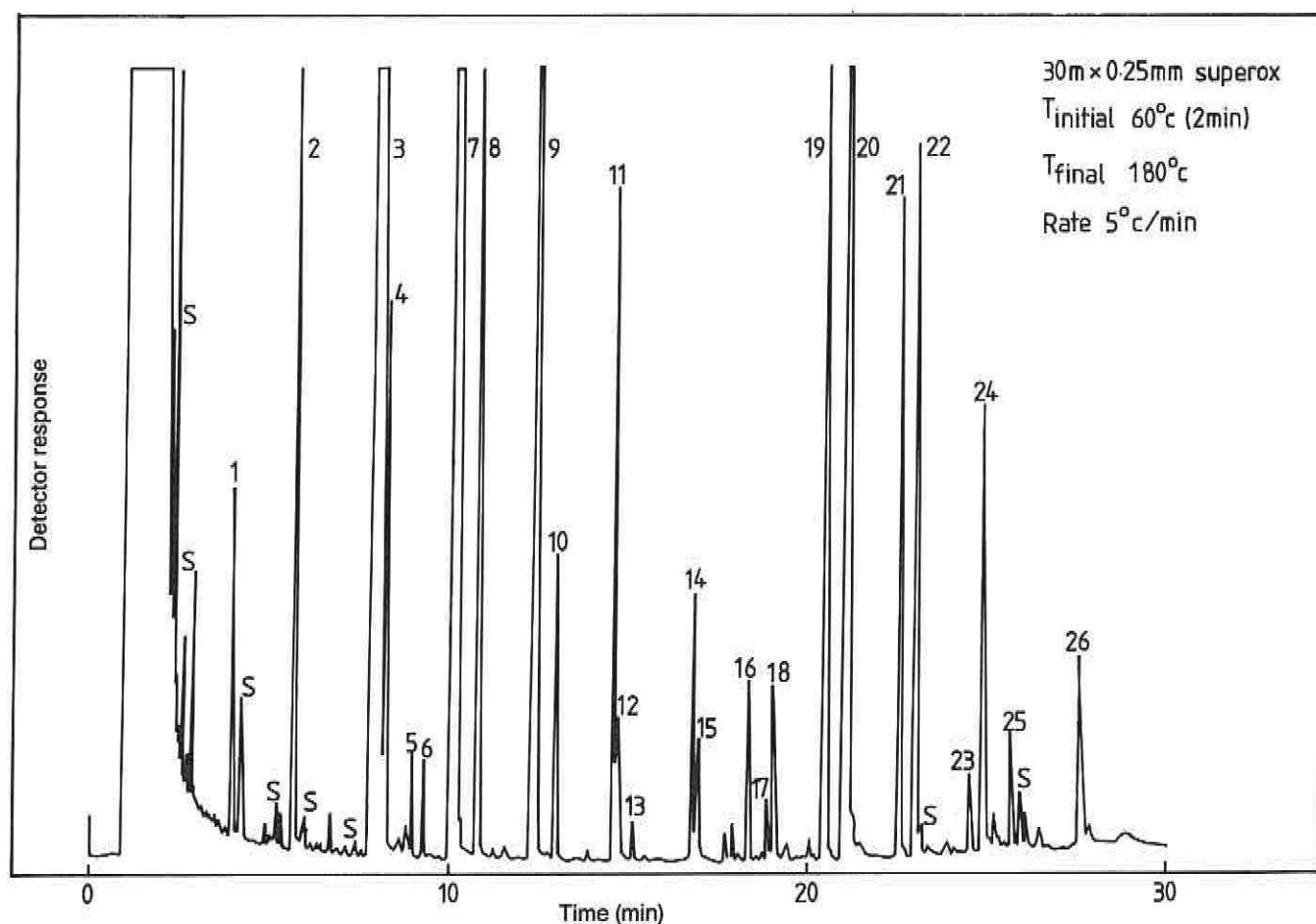


Figure 5.14 Gas chromatogram of *Commiphora rostrata* oil (hexane solution).

components are 2-decanone, 2-undecanone and 2-dodecanone, which make up about 65%, 24% and 5% of the volatiles, respectively. 2-octanone, 2-nonanone, 2-tridecanone, 2-tetradecanone and 2-pentadecanone occur at levels of less than 1%, and the corresponding 3-undecanone is found only in trace amounts. Three secondary alcohols (C₁₀–C₁₂ 2-alkanols) also occur at trace levels. Another series is made up of saturated long-chain aldehydes between C₁₃ and C₁₈, which occur in an approximate ratio of 1:1:1:18:5:3, with hexadecanal constituting about 1.5% of the total volatiles.

Table 5.4 Compounds identified from *Commiphora rostrata* oil

Peak no.	Compound	% in oil*	Evidence†
1	2-Octanone	t	MS, Rt
2	2-Nonanone	t	MS, Rt
3	2-Decanone	65.0	MS, Rt
4	Unknown	t	
5	Unknown	t	
6	3-Undecanone	t	MS, Rt
7	2-Undecanone	24.0	MS, Rt
8	2-Decanol	t	MS, Rt
9	2-Dodecanone	5.0	MS, Rt
10	2-Undecanol	t	MS, Rt
11	2-Tridecanone	t	MS, Rt
12	Tridecanal	t	MS, Rt
13	2-Dodecanol	t	MS, Rt
14	2-Tetradecanone	t	MS, Rt*
15	Tetradecanal	t	MS, Rt
16	2, 2-Dimethylnonanol	t	DMS
17	2-Pentadecanone	t	MS, Rt
18	Pentadecanal	t	MS, Rt
19	2, 2-Dimethyldecanol	t	DMS
20	Hexadecanal	1.5	MS, Rt
21	2, 2-Dimethylundecanol	t	DMS
22	Heptadecanal	t	MS, Rt
23	2, 2-Dimethyldodecanol	t	DMS
24	Octadecanal	t	MS, Rt

* t: trace amount, less than 1% of total oil.

† MS: full mass spectrum matching a library spectrum; Rt: identical retention times compared to authentic samples on two capillary columns; DMS: mass spectrum of trimethylsilyl ether.

A further four trace components (16, 19, 21 and 23 [Table 5.4]) form another homologous series of aliphatic alcohols. On the basis of their mass spectrometry (MS) and that of the trimethylsilyl (TMS) derivatives formed by reaction of the extract with bis-trimethylsilyl trifluoroacetamide (BSTFA), the compounds were assigned the structures of a series of 2,2-dimethyl alkanols of chain lengths C₉ to C₁₂. As far as we are aware, the 2,2-dimethyl alkanols reported here have not been isolated from other plant sources.

5.8.1 Ecological significance

It seems probable that this volatile resin plays a role in the defence of the plant against potential pests and pathogens. It has already been noted that the bark is conspicuously free from damage due to either boring insects or browsing mammals. Observations of freshly cut bark from which resin is flowing indicate that insects in the vicinity (such as ants and termites) immediately become excited and move rapidly away from the wound. The resin literally sprays or squirts

from a cut or stress point (caused by bending) and this may have an overwhelming effect on attacking predators or pathogens. After a short time a white sticky substance forms at the wound site, perhaps due to polymerization of the resin aldehydes, and this presumably acts to protect the wound and to prevent water loss.

The occurrence of large quantities of aliphatic ketones in exudates has previously been implicated in chemical defence. The wild tomato, *Lycopersicon hirsutum*, has glandular trichomes rich in 2-tridecanone, which is thought to be responsible for its resistance to the tobacco hornworm, *Manduca sexta*, an important insect pest of the cultivated tomato. 2-tridecanone is also toxic to the larvae of the tomato fruitworm, *Heliothis zea*, and is reported to be an ant alarm pheromone. 2-undecanone, which is a lesser component of the trichomes of *L. hirsutum*, increases larval mortality of *H. zea* when mixed with 2-tridecanone, and will cause deformity and mortality of *H. zea* pupae, either alone or combined with 2-tridecanone. 2-nonanone, 2-decanone, 2-dodecanone and 2-pentadecanone are also toxic to *H. zea*, while 2-tridecanone and 2-undecanone have also been found to be toxic to neonate larvae of the tomato pinworm, *Keiferia lycopersicella*, and the beet armyworm, *Spodoptera exigua*. 2-nonanone has been reported as an alarm pheromone in ants, hornets and honeybees and to have weak bactericidal activity.

Preliminary results from tests for both insect repellency and antifungal properties indicated that the oil is repellent to the maize weevil *Sitophilus zeamais* and that the oil and its three major components possess antifungal properties.

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5.9 NATURAL AND SYNTHETIC NAPHTHOQUINONES AS MOSQUITO LARVICIDES

G. V. Achieng, A. Chapya, A. Hassanali and E. Nyandat

As a follow-up to work on the mosquito larvicidal activities of plumbagin (I) isolated from *Plumbago zeylanica* and a number of synthetic naphthoquinones (1985 Annual Report), we undertook a more detailed comparison of larvicidal activities of available quinones at different doses.

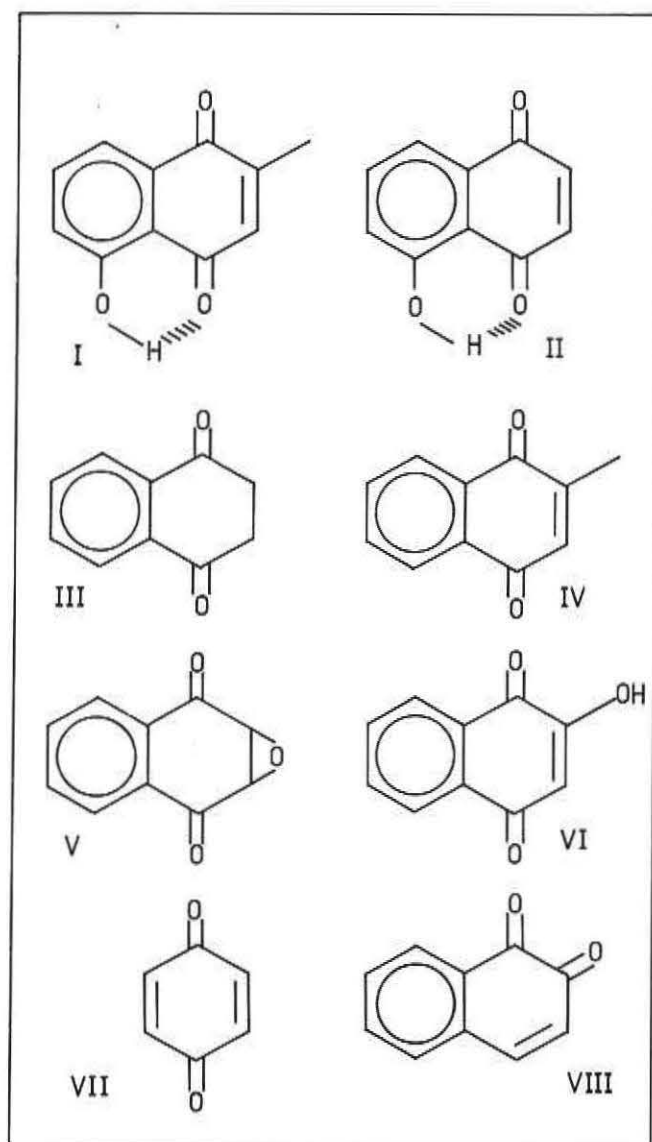
The quinones bioassayed include plumbagin (I), juglone (II), 2-methyl-1,4-naphthoquinone (III), 1,4-naphthoquinone (IV), 2,3-epoxy-1,4-naphthoquinone (V), 2-hydroxy-1,4-naphthoquinone (VI), 1,4-benzoquinone (VII) and 1,2-naphthoquinone (VIII). The bioassays were carried out on 9 replicates of 20 third-instar larvae of *Aedes aegypti* at 5–6 concentrations in the range 0–4 μ mole/20 ml solution for compounds I–V, and 0–16 μ mole/20 ml solution for compounds VI–VIII. LC₅₀ values obtained for the compounds are given in Table 5.5.

Of the quinones tested, the 5-hydroxy derivatives of 1,4-naphthoquinone (I and II) were found to be most potent. The high activity of the parent 1,4-naphthoquinone (III) relative to the 1,2-analogue (VIII) and 1,4-benzoquinone (VII) shows

Table 5.5 LC₅₀ for various quinones against third-instar *Aedes aegypti* larvae

Compound	LC ₅₀ (μmole/20 ml)	
I	0.60 ± 0.05	a
II	0.70 ± 0.05	a
III	1.15 ± 0.05	b
IV	1.82 ± 0.05	c
V	2.20 ± 0.05	d
VI	13.10 ± 0.20	e
VII	16	
VIII	17	

that the activity is largely associated with the 1,4-naphthoquinone nucleus. Substitution on the 2 position of this nucleus, however, leads to reduced activity, with the OH substituent (compound VI) causing the largest drop. Whether this drop is associated with all substituents irrespective of their electronic effects or is specifically associated with electron-donating groups will become clear when other substituted naphthoquinones are assayed.

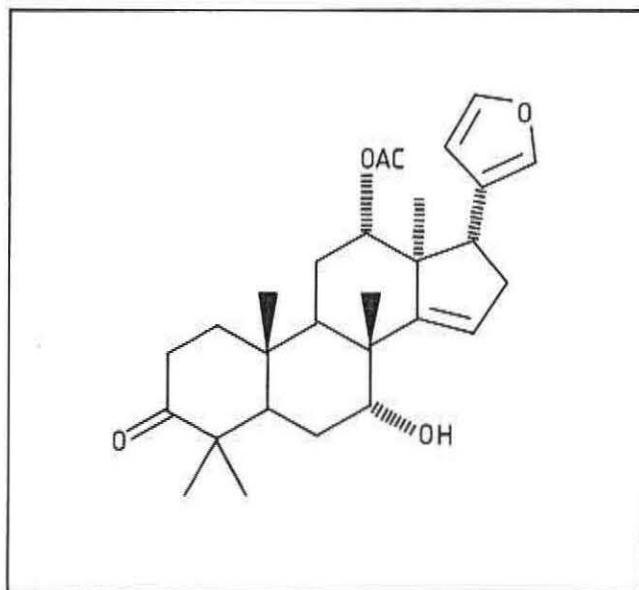
Figure 5.15 Structures of naphthoquinones, bioassayed for activity against third-instar larvae of *Aedes aegypti*.

Naphthoquinones occur widely in tropical plants and assays of a broader range of naturally occurring structural variants may lead to the identification of readily cultivable plants suitable for small-scale mosquito-control programmes.

5.10 A NEW LIMONOID ANTIFEEDANT FROM *TURREA ROBUSTA* (MELIACEAE)

M. Rajab,* A. Chapya, A. Hassanali and M. D. Bentley*

Of the various naturally occurring compounds screened in recent years, the limonoids, represented by azadirachtin and other tetranortriterpenes from the neem tree (*Azadirachta indica*), have been found to be most promising as non-conventional insect control agents. Our long-term goals are (a) to screen species of Meliaceae (and other limonoid-containing plants) indigenous to Africa for potent anti-insect limonoids, and (b) to carry out comprehensive structure-activity studies on limonoids with respect both to their antifeedant action and to their anti-hormonal, growth-disrupting activities against insects. In 1987 we undertook a study of antifeedant actions of ten limonoids with respect to the larvae of *Chilo partellus* and examined the limonoid content of the root bark of the plant *Turrea robusta* (called 'mzikoziko' in the Zaramo dialect of Tanzania), an extract of which was shown, in a preliminary test, to deter feeding by the larvae of *Maruca testulalis*, *Eldana saccharina* and *Spodoptera exempta*. The structure-activity work will be reported next year. Herein we describe the isolation and structure determination of a new limonoid, which we have called mzikonone.

Figure 5.16 Structure of a new limonoid, 'mzikonone', extracted from the root bark of *Turrea robusta*.

Extraction of air-dried root bark with methanol yielded an oil that we subjected to silica-gel chromatography with a hexane-acetone gradient followed by a hexane-ethyl acetate gradient to yield mzikonone (I) as a white, microcrystalline solid (mp 99°–101° C). High-resolution mass spectrometry (MS) resulted in a parent ion of the molecular formula

$C_{28}H_{38}O_5$. The IR spectrum (KBr) indicated the presence of an OH (3570 cm^{-1}), an ester (1740 cm^{-1}), a ketone (1715 cm^{-1}) and a C-O (1250 cm^{-1}). The presence of the ketone and ester was further substantiated by ^{13}C nuclear magnetic resonance (NMR) absorptions at 217 and 171 ppm, respectively. A 3 proton singlet at 1.91 ppm in the ^1H NMR spectrum allowed assignment of the ester as an acetate. Application of ^{13}C NMR attached proton test (APT) techniques resulted in assignment to mzikonone of 6 methyls, 5 methylenes, 5 saturated methines, 4 unsaturated methines, 4 quaternary saturated carbons and 2 quaternary unsaturated carbons. Multiplet absorptions at 6.27, 7.23 and 7.35 ppm in the 500 MHz ^1H NMR spectrum indicated the presence of a β -substituted furan. A triplet at 4.01 ppm ($J = 2.8\text{ Hz}$) was consistent with assignment of the OH to the 7- α position. The vinyl proton absorption at 5.68 ppm (dd, $J = 2,3$) was observed to shift upfield 0.2 ppm upon acetylation of the OH, consistent with assignment of the OH to the 7-position and the double bond to the 14-position. A triplet at 5.10 ($J = 9$) led to assignment of the acetate of (I) to the 12-position. Treatment of (I) with benzene seleninic anhydride resulted in

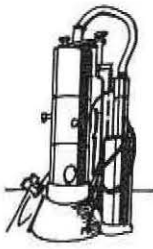
an α, β -unsaturated ketone having ^1H NMR absorptions at 7.05 ppm (d, $J = 12$) and 5.90 ppm (d, $J = 12$), consistent with assignment of the ketone to the 3-position. ^1H COSY techniques verified coupling of H-17 and H-15 with H-16, of H-9 and H-12 with H-11, of H-22 with H-21 and H-23, of H-1 with H-2, and of H-6 with H-5 and H-7. Stereochemical assignments were verified with NOESY techniques. Particularly relevant were correlations between H-21, H-22, H-23 and the acetate methyl. These, in addition to an H-12 to H-17 correlation, proved that the furan and the acetate were both either α or β . The 30-methyl was identified by correlation with H-7 and H-15. Correlation of H-12 with the 30-methyl confirmed α -stereochemistry for both the furan and the acetate.

Although mzikonone represents a relatively simple limonoid, the presence of an α -acetate group introduces an interesting feature into the skeleton, and the limonoid is a useful addition to our structure-activity work.

**Chemistry Department, University of Maine, USA.*

Histology and Fine Structure Research Unit

- 6.1 Accomplishments of HFSRU **97**
- 6.2 Morphological and functional changes associated with virus infection in male *Glossina morsitans morsitans* **98**
- 6.3 Ultrastructural evidence for transmission of the DNA virus of tsetse, *Glossina pallidipes* **101**
- 6.4 The functional morphology and biochemistry of male accessory glands and their secretions in *Glossina morsitans* **103**
- 6.5 Studies on the excretory systems and uterus in tsetse, *Glossina morsitans morsitans*, and stablefly, *Stomoxys calcitrans* **106**



6 Histology and Fine Structure Research Unit

The primary role of the Histology and Fine Structure Research Unit (HFSRU) is to assist and provide support services to ICIPE's core programmes by carrying out morphological and functional studies, at organ and cellular levels, of insect pests of ICIPE's target crops and the disease vectors of man and livestock. HFSRU both responds to specific questions raised by programmes and undertakes long-term research in areas of mutual concern.

HFSRU staff examine the functions of cellular and subcellular components to elucidate the mechanisms of the components' regulatory physiology under normal and impaired conditions. Our aim is to identify techniques or procedures that might be useful in managing and controlling insect pests, as well as to provide information that will help us understand the cellular biology of insects and their hosts. In 1987 HFSRU studies included the following.

- *The purification and characterization of the virus from hypertrophied salivary glands of wild *Glossina pallidipes* and *G. morsitans* and the development of immunological tests for the identification of the virus in tsetse and non-target animals.*
- *The characterization of antigens (proteins) common to accessory glands and spermatophore and analyses of the process of biosynthesis and the export of accessory reproductive gland proteins.*
- *Examinations of the infection processes of tsetse by entomopathogenic fungi and study of the effects of antibacterial factors on the reproductive performance of pests.*
- *A study of the distribution of regulatory neurohormones.*
- *Determination of neurotransmitter(s) present in the sensory nerve terminals.*

6.1 ACCOMPLISHMENTS

E. D. Kokwaro

The Histology and Fine Structure Research Unit continued its collaborative research with four ICIPE research programmes.

In collaboration with the Livestock Ticks Research Programme, SEM and TEM techniques were used to investigate blood cells of people with a history of fever, irritability and an itchy rash over the face and upper limbs. The same parasite was examined after passage in rodents. In smears of peripheral blood, the red cells appeared disfigured and had conspicuous round to oval bodies at the edge and face view of the erythrocyte. There was variation in the number of bodies: some erythrocytes had less than ten bodies; others had many over the surface. Using the transmission electron microscope, membrane-bound bodies with electron-dense material were observed. The morpho-

logical features of the organism as revealed by the TEM and SEM suggests that the rashes were due to Bartonella (haematropic bacterium) infection.

In a study of artificial tick feeding, we examined the mechanisms used by ticks during feeding to keep themselves on their hosts. In collaboration with the Sensory Physiology Research Unit (SPRU), scanning electron microscopy techniques were employed to examine details of tick attachment on a reinforced boudruche membrane. Ticks were shown to make an incision through the artificial membrane by cutting movements of the cheliceral digits. As the hood is forced into the lesion, a cement cone is formed. The cement cone is made of polysaccharide material secreted by the tick as soon as the attachment process begins. The cone material permeates and flows into the lesion, forming a thick coating on the inside of the membrane at the attachment site. The cheliceral hood and the hypostome get firmly anchored in the cone material, which forms a well-defined structure with a crater-like opening through which

the tick sucks fluid. The cheliceral digits are able to move in and out of this opening and feed in a similar manner to normal feeding (for details, see the SPRU Chapter of this Report).

Of particular interest for tsetse population control are the cytodifferentiation events taking place during the development of the various functional units, such as the reproductive organs. Ultrastructural development of accessory glands of *G. morsitans* has been investigated during the reproductive maturation age of 0–7 days.

The secretory cells of the male accessory reproductive glands (ARG) are fully differentiated at the time of eclosion and exhibit rapid synthesis of secretory products during the first week of adult life, which corresponds to the period of high reproductive activity in the male flies. The early production of functional spermatozoa appears to be paralleled by an early ultrastructural differentiation of the accessory gland secretions. This explains why male tsetse are able to mate and transfer spermatozoa immediately after emergence. The spermatophore, which enhances the transfer of spermatozoa in *G. morsitans*, has all the structural proteins commonly seen in secretions of sexually mature and newly emerged adults.

We have examined morphological and functional changes associated with virus infection in male *Glossina morsitans morsitans*. Lesions in the tsetse of virus-infected *G. morsitans* depict progressive degeneration of spermatozoa associated with degenerate mitochondrial derivatives (nebenkern) in some cases, and complete arrest of spermatogenic development and differentiation in others. While the degeneration of the nebenkern (as a store of energy for sperm motility) adversely affects the functional competence of spermatozoa and results in a significant reduction in insemination rates and fecundity, sperm necrosis and arrested development culminate in sterility, thus making the virus a possible biological control agent for tsetse.

Ultrastructural and autoradiographic studies established structural and functional specialization in *G. m. morsitans* Malpighian tubules and the occurrence of three cell types: secretory, transitional and proximal (absorptive). Autoradio-

graphic observations indicated the similarity between the distal and transitional cells.

Ultrastructural observations established the similarities and differences between the Malpighian tubules of *S. calcitrans* and those of *G. m. morsitans*. As in *G. m. morsitans* tubules, *S. calcitrans* tubules exhibited regional specialization. The tubules in the latter, however, have more lysosomes and lipid droplets in the epithelial cell cytoplasm.

Cytochemical observations localized acid phosphatase in lysosomes and some of the multivesicular bodies close to Golgi complexes and rough endoplasmic reticulum. The presence of polysaccharides with 1,2-glycol groups was also demonstrated.

Ultrastructural observations made on the hindgut and rectum of *G. m. morsitans* showed their involvement in fluid and ionic transport.

Autoradiographic observations showed uptake of (^3H)-glucose by the uterus in *G. m. morsitans* following injections of the radiochemical into the haemolymph of the fly.

6.2 MORPHOLOGICAL AND FUNCTIONAL CHANGES ASSOCIATED WITH VIRUS INFECTION IN MALE *GLOSSINA MORSITANS MORSITANS*

W. G. Z. O. Jura

Virus particles pathogenic to tsetse have been reported only in *Glossina pallidipes*, where the particles occur in males and females and in young and adult, causing extensive proliferation and vacuolation of salivary gland epithelial cells and abnormal reproductive performance, which is characterized, histologically, by the arrest of sperm development in the male, by gonadal degeneration and necrosis in both sexes and by the presence of virogenic stromata in germarial cells of the female ovariole.

In this study, testes obtained from *Glossina morsitans morsitans* with virus-infected hypertrophied salivary glands (HSG) have been processed to araldite and examined to

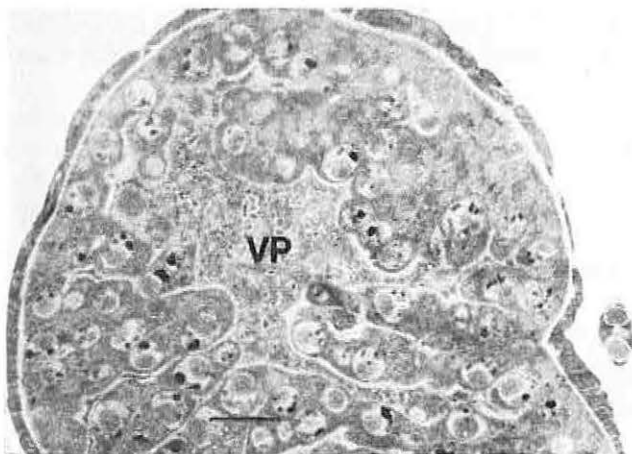


Figure 6.1 A photomicrograph of a hypertrophied salivary gland (HSG) of a teneral *Glossina morsitans morsitans*, depicting epithelial cell proliferation and occlusion of glandular lumen with hyperplastic cells and masses of virus particles (VP). Bar = 19.1 μm .

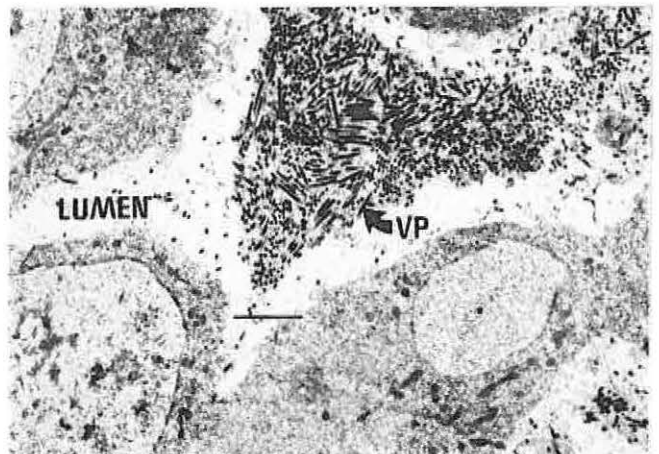


Figure 6.2 An electron micrograph of an HSG of a teneral male *G. m. morsitans*, showing virus particles within epithelial cells and in the lumen. Bar = 1.82 μm .

determine the associated morphological and functional alterations.

The male *G. m. morsitans* whose testes were examined in this study had HSG, in which the epithelial cells had proliferated extensively and occupied a greater part of the granular lumen (Figure 6.1). Masses of virus particles were depicted within the lumina of the salivary glands (Figure 6.2), intranuclear, intracytoplasmic and within the interstices of hyperplastic epithelial cells.

Sections of testes follicles of normal *G. m. morsitans* teneral contained distinct cysts of densely packed spermatozoa (Figure 6.3) embedded in large, polyvalent epithelial cells (Figure 6.4). The spermatozoa of each cyst were identical and generally contained two highly ordered, arc-shaped components of the nebenkern symmetrically situated adjacent to the flagellum of each spermatozoon (Figure 6.4).

Testes lesions manifested by the virus-infected *Glossina morsitans morsitans* teneral with HSG fell into three

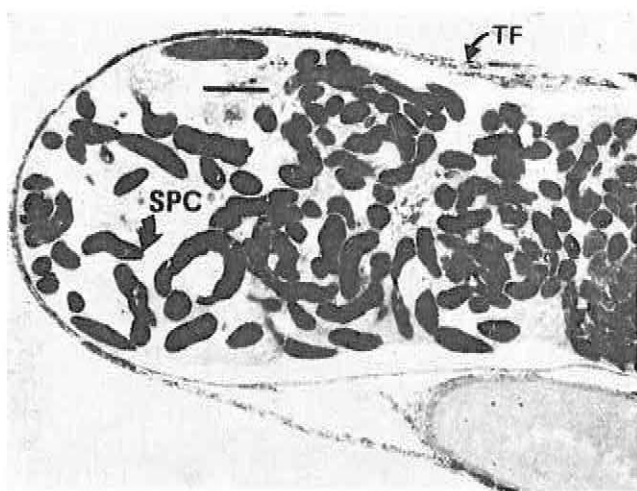


Figure 6.3 A photomicrograph of a testis follicle of normal, teneral male *G. m. morsitans*, showing distinct cysts of spermatozoa. SPC: sperm cysts; TF: testis follicle. Bar = 24.8 μm .

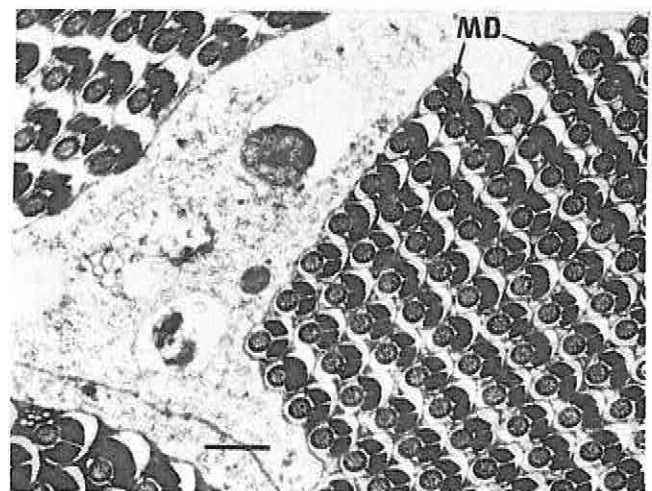


Figure 6.4 A section of a testis follicle of normal, teneral male *G. m. morsitans*, showing that cysts of normal spermatozoa possess symmetrical mitochondrial derivatives (MD). Bar = 0.57 μm .

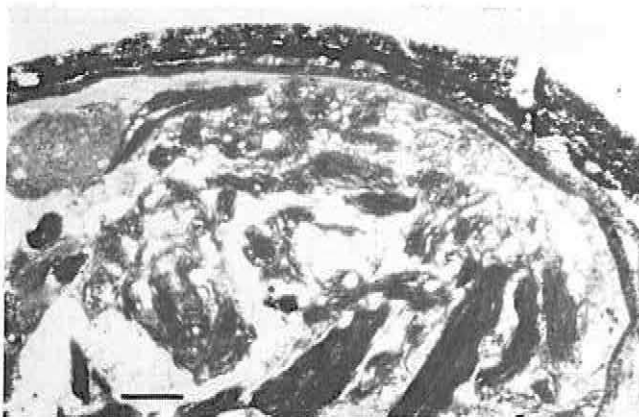


Figure 6.5 A photomicrograph of a section of testis follicle of an HSG of a teneral male *G. m. morsitans* in category I, showing diffuse vacuolation of epithelial cells and spermatolysis. Bar = 19.1 μm .

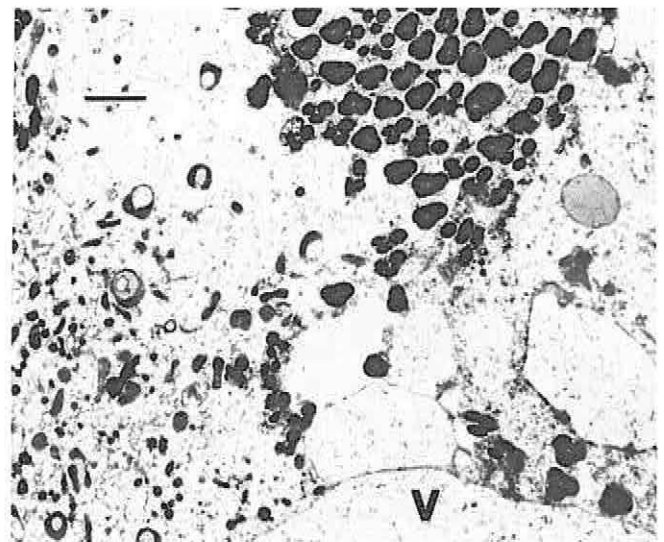


Figure 6.6 A section of a category I testis showing extensive degeneration and lysis of spermatozoa and epithelial cells. A cluster of polymorphic sperm is shown in the electron micrograph. v: vacuole. Bar = 0.88 μm .

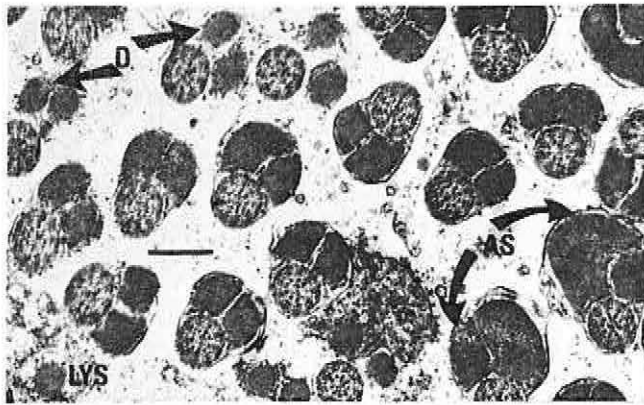


Figure 6.7 A higher magnification of the sperm cluster in Figure 6.6, depicting abnormalities of the nebenkern. D: degeneration, As: asymmetry, LYS: lysis. Bar = 0.27 μ m.

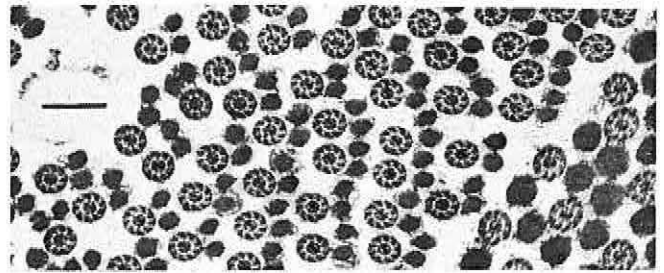


Figure 6.8 An electron micrograph of a category I testis, showing another cluster of spermatozoa with diffuse degeneration of the nebenkern. Bar = 0.40 μ m.

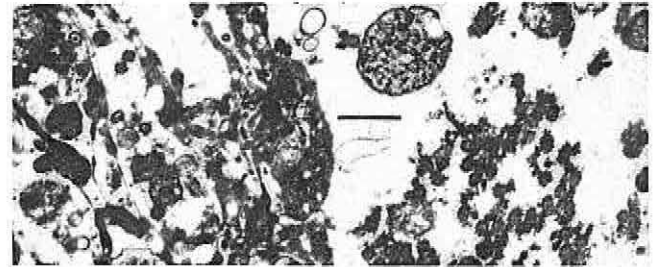


Figure 6.10 An electron micrograph of a section of category II testis follicle, showing fragmentary sperm components. Bar = 0.88 μ m.

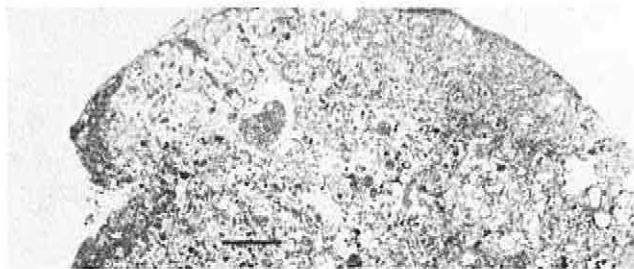


Figure 6.9 A photomicrograph of a section of a category II testis, showing complete loss of cystic structures and the presence of cellular debris instead of spermatozoa, depicting the final stages in the progressive degenerative process. Bar = 22.6 μ m.

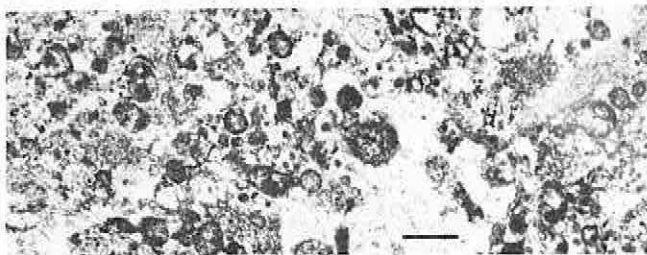


Figure 6.11 An electron micrograph of a section of testis follicle of an HSG of a teneral male *G. m. morsitans* in category II, showing necrosis of the polyvalent epithelial cells and spermatozoa. Bar = 0.57 μ m.

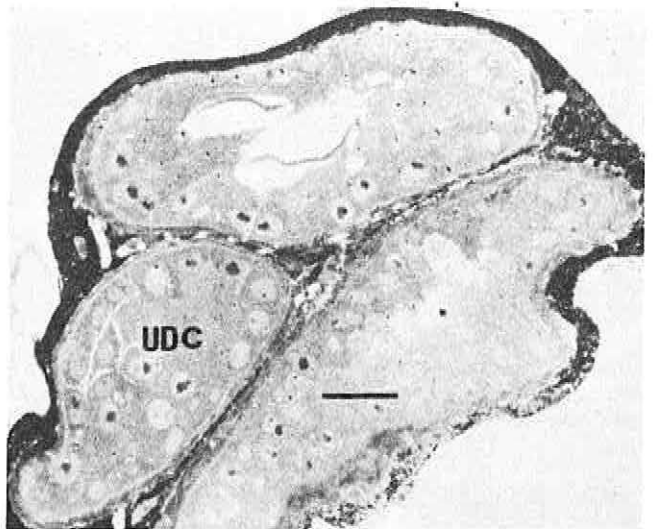


Figure 6.12 A photomicrograph of a section of category III testis follicle of an HSG of a teneral male *G. m. morsitans*, showing undifferentiated spermatogenic cells (UDC). Bar = 17.7 μ m.

Pathological changes in the testes of virus-infected *G. m. morsitans* fall into three categories: one in which the testes are only slightly shrunken and contain diffusely vacuolated polyvalent epithelial cells and patches of spermatozoa with predominantly degenerate mitochondrial derivatives; the second in which fragmentary and caseated epithelial cell and sperm debris constitute the contents of the testes follicle; and the third showing a complete arrest of spermatogenic development and differentiation. Male *G. m. morsitans*, whose testes lesions fall in the latter two categories, are sterile. The first category would appear to represent a stage before the second category in a progressive degenerative process. Even though in the first category patches of spermatozoa are still detectable, they show severe abnormalities of the nebenkern characterized by the disruption of the outer limiting membrane, disparate sizes, degeneration and eventual lysis of the derivatives.

The nebenkern serves as a store of energy for sperm motility. Its degeneration would adversely affect the functional competence of the spermatozoa and would result in a significant reduction in insemination rates and fecundity. At the extreme, where the degenerative process is advanced and loss of nebenkern occurs, the HSG male *G. m. morsitans* so affected would ultimately be sterile, thus emphasizing the potential of the testes virus as a biological control.

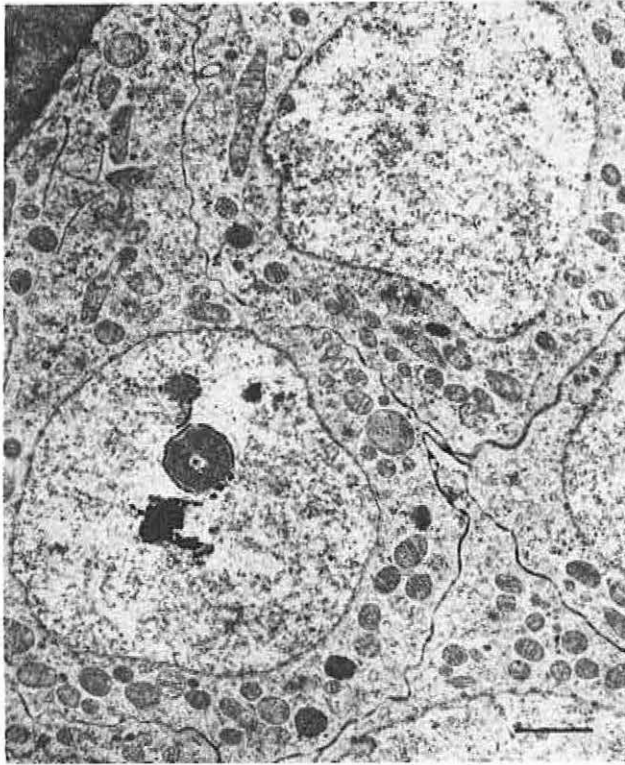


Figure 6.13 An electron micrograph of a category III testis follicle, showing premeiotic undifferentiated spermatogenic cells. Bar = 1.33 μm .

6.3 ULTRASTRUCTURAL EVIDENCE FOR TRANSMISSION OF THE DNA VIRUS OF TSETSE, *GLOSSINA PALLIDIPES*

W. G. Z. O. Jura, L. H. Otieno and
M. M. B. Chintawi

The mechanisms by which the DNA virus of tsetse is transmitted in nature among the flies are not known. When normal females are mated to either normal males or males with HSG, the resultant progeny is, in general, normal. Ultrastructural examinations of testes of males with hypertrophied salivary glands, and ovarioles from similarly affected female *G. pallidipes*, have demonstrated that no virus particles reside within testicular tissue and that germ cell cystocyte clusters within ovarioles contain virogenic stromata.

The above observations imply that the tsetse virus, which causes hyperplasia and hypertrophy of salivary gland epithelial cells, is not transmitted from an infected father to progeny, but that the particles are likely passed on vertically from mother to offspring.

The specific objectives of this study were to determine the incidence of virus infection in very young, field-caught, virgin female *G. pallidipes* of ovarian age category OA and to examine, by electron microscopy, the distribution of the virus particles within ovarian tissues of infected females in general, in order to demonstrate vertical transmission as a method of dissemination of the DNA virus of *Glossina pallidipes*. *Glossina pallidipes* used in this study were trapped from Ruma and Riamakanga study sites in Lambwe Valley,

Nyanza Province ($0^{\circ} 33' \text{S}$, $34^{\circ} 15' \text{E}$) using biconical traps. Only females were used in the study to determine the incidence of HSG among teneral *G. pallidipes* because the ovarian age category method is more accurate for identifying tenerals. The 'wing-fray' method, the only method available to determine the age of male tsetse, is used to compare mean

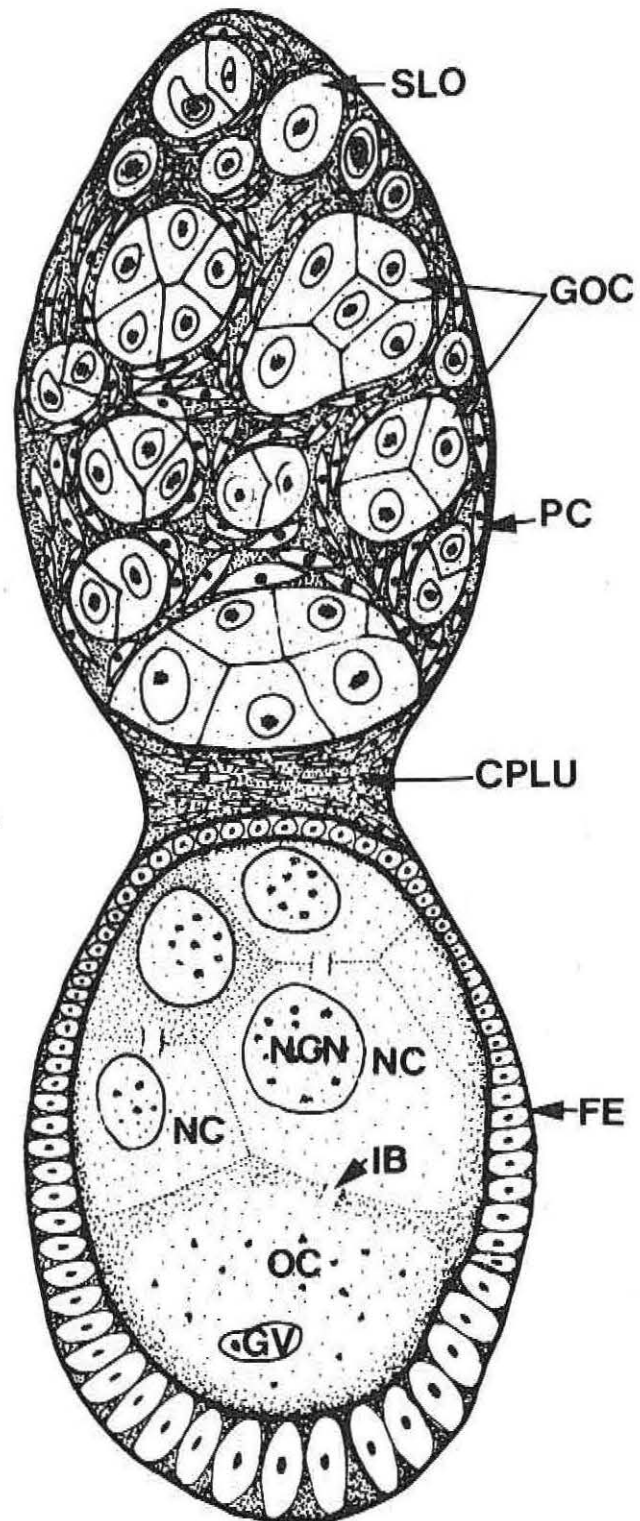


Figure 6.14 A diagram of structural components of an ovariole of tsetse. SLO: stem oögonia, GOC: germarial oögonial cyto blasts, PC: pre-follicular cell, CPLU: cellular plug, OC: oocyte, NC: nurse cell, IB: intercellular bridges, FE: follicular epithelium, GV: germinal vesicle, NCN: nurse cell nucleus.

ages of populations but is unreliable for determining the age of individuals. A record of OA-females with HSG was made. Furthermore, ovaries retrieved from infected females of all age groups were fixed in 2.5% glutaraldehyde and processed by standard techniques for electron microscopy.

The incidence of HSG among the OA-female *G. pallidipes* at both Ruma and Riamakanga study sites is shown in Table 6.1. About 2.1% of the teneral flies had virus infection, as shown by the presence of hypertrophied salivary glands. Various structural components of an ovariole of tsetse are illustrated diagrammatically (Figure 6.14). The germarium is located anteriorly and consists of clusters of oogonial cytotoblasts or cystocyte units. Each unit subsequently differentiates, with one compartment forming the oocyte and the rest nurse cells. Centrally, pre-follicular cells grow between the cystocyte clusters while in the posterior region of the ovariole, the typical egg-chamber or follicle is formed with oocyte located posterior to the nurse (15 nurse cells in total), the oocyte-nurse cell complex being surrounded by a

Table 6.1 Incidence of hypertrophied salivary glands (HSG) among the OA-female *G. pallidipes* at Ruma and Riamakanga study sites, Lambwe Valley

Site	Number of OA-females examined	Number of OA-females with HSG
Ruma	305	7
Riamakanga	220	4
Total	525	11

follicular epithelium of mesodermal origin. An examination of sections of germaria of ovarioles of *G. pallidipes* with HSG depicted virogenic stromata with virions within nuclei (Figure 6.15) and cytoplasm (Figure 6.16) of germ cell daughter cytotoblasts. In the egg-chamber or follicular tissues, very few virus particles were observed within nurse cells (Figure 6.17) and fewer still in the oocyte cytoplasm (Figure 6.18).

We have shown in this study that 2.1% of very young, unfed, virgin *G. pallidipes* are already infected by the virus and have, at this early stage, developed markedly hypertrophied salivary glands. The presence of such highly enlarged salivary glands in teneral tsetse suggests that the flies acquired the virus infection much earlier during their development, most likely in the mother.

This study also demonstrates virus particles both within oogonia and their daughter cystocyte clusters and in the oocyte and the interconnected nurse cells. In meroistic polytrophic ovaries such as in tsetse, the oocyte and the associated nurse cells develop as a syncytial unit or cystocyte cluster derived from one origin, the stem line oogonium, by synchronous divisions within the germarium of an ovariole. The observed presence of virus particles within oogonia and their derivatives (the oocyte and nurse cells) demonstrate that within a virus-infected female *G. pallidipes*, the germ cell acts as the perpetual source of virions within the ovarian tissue and that the virus is transmitted to progeny through the egg cytoplasm (trans-ovum transmission).

In an earlier study it was shown that the presence of large numbers of virus particles within the germaria of ovarioles of

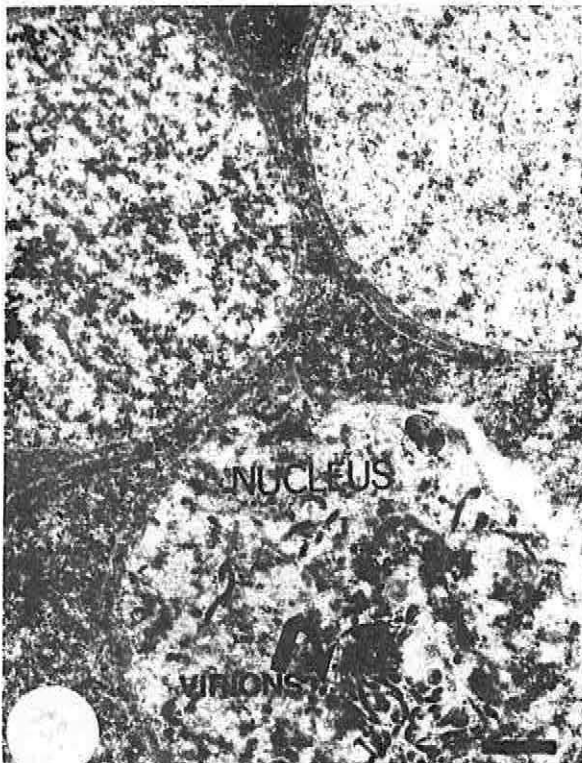


Figure 6.15 A section of a germarium of an ovariole of a female *G. pallidipes* with hypertrophied salivary glands (HSG), depicting virions within the nucleus of an oogonial daughter cytotoblast. Magnification: $\times 20\ 000$. Bar = $0.5\ \mu\text{m}$.

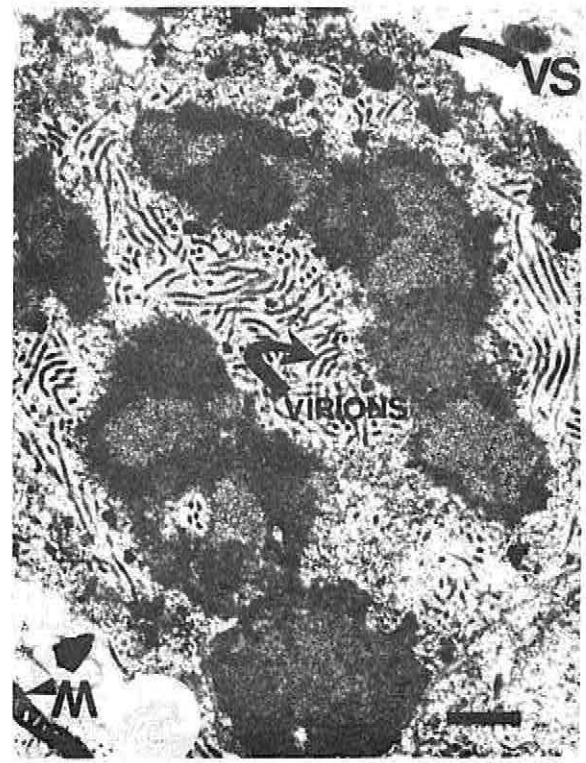


Figure 6.16 A section of a germarium of an ovariole of an HSG of a female *G. pallidipes*, depicting a virogenic stroma with numerous virions within the cytoplasm of a germ cell. VS: virogenic stroma, M: mitochondrion. Magnification: $\times 30\ 000$. Bar = $0.33\ \mu\text{m}$.

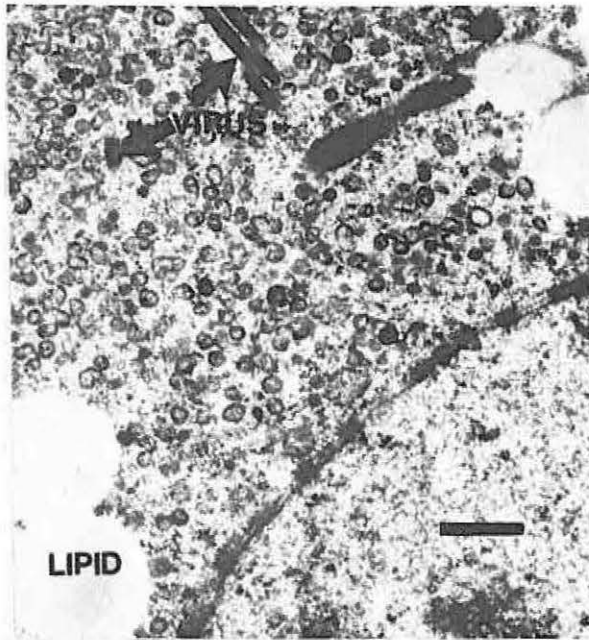


Figure 6.17 A section of a follicle of an ovariole of an HSG of a female *G. pallidipes*, showing few virus particles within nurse cell cytoplasm. Magnification: $\times 30\,000$. Bar = $0.33\ \mu\text{m}$.

female *G. pallidipes* with HSG produced severe caseous necrosis and degeneration of germ cells. However, it is interesting that in this study only an occasional virus particle was found within the ooplasm and that the affected oocytes appeared perfectly intact, without pathological changes. We suggest that the presence of extremely few virus particles in the oocyte cytoplasm precludes deleterious effects of the virus on the ovum and ensures normal development of the egg into an offspring.

6.3.1 Conclusions

- Progeny of virus-infected, female *G. pallidipes* with HSG acquire the virus infection in the mother.
- In such infected females, stem line oogonia act as a perpetual source of virions within the ovarian tissue and the virus is transmitted to progeny through the egg cytoplasm (trans-ovum transmission).
- Sparse distribution of virus particles within the ooplasm precludes deleterious effects of the virus on the ovum and ensures normal development of the egg into an offspring.

6.4 THE FUNCTIONAL MORPHOLOGY AND BIOCHEMISTRY OF MALE ACCESSORY GLANDS AND THEIR SECRETIONS IN *GLOSSINA MORSITANS*

E. D. Kokwaro and J. K. Murithi

The male tsetse fly, *Glossina morsitans*, may copulate several times and transfer several spermatophores during its life. This suggests that the accessory glands are extremely active in the synthesis of spermatophore components. In a previous study we examined the histology, histochemistry and ultrastructure of seven-day-old accessory glands in relation to spermatophore formation. These studies revealed the

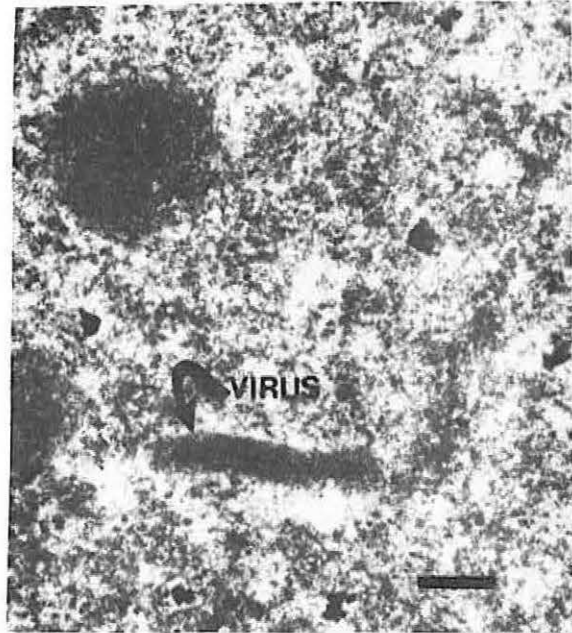


Figure 6.18 A section of an oocyte of an HSG of a female *G. pallidipes*, showing a lone virus particle in the ooplasm. Magnification: $\times 60\,000$. Bar = $0.17\ \mu\text{m}$.

presence of one type of secretory cells and the cell showed a high activity of protein synthesis. The gland lumen in the seven-day-old male fly accumulates a large quantity of complex secretion. In a recent study we have produced biochemical and immunological evidence suggesting that secretory proteins formed originally in the accessory glands are used to form the spermatophore. There was also immunological cross-reactivity between accessory glands and spermatophore proteins, indicating the presence of common antigenic determinants.

From these observations it was not clear to what extent functional maturation of the accessory glands is paralleled by cellular differentiation at the ultrastructural level during the 0–7 days of adult development. We also wanted to know how the synthesis of secretory products correlated with the reproductive age of the adult male.

The structure of the accessory glands in the teneral adult immediately following eclosion is shown in Figure 6.19. The epithelial layer of these tubular glands is composed of a single type of cuboidal cells that show a clear, pronounced secretory activity. Densely staining secretory granules are detectable in the gland lumen and cytodifferentiation of the epithelial cells is fully complete.

The cells are linked by septate desmosomes, which are often found in long continuous stretches. In the newly emerged fly, the epithelial cells are functionally differentiated and show a high synthetic activity, as can be seen from the appearance of such organelles as the rough endoplasmic reticulum, Golgi complex, randomly distributed mitochondria, fairly abundant free ribosomes most often clustered in small polysomal units and some microtubules. The Golgi complexes, located primarily in the perinuclear region, have inflated lamellae and give rise to small and large electron transparent vesicles and a few condensing vacuoles, which contain membranous immature secretory products (Figure 6.20). This characteristic export protein population is

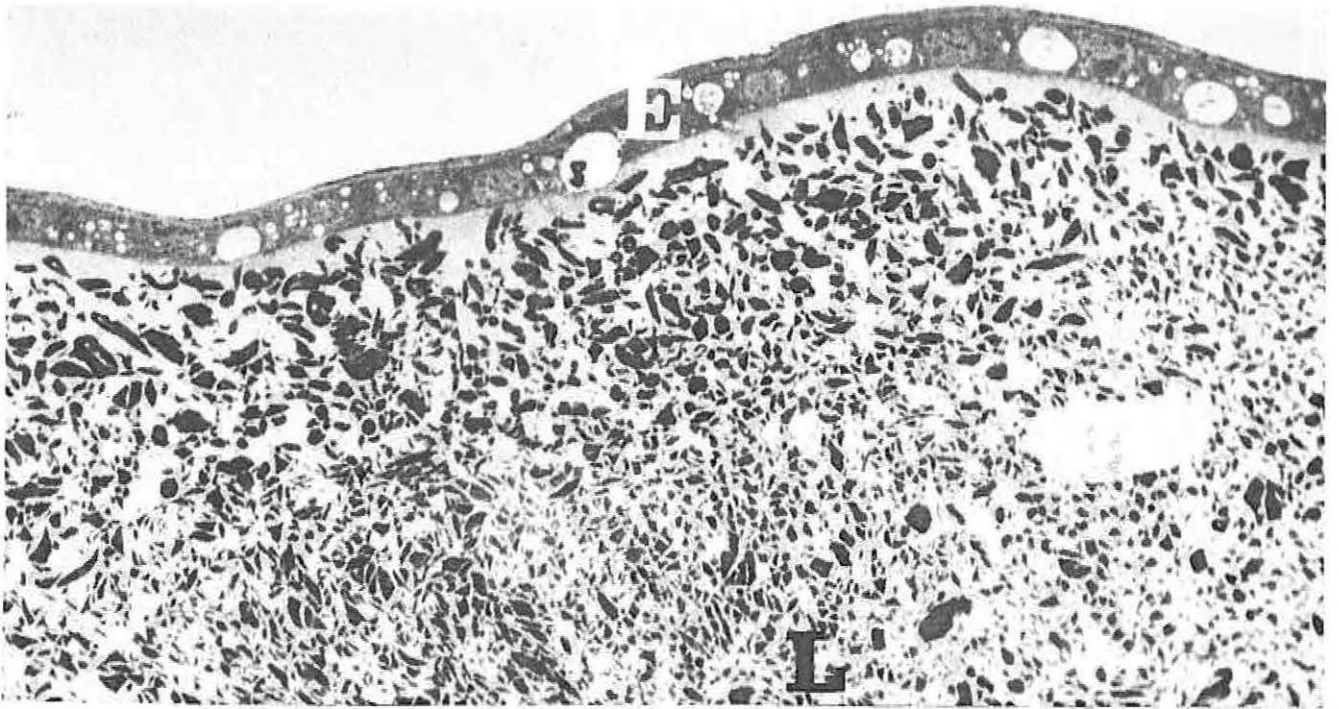


Figure 6.19 A photomicrograph of a thick longitudinal section through the accessory reproductive gland of a teneral fly stained by toluidine blue-borax. The epithelium (E) surrounds a lumen (L) containing irregularly shaped, densely stained secretory material.

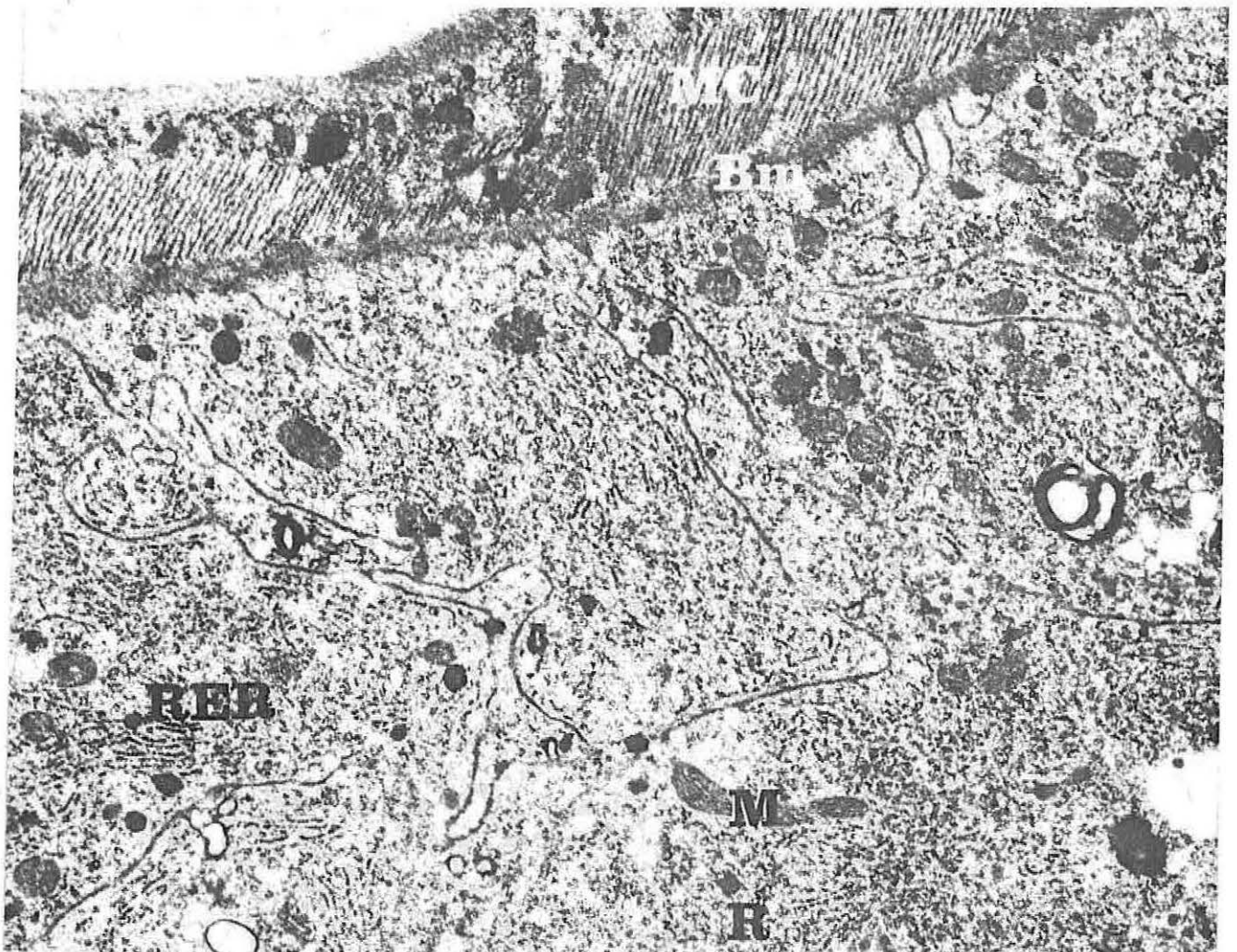


Figure 6.20 A micrograph of a cell from the teneral fly. Basically, the gland is invested with a sheath of muscle (MC) and basement membrane (Bm). Much of the cytoplasm is made up of rough endoplasmic reticulum (RER), free ribosomes (R), small Golgi complexes (GC) and mitochondria (M).

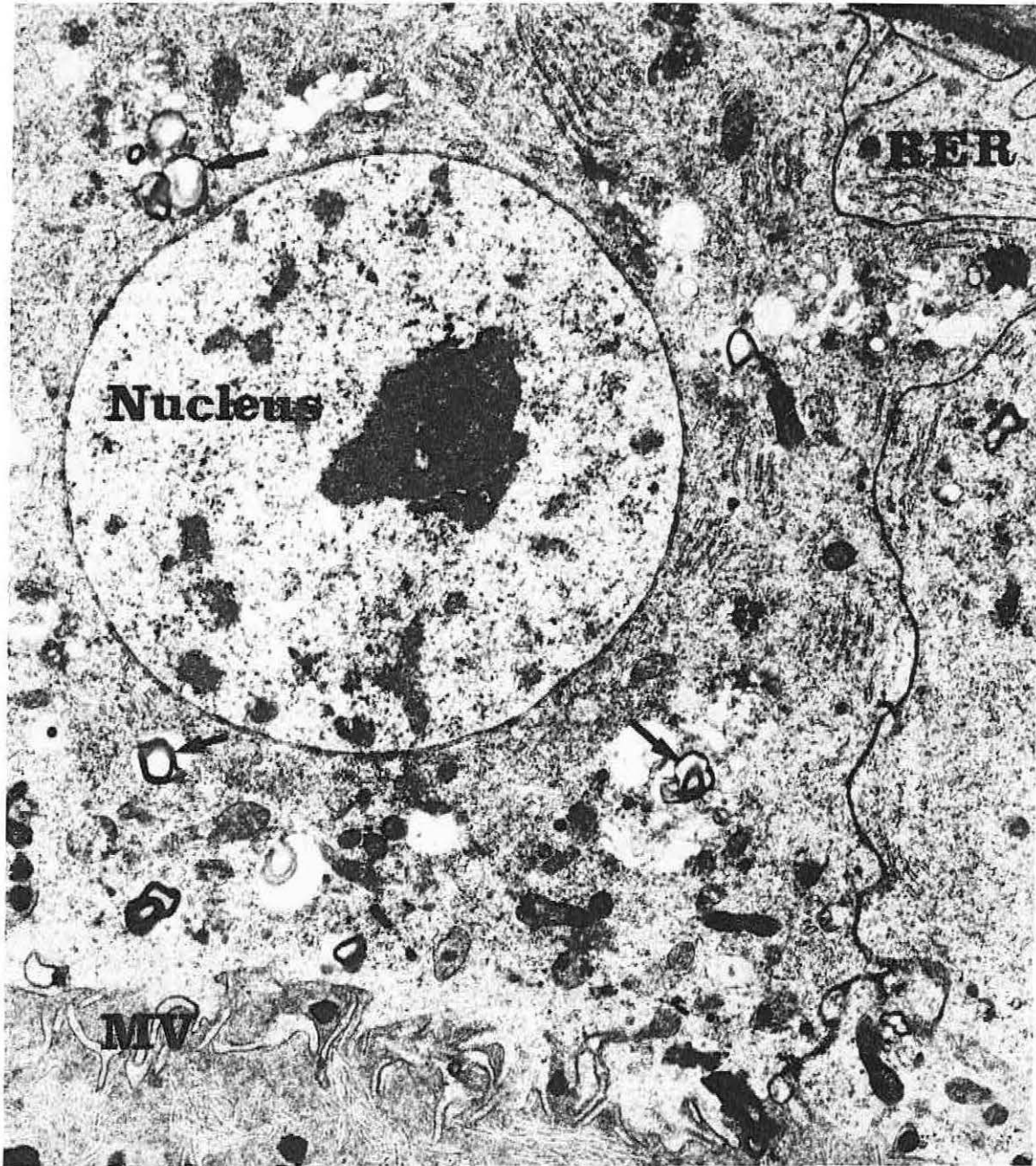


Figure 6.21 An electron micrograph obtained from six-day-old ARG shows the characteristic features of the 1–6 day period. The cytoplasm contains organelles similar to those found in the teneral fly. The basal, middle and apical parts of the cell contain few immature secretory granules with intervening membranous structure (arrow). Electron-dense bodies at the microvillous border (MV) are being exocytosed into the lumen.

similar to those in the secretory vesicles of young glands 1–6 days old. At the microvillous border, the membranous immature secretory products mature into electron-dense bodies in readiness for exocytosis into the lumen (Figure 6.21).

As the male tsetse becomes reproductively mature, the rough endoplasmic reticulum becomes more extensive and there are more ribosomes bound to the cisternae. Free ribosomes and polyribosomes remain abundant. The Golgi complex becomes more distended and gives rise to condensing vacuoles containing electron-dense secretory material. The secretory vesicles fuse to form large vesicles that contain large secretory bodies that have been packaged in the Golgi system. The gland epithelium is greatly flattened

due to the large accumulation of secretory materials in the lumen.

Preliminary attempts have been made to correlate accessory gland development with electrophoretic patterns of proteins. More than 27 protein bands were observed; of these 13 were major protein bands. Secretions of accessory glands and spermatophore each contain a distinct complement of proteins. The accessory glands continued to make the same proteins, except on days 5, 6 and 7, when there was a common distinct band not observed in young glands (0–4 days). Studies on the spermatophore also showed the presence of a protein pattern similar to that at age 5–7.

The present study has shown that the ARG glands are fully differentiated at the time of eclosion. Rapid synthesis of the

secretory products has been observed during the first week of adult life, which corresponds to the period of high reproductive activity of the male flies.

The occurrence of secretion in the gland lumen as early as that in teneral flies suggests that secretory products are found in the gland lumen prior to eclosion.

That secretion from accessory glands of age 5–7 have at least one protein band not found in those of age 0–4 is evidence for age-dependent qualitative and quantitative alterations of the secretory proteins that are significant in spermatophore formation. It is at the age of 5–7 that the glands are particularly active in the synthesis and accumulation of proteins that are transferred during mating as part of the spermatophore.

It appears that, regarding biochemical properties, the male accessory glands from adult flies 0–7 days old exhibit some diversity, such as in their ultrastructural morphology features.

Our plans are to purify and identify by immunological assay the specific protein band observed in five- to seven-day-old adult flies and in the spermatophore.

6.5 STUDIES ON THE EXCRETORY SYSTEMS AND UTERUS IN TSETSE, *GLOSSINA MORSITANS MORSITANS*, AND STABLEFLY, *STOMOXYS CALCITRANS*

J. A. Kongoro

Two major aspects of tsetse (*Glossina morsitans morsitans*) biology investigated were the excretory system comprising the Malpighian tubules, the hindgut rectum and the function of uterine secretion in tsetse reproduction. The Malpighian tubules of the stablefly, *Stomoxys calcitrans*, were also studied. *S. calcitrans*, like tsetse, has been implicated in the transmission of trypanosomes.

6.5.1 Studies on the Malpighian tubules

The objective of the studies on *G. m. morsitans* and *S. calcitrans* tubules was to elucidate functional variations that might exist in the Malpighian tubules of the two haematophagous species that have differences in reproductive behaviour.

Ultrastructure of the Malpighian tubules in G. m. morsitans. Earlier observations (1986 Annual Report) indicate the presence of two cell types—proximal (absorptive) and distal (secretory)—in *G. m. morsitans* Malpighian tubule proximal and distal regions, respectively. Since then, a third cell type—the transitional cell—has been observed in the transitional tubule region. This cell has ultrastructural characteristics similar to those of the distal cell. Autoradiographic observations indicated that the transitional and distal regions had similar patterns of incorporation of (³H)-glucose, thus pointing to similarity in function to the two regions.

Further studies are in progress to elucidate the functions of the enlarged distal region in *G. m. morsitans* Malpighian tubules.

Acid phosphatase and periodic acid Schiff reaction in the Malpighian tubules of G. m. morsitans. The acid

phosphatase and periodic acid Schiff (PAS) tests were used to establish the basic cytochemistry of *G. m. morsitans* Malpighian tubules, which will be used as a reference in elucidating changes in cellular function by observing variations in cellular and intracellular sites of reaction. Cytochemical observations localized acid phosphatase activity in lysosomes multivesicular and some multivesicular bodies in close proximity to Golgi complexes and rough endoplasmic reticulum in the cell cytoplasm of *G. m. morsitans* Malpighian tubules.

Acid phosphatase activity was also localized in the microvillar region of the proximal tubules region. The PAS reaction indicated the presence of polysaccharides with 1,2-glycol groups in the epithelial cell cytoplasm of the Malpighian tubules.

Ultrastructure of S. calcitrans Malpighian tubules. The dorsal pair of the four Malpighian tubules in *S. calcitrans* were enlarged distally. The degree of enlargement was much greater than that in *G. m. morsitans* tubules. As in *G. m. morsitans* tubules, there was regional structural specialization in *S. calcitrans* tubules. There were three cell types—proximal, transitional and distal—that had similar characteristics to their counterpart cells in *G. m. morsitans* Malpighian tubules. The cytoplasm of the distal cells in *S. calcitrans* tubules, however, had more lipid droplets lysosomes and greater elaboration of the lateral and basal cell membranes. These observations imply that there is greater synthetic activity in the Malpighian tubule distal cells of *S. calcitrans* than in those of *G. m. morsitans*.

6.5.2 Ultrastructure of the hindgut and rectum in *G. m. morsitans*

The objective of these studies was to elucidate the involvement of the hindgut in excretion and cellular organization in the rectum in tsetse. The hindgut wall has a simple epithelium of cuboidal cells. The latter, which are apically bordered by a thin cuticle, rest on a thin basal lamina. The lateral membranes form deep, tortuous infoldings, which are associated with mitochondria, thus indicating the involvement of the hindgut cells in fluid and ionic transport.

The principal cells in *G. m. morsitans* rectum are large and columnar and have the structural characteristics associated with fluid and ionic transport. These include an elaborate series of lateral membrane stacks that are closely associated with mitochondria. There are numerous lipid droplets in the cell cytoplasm, indicating that these cells are probably also involved in synthetic activity.

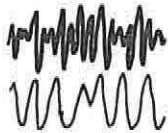
6.5.3 Autoradiographic studies on the uptake of (³H)-glucose by the uterus *G. m. morsitans*

The aim of this study was to label the secretion in the lumen of *G. m. morsitans* uterus in order to trace its contribution in tsetse reproduction. Autoradiographic results show that although the different cell types in the uterus of a two-day-old virgin female *G. m. morsitans* are labelled, the secretion in the uterine lumen is not labelled. This observation implies that the uterine secretion is formed before the incorporation of the radiochemical into the cells of the uterus. Further studies will be conducted on uteri of flies that are younger than two days.

Sensory Physiology Research Unit

- 7.1 Analysis of spike patterns from taste receptors **109**
- 7.2 Attachment and feeding of the brown ear tick, *Rhipicephalus appendiculatus*, on an artificial membrane **109**
- 7.3 *Chilo partellus* sex pheromone system **111**
- 7.4 Electroantennogram (EAG) responses of *C. partellus* to air-borne volatiles from *Sorghum bicolor* **113**
- 7.5 Behavioural and electrophysiological responses of tsetse flies, *G.m. morsitans* and *G. pallidipes*, to various phenols **113**
- 7.6 Antennal responses of tsetse to analogues of the attractant 1-octen-3 ol **114**

7



Sensory Physiology Research Unit

The main responsibility of the Sensory Physiology Research Unit (SPRU) is to carry out collaborative research with ICIPE's research programmes and units, especially in areas concerning sensory biology and the chemical basis of insect behaviour. The techniques employed are designed to study insect behaviour and sensory coding for various environmental stimuli.

*Since the last report, emphasis has been placed on the sex pheromone and host plant stimuli of the stem borer *Chilo partellus* and chemocommunication of tsetse flies *Glossina morsitans morsitans* and *G. pallidipes*. Furthermore, artificial feeding of the brown ear tick, *Rhipicephalus appendiculatus*, was started. This is basically a continuation of the previous year's work. Brief reports on accomplishments in the various areas of work are given below.*

7.1 ANALYSIS OF SPIKE PATTERNS FROM TASTE RECEPTORS

S. M. Waladde, S. A. Ochieng and H. A. Kaharo

The method widely used for spike patterning analysis has been based on filming electrophysiological responses with an oscilloscope camera and then manually counting the spikes as a function of time. Furthermore, spikes from different cells were distinguished visually. This is a tedious and time-consuming process, especially when dealing with large amounts of data. To overcome this problem, automated acquisition and analysis of electrophysiological data has been adopted. In this approach we are using an IBM PC XT computer equipped with a Metrabyte DASH-16 A/D card and a spike analysis programme developed at the University of Alberta and made available to us by Professor B. K. Mitchell. Spike analysis is based on the size of the spikes. Usually different cells will have action potentials which differ in height, width or time constant of the return to the baseline by the negative phase. The parameter most often used to discriminate between spikes is the total amplitude (peak-peak) height.

With the available hardware and software it is possible to acquire and digitize 10 000 data points per 1000 ms. The pattern of activity from the responding cells can be expressed as frequency histograms of spike heights assigned among 25 equal-sized bins. All spike heights in a set of picked spikes are scaled to the largest spike. With this approach, the overall activity evoked by a given stimulus applied on the sensilla of insects may be compared with recording from sensilla treated with different stimuli.

This technique has been used to examine the spike types evoked in *Chilo partellus* and *Eldana saccharina styloconica* sensilla. The stimuli were sucrose and aqueous extract components from Inbred A and MP 704 maize varieties. Earlier observations showed that the dose-response curves to aqueous extracts from the two materials were different, especially in the lateral sensilla (Figure 7.1). This suggested that the receptor cells can detect chemical differences between the two varieties. This was further confirmed by the analysis of frequency histograms (Figure 7.2) using the computer-based program outlined above. The frequency histogram of a given stimulus represents a sample of the input evoked by the stimulus. The central nervous system integrates that input to evoke a particular behavioural response. Therefore a significant difference between the frequency histograms reflecting the effects of susceptible and resistant aqueous extracts (Table 7.1) indicates potential differences in the behavioural responses of the stem-borer larvae to the two maize varieties.

7.2 ATTACHMENT AND FEEDING OF THE BROWN EAR TICK, *RHIPICEPHALUS APPENDICULATUS*, ON AN ARTIFICIAL MEMBRANE

S. M. Waladde, S. A. Ochieng and E. D. Kokwaro

A baudruche membrane was made leak-proof by coating both surfaces with a thin layer of rubber cement. One surface was treated with rubber cement mixed with pieces of rabbit hair giving a rough surface which provided the tactile

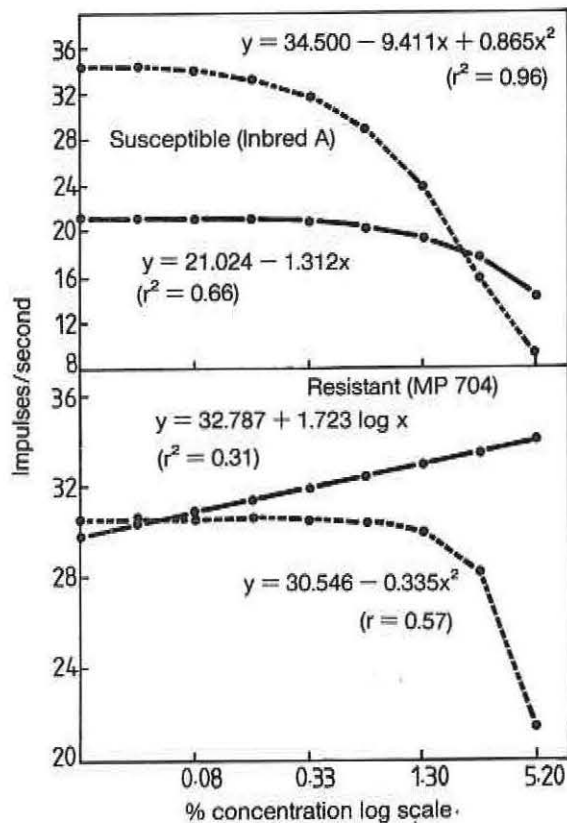


Figure 7.1 Dose-response curves of *Chilo partellus* lateral (solid line) and medial maxillary styloconica sensilla (broken line) stimulated with aqueous extracts from shoots of susceptible (Inbred A) and resistant (MP704) maize varieties.

Table 7.1 Comparison of cumulative spike frequencies for various stimuli (aqueous sucrose, and extracts of susceptible maize and resistant maize varieties)

Treatments	K-S statistic*	Level of significance
Lateral sensillum (<i>C. partellus</i>)		
Sucrose vs susceptible	0.55	$P < 0.001$
Sucrose vs resistant	0.24	ns
Susceptible vs resistant	0.34	ns
Medial sensillum (<i>E. saccharina</i>)		
Sucrose vs susceptible	0.42	$P < 0.01$
Sucrose vs resistant	0.31	$P = 0.05$
Susceptible vs resistant	0.14	ns
Lateral sensillum (<i>E. saccharina</i>)		
Sucrose vs susceptible	0.53	$P < 0.001$
Sucrose vs resistant	0.56	$P < 0.001$
Susceptible vs resistant	0.07	ns

* K-S: Kolmogorov-Smirnov test, ns: not significant.

stimulation necessary for tick attachment. With this surface facing upwards, the membrane was fixed over a reservoir, 520 mm in diameter and 10 mm deep, loosely packed with glasswool. This formed a feeding chamber, the interior membrane surface abutting the glasswool. The chamber had inlet and outlet orifices fitted with 2-mm internal diameter Teflon tubing through which fluids could be circulated. The

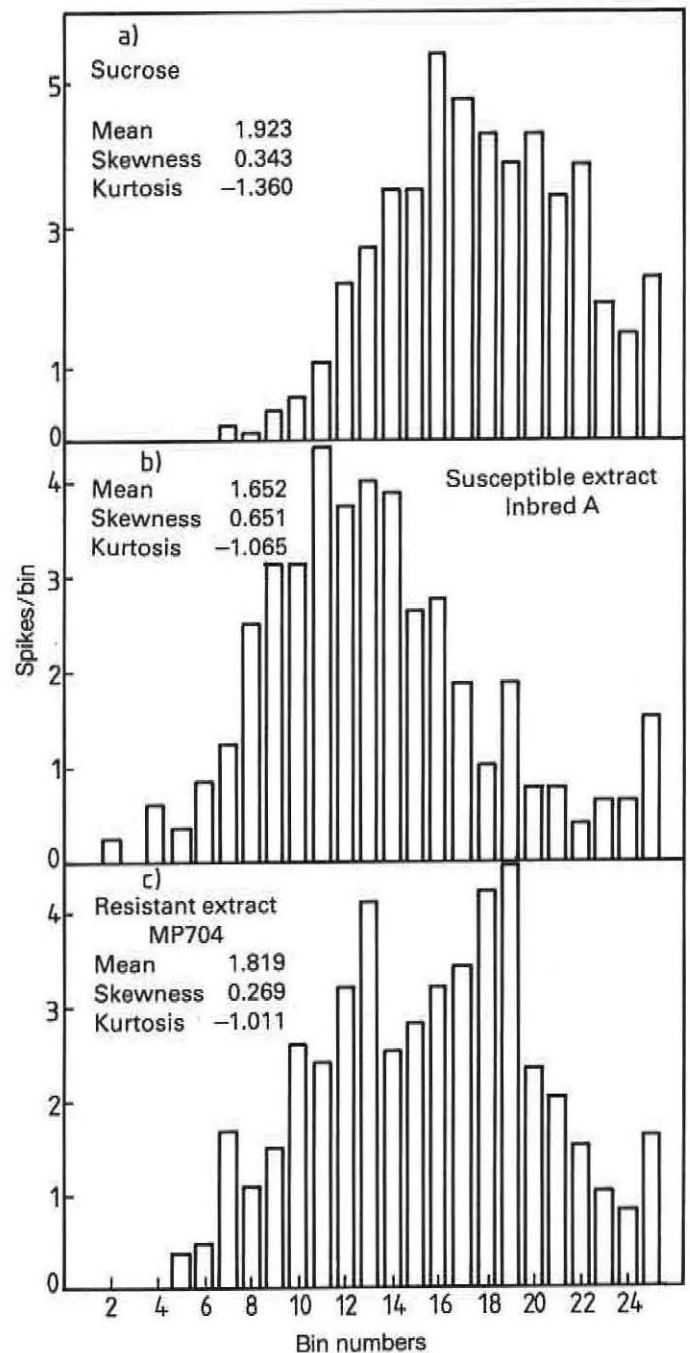


Figure 7.2 Frequency histograms showing different patterns of spike distributions evoked by stimulating the lateral styloconica sensilla of *Chilo partellus* with (a) sucrose and aqueous extracts from (b) Inbred A and (c) MP 704 maize varieties.

assembled feeding chamber was sterilized under an ultraviolet lamp before use. Cattle blood serum was used as the feeding fluid, sterilized by filtering it through a 0.45- μ m millipore filter before it was introduced into the feeding chamber. Before the ticks were placed on it, the external membrane surface was smeared with a concentrated cattle ear-wash, which provided an olfactory stimulus that appeared to arrest tick movement and all the ticks assumed a probing posture. The apparatus was maintained at 35°–37° C, which simulated host body temperature. Adults of *R. appendiculatus* confined on such a membrane were able to attach themselves to the membrane within twelve hours.

Successful attachment is preceded by the secretion of a cement cone in which the hypostome and the cheliceral hood are embedded (Figure 7.3a). The cheliceral digits penetrate the membrane, thus making an opening through which the cement material is deposited on the undersurface of the membrane (Figure 7.3b). The cement thus seals the hole made by the cheliceral digits, preventing the feeding fluid from leaking out. Feeding fluids, if they ooze out of the hole made by the cheliceral digits, can drown a tick. Below the membrane the cement cone has one opening at the position of the cheliceral digits, through which the tick imbibes the fluids (Figure 7.3c).

7.3 CHILO PARTELLUS SEX PHEROMONE SYSTEM

S. M. Waladde and P. G. McDowell

Although the major components of *Chilo partellus* pheromone (*Z*-11-hexadecenal and (*Z*-11-hexadecen-1-ol) were identified in the Indian strain of this insect, no synthetic pheromone blend adequately attracts African *C. partellus* males in the field. This suggests that the available pheromone blend is lacking an important component and is therefore not useful in monitoring *C. partellus* populations in the field.

Our purpose is to re-examine the major and minor chemical components in the sex pheromone gland of *C.*

partellus. We are using a gas liquid chromatograph (GLC) coupled to an electrophysiological recorder. The active compounds identified with this method are bioassayed using electrophysiological and behavioural methods. We present the pheromone gland profiles obtained by GLC and the electroantennogram (EAG) response curves and single cell response patterns to standard pheromone components that we obtained.

7.3.1 Coupled gas liquid chromatography/ electrophysiology

A Hewlett Packard HP 5890A GLC was fitted with an oven capillary column and a flame ionization detector (FID). Hydrogen was used as the carrier gas. The interface between the GLC and the recording apparatus was modelled after that used by the Pheromone Group at the University of Lund, Sweden. It has an effluent splitter with one arm of the tubing connected to the gas chromatograph (GC) detector in the normal fashion, while the other transfer arm was led through the side wall of the oven and oriented in such a way that its effluents were delivered into a glass tube carrying a continuous air stream flowing directly to the insect preparation.

The design ensured the simultaneous arrival of equal volumes of the effluent components at the FID and the male moth antenna preparation. EAG responses and signals from the FID were recorded simultaneously. Standard electrophysiological procedures using a Grass P16 AC/DC

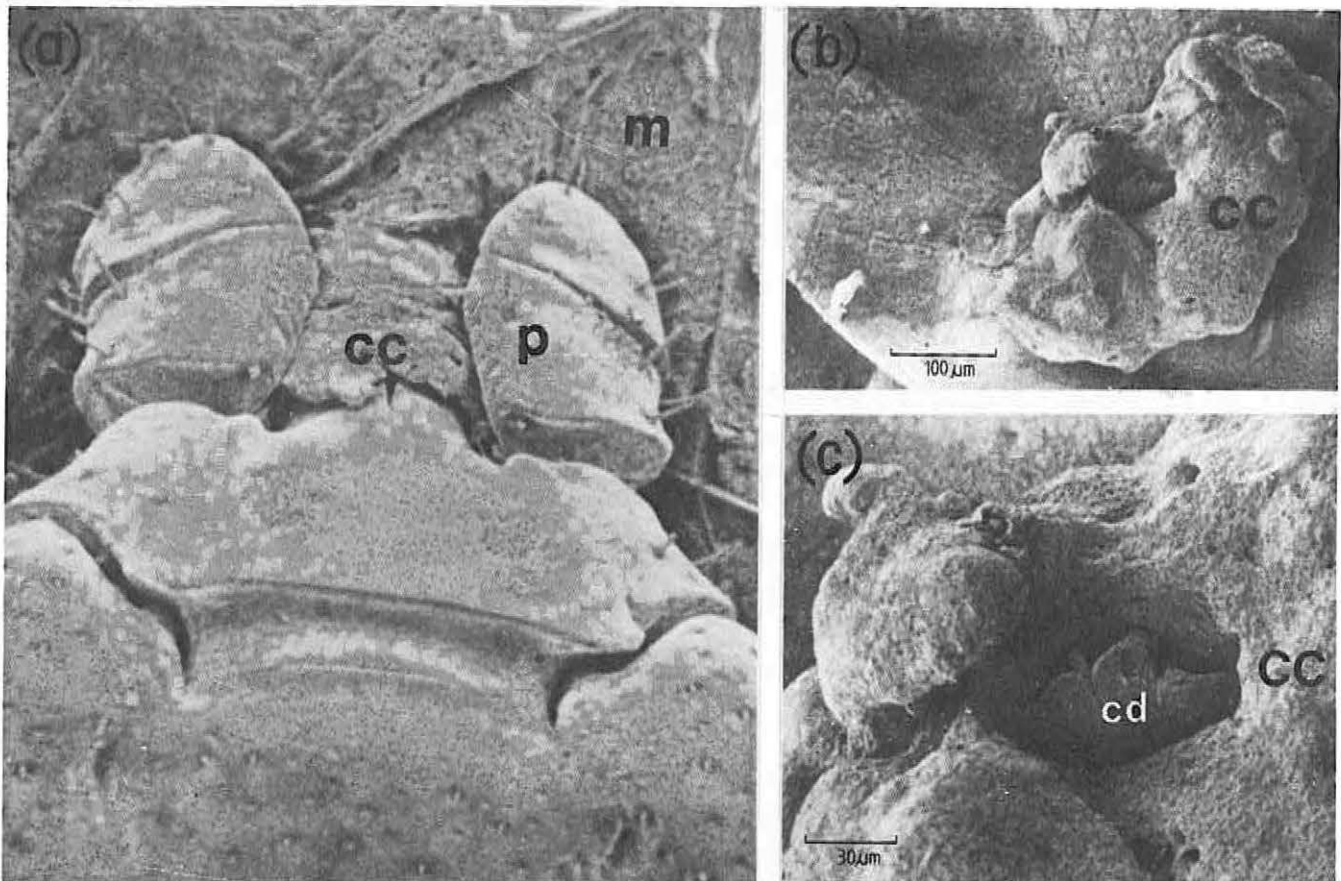


Figure 7.3 Attachment of *Rhipicephalus appendiculatus* female to artificial membrane. (a) Dorsal view: chelicerae penetrating the membrane (m), are covered with cement cone (cc) deposited between palps (p). (The broad horizontal structure, the basis capituli, is 0.46 mm wide.) (b) Undersurface of membrane showing cement cone plaque with a well-defined lumen for fluid uptake. (c) Enlarged view of cement cone with tips of cheliceral digits (cd) protruding through membrane.

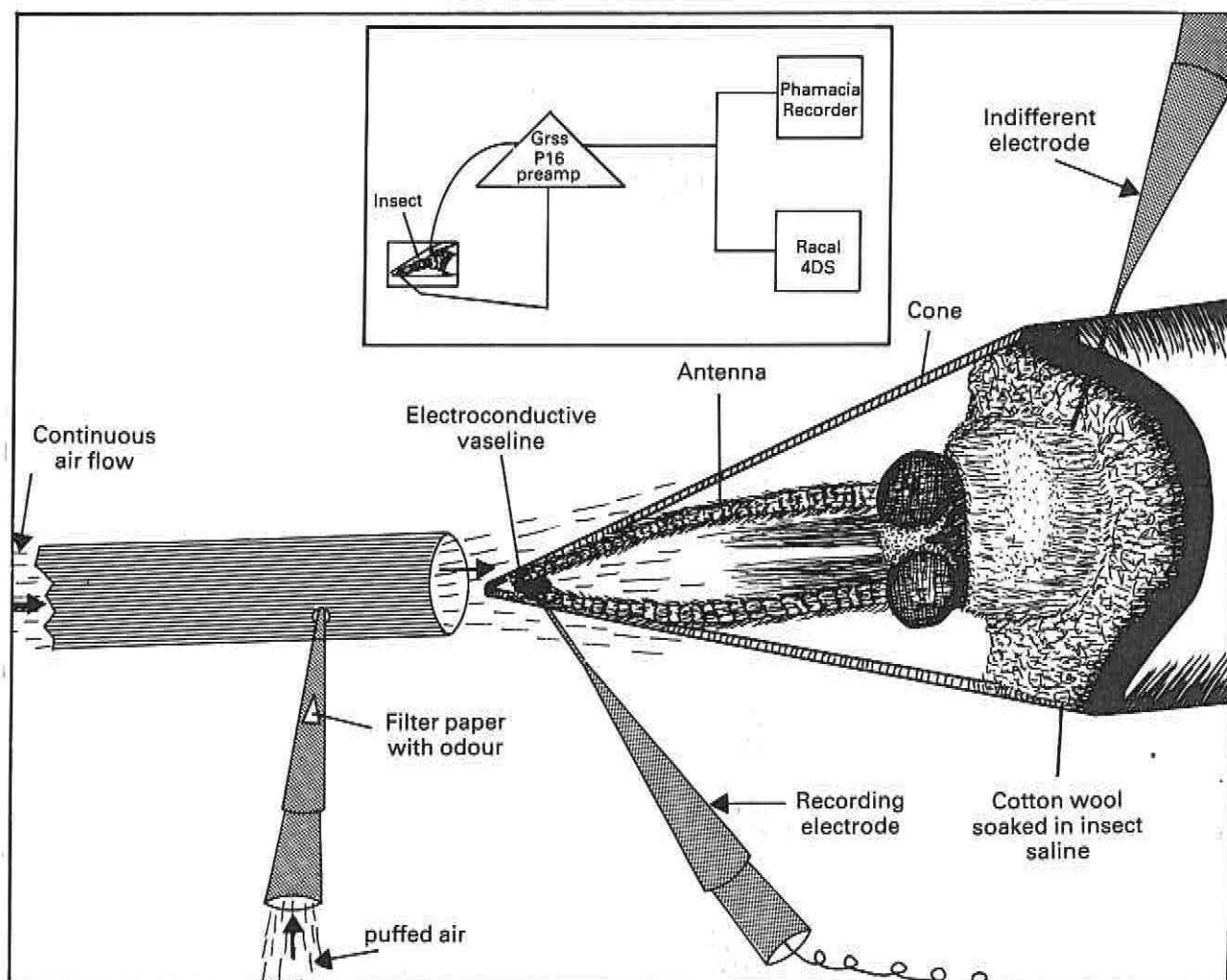


Figure 7.4 Antennal preparation and accessories used in recording EAG.

preamplifier were employed to obtain and amplify the EAG responses from the antennal preparation.

7.3.2 EAG and single-cell responses

This apparatus was used to assay several pheromone standards and also *C. partellus* pheromone gland extracts. The 'puffing' method for measuring EAG responses to standard pheromone components was used by blowing various concentrations of the test compounds directly into the airstream directed towards the antenna (Figure 7.4).

The compounds with detected EAG activity are listed in Table 7.2. Some, especially numbers 2, 3, 5 and 6, were

identifiable in the pheromone gland extract. EAG response curves have confirmed that number 5, (*Z*)-11-hexadecenal, is a better stimulant than number 6, (*Z*)-11-hexadecen-1-ol, and is a major component evoking the best EAG responses. This agrees with observations made by other workers on the Indian strain of *C. partellus*.

Electron microscopy studies showed that the pheromone sensitive sensilla (Figure 7.5a) has two receptor cells (Figure 7.5b) that adapt fairly rapidly when stimulated with the appropriate pheromone component. One cell is sensitive to the alcohol component and the other is sensitive to the aldehyde component (Figure 7.5c).

The difference between the pheromones of the African and the Indian *C. partellus* may be in the minor components observed in the GC profiles. Work on the identification of those components is in progress.

Table 7.2 Compounds with which electroantennogram activity were detected using preparations of male *Chilo partellus* antennae

Compound	Structural notes
(<i>Z</i>)-9-tetradecanal	Z9-14: aldehyde
(<i>Z</i>)-7-hexadecanal	Z7-16: aldehyde
(<i>Z</i>)-9-hexadecanal	Z9-16: aldehyde
(<i>Z</i>)-13-octadecanal	Z13-18: aldehyde
(<i>Z</i>)-11-hexadecanal	Z11-16: aldehyde
(<i>Z</i>)-11-hexadecen-1-ol	Z11-16: OH

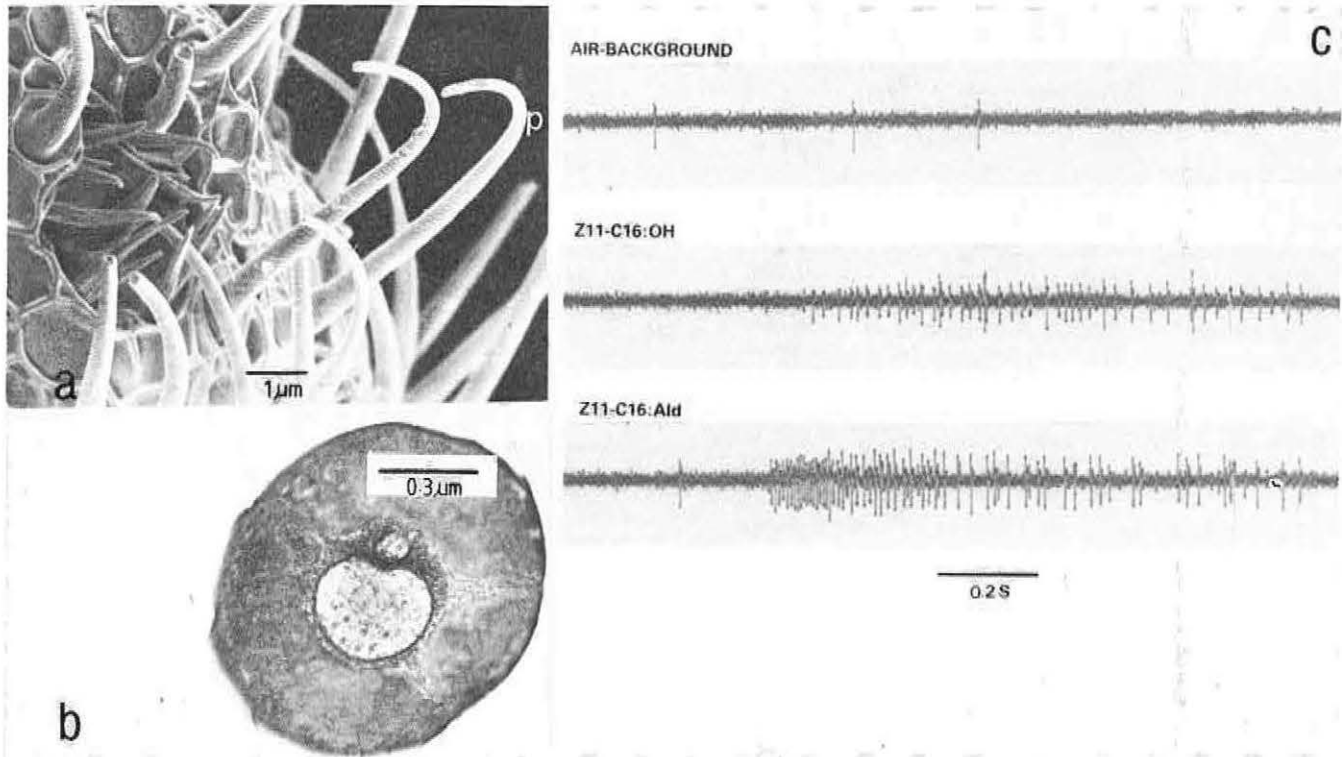


Figure 7.5 Electroantennogram responses of *Chilo partellus* males. (a) Sensilla (b) section of sensillum (c) single cell responses from grooved basiconica sensilla, innervated by two receptor cells. Background (top), response to the alcoholic component (middle) and to the aldehyde (lower).

7.4 ELECTROANTENNOGRAM (EAG) RESPONSES OF *C. PARTELLUS* TO AIR-BORNE VOLATILES FROM *SORGHUM BICOLOR*

S. M. Wallade and W. Lwande

The details of the chemistry done in this study are given in the Chemistry and Biochemistry Research Unit Chapter of this Report. Here we report the effectiveness of the compounds isolated in inducing EAG responses in both male and female *C. partellus* antennae. It was observed that the males are more sensitive to the plant odours. Furthermore, those compounds found in low concentrations in the plant evoked the best EAG responses (Figure 7.6).

7.5 BEHAVIOURAL AND ELECTROPHYSIOLOGICAL RESPONSES OF TSETSE FLIES, *G. M. MORSITANS* AND *G. PALLIDIPES*, TO VARIOUS PHENOLS

R. K. Saini and A. Hassanali

The most active fraction of buffalo urine, a potent attractant for *Glossina* species, is composed of seven phenolic compounds (Table 7.3). Behavioural (antennal movement) and electrophysiological (electroantennogram) experiments were undertaken to determine the relative importance of these phenols in conferring activity to the blend. The most stimulating of the seven phenols was 4-cresol which elicited responses ranging from 1.5 to 4.0 times that of the control.

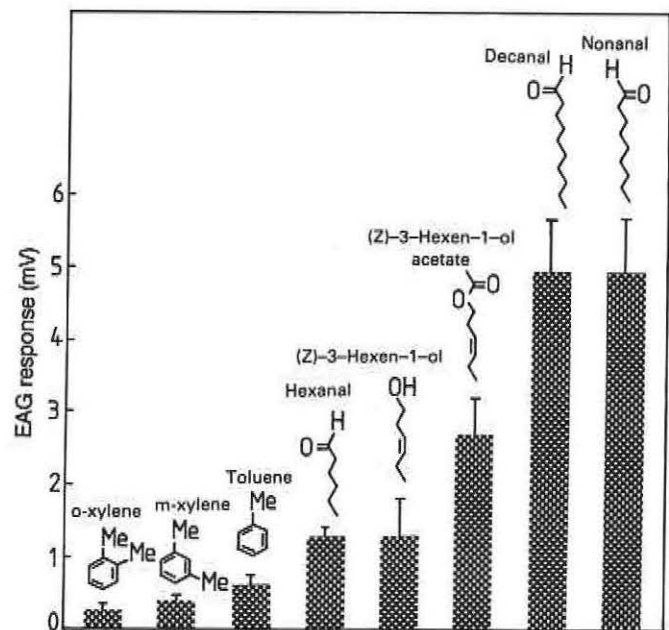


Figure 7.6 Histograms showing effectiveness of odour components in air-borne volatiles from four-week-old *Sorghum bicolor* plants in eliciting EAG responses of *Chilo partellus*. Bars = ± 1 S.E.

With increasing concentration, responses of *G. m. morsitans* to 4-cresol increased, while those of *G. pallidipes* decreased. On the other hand, 3-cresol elicited a response about twice that of the control in both species.

Table 7.3 Comparison of the mean percentages of antennal behavioural responses of tsetse flies to seven phenols tested

<i>G. m. morsitans</i>		<i>G. pallidipes</i>	
Chemicals tested	Mean % response	Chemicals tested	Mean % response
4-cresol	22.85 ^a	4-cresol	22.18 ^a
3-cresol	13.11 ^b	3-propylphenol	17.01 ^b
3-propylphenol	12.08 ^b	4-ethylphenol	16.96 ^b
3-ethylphenol	11.56 ^b	3-cresol	16.06 ^b
4-ethylphenol	10.21 ^{bc}	3-ethylphenol	14.71 ^{bc}
4-propylphenol	7.70 ^c	4-propylphenol	12.08 ^c
Phenol	7.50 ^c	Phenol	8.86 ^d

Means in a column followed by a different superscript are significantly different $P < 0.05$.

Both species were the least stimulated with phenol itself. In fact there was no significant change in response to dose in *G. m. morsitans* and only a slight (two-fold) response in *G. pallidipes*.

Electroantennograms (EAGs) also provide an insight into the sensitivity of olfactory receptors to odours. This was well illustrated by the steadily increasing response of a male *G. m. morsitans* to increasing doses of 4-cresol.

EAG responses to equimolar concentrations of these phenols indicate that, as in the behavioural studies, 4-cresol was more stimulatory than 3-cresol. *G. m. morsitans*, however, gave EAGs with significantly larger amplitudes than *G. pallidipes* did, indicating that the sensitivity of the two species to 4-cresol differs. 3-propylphenol was significantly more stimulatory than 4-propylphenol in both species and was the most stimulating of all the phenols for *G. pallidipes*. Both ethylphenols were equally stimulatory for *G. m. morsitans*, but 3-ethylphenol was significantly more active than 4-ethylphenol for *G. pallidipes*. EAG responses to phenol differed between species, and it was the least stimulatory to *G. pallidipes*, although in *G. m. morsitans*, EAG amplitude increased with concentration.

Electrophysiological responses at the receptor level support antennal behavioural responses and confirm that the responses to various phenols differ between species. Our results also suggest that 4-cresol and 3-propylphenol may be important in conferring activity to the blend. Further investigations of the responses of tsetse flies to various odours are in progress in a flight tunnel. We hope this will give us a better understanding of the type and interplay of responses to individual chemicals and their combinations. Such studies may suggest the means by which odours may be used more effectively.

7.5.1 Chemical communication in tsetse flies

Our behavioural and electrophysiological investigations of the role of various chemical stimuli in tsetse behaviour have continued in collaboration with the Chemistry and Biochemistry Research Unit and the Tsetse Research Programme.

7.6 ANTENNAL RESPONSES OF TSETSE TO ANALOGUES OF THE ATTRACTANT 1-OCTEN-3-OL

R. K. Saini, A. Hassanali and R. D. Dransfield

Investigations of structure-activity relationships of bioactive compounds have proved to be important for understanding the mechanism of odour perception and for designing and synthesizing new potent chemicals. Antennal responses of male *G. m. morsitans* to 12 analogues of the tsetse attractant 1-octen-3-ol (Figure 7.7) were investigated with two major objectives in mind: (1) to study the effect of the length of the hydrocarbon chain on activity in a homologous series of seven alcohols (see Figure 7.7a) and (2) to study the effect of variations on the functional end of the parent kairomone molecule in six related compounds (Figure 7.7b).

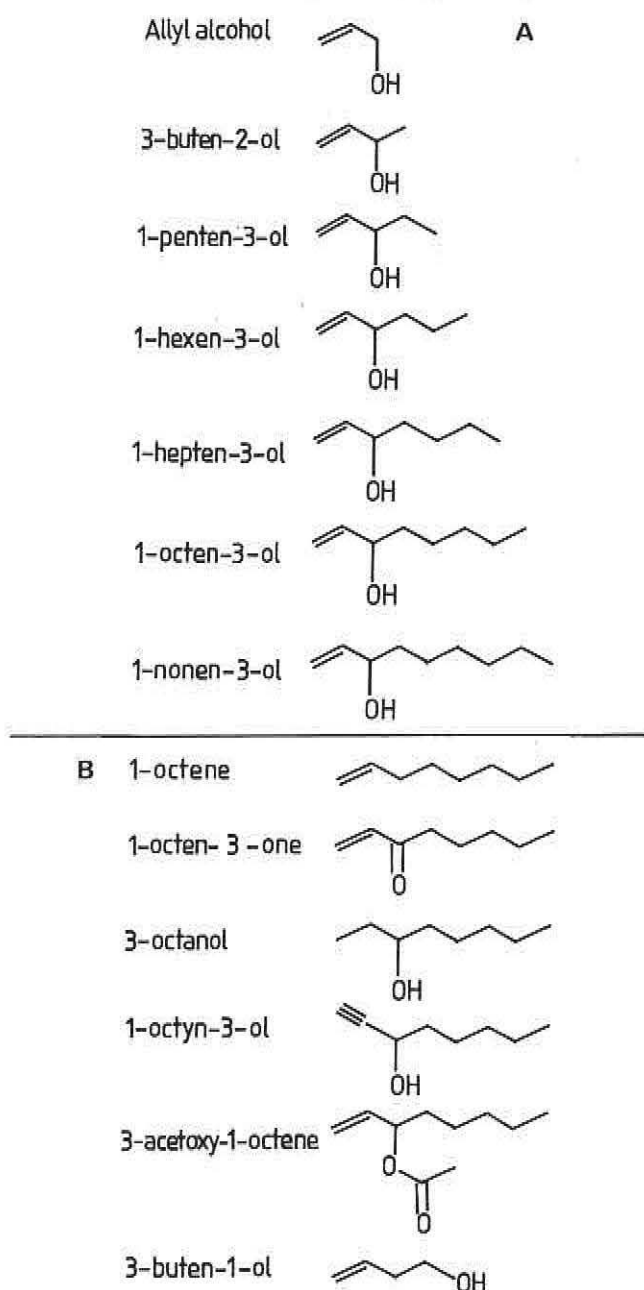


Figure 7.7 Chemical structures of 1-octen-3-ol analogues tested; (a) for the effect of chain length on activity and (b) for the effect of variations in the functional end of the parent kairomone molecule.

When the antennal behavioural responses to the compounds were tested in relation to the control, the mean percentage of male response to the control was $14.4 \pm \text{SE } 0.23$. The various analogues tested evoked antennal responses decreasing in the order shown in Table 7.4. These results indicate that activity was dependent on the length of the alkyl chain and that homologues with odd-numbered carbon alkyl chains, such as 3-buten-2-ol, 1-hexen-3-ol and 1-octen-3-ol, evoked higher antennal responses than homologues with even-numbered alkyl chains, such as 1-nonen-3-ol, 1-hepten-3-ol and 1-penten-3-ol.

A comparison of the activities of the 8-carbon structural variants with 1-octen-3-ol (Table 7.4) showed that the structural requirements for activity of the functional end of the molecule may not be very rigid. Thus 1-octyn-3-ol elicited significantly more responses than 1-octen-3-ol. Acetylation of the hydroxyl group of 1-octen-3-ol to 3-acetoxy-1-octene, or its oxidation to 1-octen-3-one (Table 7.5), did not lead to any significant change in activity. On the other hand, significantly lower responses to 1-octene and 3-octanol showed that both the double bond as well as the oxygen function were important for eliciting high antennal responses.

Table 7.4 Comparison of overall mean percentages of antennal behavioural responses (response to test chemical—response to control) to nine test chemicals for male *G. m. morsitans*

Chemicals tested	Mean % response
1-octyn-3-ol	19.50 ^a
3-buten-2-ol	17.90 ^{ab}
3-acetoxy-1-octene	17.02 ^{abc}
3-buten-1-ol	15.38 ^{bc}
1-octen-3-ol	14.62 ^{cd}
1-hexen-3-ol	12.25 ^{de}
1-nonen-3-ol	10.40 ^{ef}
1-hepten-3-ol	10.28 ^{ef}
1-penten-3-ol	8.77 ^f

Means followed by a different superscript are significantly different $P < 0.05$.

High responses to some of the octenol variants discussed indicate that the chemoreceptors involved in the perception of this set of kairomones may not be highly specific. This is

Table 7.5 Comparison of mean percentages of antennal behavioural responses (response to test chemical—response to control) to 10 μl of six structural analogues of 1-octen-3-ol

Chemicals tested	Mean % response
1-octen-3-one	24.27 ^a
1-octen-3-ol	20.67 ^a
allyl alcohol	18.75 ^a
3-acetoxy-1-octene	18.48 ^a
3-octanol	13.36 ^b
1-octene	13.20 ^b

Means followed by a different superscript are significantly different at $P < 0.05$.

further borne out by the comparison of responses to 3-buten-2-ol and 3-buten-1-ol. In the former the hydroxyl and unsaturated groups are in the same relative positions as in 1-octen-3-ol; while in the latter they are separated by a methylene bridge (Figure 7.7). However, the differences in the activities of the two compounds were not statistically significant at all the four doses tested.

Earlier studies on antennal responses of tsetse to various synthetic and excretory products showed that the antennal responses observed in laboratory bioassays correlated well with field observations, since chemicals that elicited antennal responses 2–3 times more than the control also increased trap catches in the field. In the present investigation, 1-octyn-3-ol, 3-buten-2-ol, allyl alcohol and 1-octen-3-one evoked antennal responses that were about 2.5 times more than that evoked by the control, suggesting that these compounds might have attractive properties. Preliminary field trials at Nguruman, in which some of these chemicals were tested using blue and white biconical traps, showed that 3-buten-2-ol and allyl alcohol, dispensed at 10 $\mu\text{g/h}$, significantly increased the catches of male *G. pallidipes* with an index of increase of 1.8 \times . Catches of females with these chemicals showed the same trends. However, when 3-buten-2-ol was tested in conjunction with acetone and cow urine using NG2B traps, it did not give a significant increase in catch, but an optimal dose may not have been used. It would be useful to determine whether some of the other more potent analogues identified in this study would be useful additives or substitutes for attractants currently in use.

Biostatistics and Computer Services Unit
8.1 Accomplishments 119

8



Biostatistics and Computer Services Unit

8.1 ACCOMPLISHMENTS

S. Nokoe

In 1987 the Biostatistics and Computer Services Unit (BCSU) was given a mandate to provide statistical modelling services to ICIPE's scientists and specialists in the African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests, to provide training in database management and analytical methodology for the interpretation of data and to develop ICIPE's capacity to use remote-sensed data and process geo-coded information.

8.1.1 Staff movement

Two Senior Research Scientists, a Computer Engineer and a Graphics Technician were recruited this year. One scientist was assigned to BCSU (for environmental monitoring and modelling) and one to the Livestock Ticks Research Programme (for tick population modelling). One Senior Scientist was posted to ICIPE's Mbita Point Field Station to strengthen biostatistics and computer service activities there, as well as to provide input in crop-pest modelling.

8.1.2 VS80 computer

The VS80 computer was moved from Duduville to Chiromo, where our main work was to maintain applications already installed on the computer. Resource constraints and lack of back-up storage on the VS80 meant system maintenance took more time than normal. Files had to be re-organized more often than normal and paging and memory use had to be regularly monitored.

8.1.3 Microcomputers

Use of the VS80 decreased during the year with the

commissioning of the first batch of IBM-compatible microcomputers. Altogether, 20 computers as well as peripheral equipment, such as dot matrix and laser printers, an optical scanner and logical connections, were installed and commissioned. A computer-operated device using a polaroid palette for the production of slides was commissioned in the latter part of the year.

8.1.4 Short-term courses

Courses were conducted in 1987 introducing staff and students in ICIPE's African Regional Postgraduate Programme in Insect Science to the microcomputers, to the Lotus 1-2-3 software program and to an introductory version of SAS, a statistical software program. By the end of the year almost 100 scientists, technicians and administrative staff had attended these courses, which will continue into 1988, when more advanced courses in SAS, MS-Word and dBase will be offered.

Two students from the Nairobi Polytechnic were given an industrial attachment in computer science and programming in BCSU under the supervision of the Senior Application Specialist (Computer Programmer).

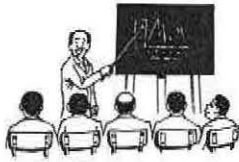
8.1.5 Electronic mail

BCSU installed a computer link between ICIPE's Mbita Point Field Station and the Chiromo Headquarters. The link-up was active for two hours of each day and was used primarily to despatch messages and documents. At the same time, ICIPE became part of a world-wide computer mail network operated by CGNET. This network caters for international agricultural research centres, donor agencies and individuals active in international agricultural work.

Outreach and Training Unit

- 9.1 Overview of activities **123**
- 9.2 CORD: Collaborative Research and Development **124**
- 9.3 PESTNET: African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests **124**
- 9.4 ARPPIS: African Regional Postgraduate Programme in Insect Science **125**
- 9.5 Postdoctoral research fellows **127**
- 9.6 FAMESA: Special Project on Financial and Administrative Management of Research Projects in Eastern and Southern Africa **127**
- 9.7 IPTIS: International Programme for Specialized Short-Term Training in Insect Science **127**
- 9.8 In-service training **129**
- 9.9 Staff development training **129**

9



Outreach and Training Unit

The mandate of the Outreach and Training Unit (OTU) is to strengthen the scientific leadership and technological capability of tropical developing countries in applied insect science through collaborative research and training programmes. The long-term aim of OTU is to establish sustainable, collaborative links and facilitate the process of strengthening national scientific capabilities that will ensure effective use of insect science for the improvement of human health and food production levels in the tropics.

9.1 OVERVIEW OF ACTIVITIES

Z. M. Nyiira

In 1987 ICIPE continued to exchange information and material with international agricultural research centres and universities throughout the world.

The Centre increased its collaborative work with other institutions and explored new collaborative ventures with the Food and Agriculture Organization of the United Nations, the International Atomic Energy Agency and the Overseas National Development Research Institute. Invitations for collaborative research and development work were received from various countries, including the Philippines, India, Mozambique, Guinea Bissau, Guinea (Conakry) and Mexico, stronger links were made with the International Rice Research Institute and collaborative work with the International Institute of Tropical Agriculture continued.

Collaborative research is being conducted at ICIPE by two Ivorian scientists and collaborative agreements were signed with the Philippine Council for Agriculture and Resources Research and Development (the Philippines) and Zambia's Ministry of Agriculture and Water Development. During the year ICIPE also increased its involvement in the activities of institutions associated with the development of third world science and technology and, more specifically, institutions associated with insect science and its application, such as the Third World Academy of Sciences and the African Association of Insect Scientists.

Training activities during the year fell under the African Regional Postgraduate Programme in Insect Science (ARPPIS), collaborative graduate studies for a Master of Philosophy in biological control, short-term specialized and general group training courses, in-service training, research associateships and postdoctoral research fellowships. The graduate studies programme was strengthened by

recommendations of a special task force mandated to review its structure and future focus as well as by the excellent performance maintained by the students enrolled in ARPPIS. Four integrated group training courses were given during the year to participants who came from around the world, and responses to ICIPE's offer of postdoctoral research fellowships from scientists from non-African countries increased.

The special project on Financial and Administrative Management of Research Projects in Eastern and Southern Africa (FAMESA) suffered a minor setback when its Coordinator left in the early part of the year, but the project achieved much in the year, even so. The draft for a fourth FAMESA training manual on project planning, monitoring and control was completed and approved and arrangements were planned for future national training workshops in research and development management.

OTU's relations with funding agencies, particularly with the International Development Research Centre and the Ford Foundation, remained excellent.

The African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests (PESTNET) was successfully established along the recommendations of the International Working Group on the Implementation of PESTNET. Activities were started in Kenya, Rwanda and Zambia, while Somalia continued to benefit from PESTNET training. The Network's newsletter, *PESTNET TODAY*, gained a wider readership both in and out of the PESTNET zone.

Several changes occurred in the personnel of OTU. Dr. C. P. F. De Lima, Head of the Unit, and Dr. Luka O. Abe, the FAMESA Coordinator, left the Unit this year. They were replaced by Dr. Z. M. Nyiira (Head of Unit) and Dr. V. O. Musewe (FAMESA Coordinator). Later in the year, Dr. E. O. Omolo was made PESTNET Scientific Coordinator and

Dr. Z. M. Nyiira took over as FAMESA Coordinator in addition to his role of Head of the Unit.

9.2 COLLABORATIVE RESEARCH AND DEVELOPMENT (CORD)

Z. M. Nyiira

Interactive research and development work was maintained with the Centre's traditional collaborators, including universities and research centres in Europe and North America and other institutions belonging to the Consultative Group on International Agricultural Research, such as the International Institute of Tropical Agriculture, the International Rice Research Institute, Centro Internacional de Mejoramiento de Maiz y Trigo, the International Laboratory for Research on Animal Diseases, the International Livestock Center for Africa and the International Crops Research Institute for the Semi-Arid Tropics, as well as related institutions, such as the International Council for Research in Agroforestry.

An agreement was reached between ICIPE and the Philippine Council for Agriculture and Resources Research and Development, in the Philippines, for collaborative research, development and training. ICIPE and the International Rice Research Institute also signed a new collaborative agreement that will facilitate further cooperation between the two institutions.

An exploratory mission consisting of two ICIPE officials visited Guinea Bissau in May to examine the national research system, identify areas of possible collaboration and assess what role ICIPE might play in strengthening that country's national pest management research and agricultural extension systems. ICIPE's officials also visited Ethiopia and Tanzania to talk with national officials and explore the possibilities of collaborative research on tsetse and banana weevils, respectively.

Requests were received from Mozambique and Malawi for collaborative work in the crop and livestock sectors. This work will fall under PESTNET, since these two countries are members of the Network.

New proposals were presented for collaborative projects to develop technology for the integrated pest management of stem borers of staple cereal crops (the Food and Agriculture Organization) and of tsetse in the Kagera River Basin (United Nations Development Programme/Economic Commission for Africa). ICIPE and the Overseas Development National Research Institute discussed possible collaboration on an investigation of crop losses in cereals due to stem borers in the Sudan and Kenya. A project sponsored by the Economic Commission for Africa/ICIPE/Kenya Government, at Oyugis, in Kenya's South Nyanza District, continued for a third year to demonstrate an effective interactive approach for technology dissemination. The joint effort of the African Biosciences Network, the International Development Research Centre, the United Nations Environment Programme, the United Nations Development Programme and ICIPE continued to provide support for the African Association of Insect Scientists, the institution behind the current ICIPE-supported bimonthly journal,

Insect Science and Its Application. Finally, the International Atomic Energy Agency and the European Economic Commission continued to support collaborative projects on training in the use and safe handling of radioisotope material and on a group training course on tsetse and tick ecology, respectively.

9.3 AFRICAN REGIONAL PEST MANAGEMENT RESEARCH AND DEVELOPMENT NETWORK FOR INTEGRATED CONTROL OF CROP AND LIVESTOCK PESTS (PESTNET)

Z. M. Nyiira

PESTNET activities were consolidated in 1987. Formal government commitment to participate in PESTNET was received from the government of the Republic of Zambia, in addition to the longstanding agreement between ICIPE and the Democratic Republic of Somalia. An exploratory mission visited Rwanda in October to follow up the interest expressed by that country in collaborating with ICIPE in pest management research and training, to develop a framework for the collaboration and to assess what role ICIPE will play in the strengthening of national pest management research and extension systems. Other member countries continued to provide positive support to the Network. Sudan appointed a national PESTNET Coordinator. All other member countries maintained link persons to take charge of PESTNET tick, tsetse and crop activities in their countries and to coordinate in-country research and development programmes for the Network.

Mozambique and Ethiopia both asked to collaborate in PESTNET activities and extensive discussions were held between ICIPE and the Overseas Development National Research Institute on a proposed collaborative project to estimate crop losses.

9.3.1 Publications

The organization's newsletter, *PESTNET TODAY*, a vehicle for the exchange of information and experiences among the Network scientists and institutions, was published on schedule. A number of documents were also prepared by the Secretariat. These include *PESTNET Profile*, *Progress Report*, *Proceedings of the First Annual Conference of the African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests (PESTNET)*, *Report of the ICIPE Mission to Zambia to Explore Areas of PESTNET Collaboration and Mode of Implementation of the Network's Activities in Zambia*, *The African Regional Pest Management Research and Development Network (PESTNET): Priorities and Programme and Proceedings of the National PESTNET Workshop on Methodology for On-Farm Evaluation of Maize Stem-Borer Infestation and Damage*.

9.3.2 Training

The first Cooperative National PESTNET Training Workshop on Methodologies for Assessment of Stalk-Borer Infestation and Damage Levels on Maize took place in Zambia in June. Under the Network Research Associate Scheme, the first candidate was selected from Zambia for a

tenure of three months with ICIPE's Crop Pests Research Programme at Mbita Point Field Station. A Master of Science candidate from Somalia, registered with Sokoine University of Agriculture and funded by the Canadian International Development Research Centre, successfully completed his training.

For short-term training, invitations were sent to PESTNET member countries for an International Group Training Course in August. An in-service training course was held from April to June for two technical officers from Uganda's Crop Research Division.

9.4 THE AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE (ARPPIS)

M. E. Smalley

We reported last year that ARPPIS now includes two programmes: a Ph.D. programme in the insect sciences, organized jointly by ICIPE and 14 African universities, and a Master's degree programme specializing in the biological control of agricultural pests and run in conjunction with Rivers State University of Science and Technology (RSUST), Nigeria. The latter is a special ICIPE activity financed through the Africa-wide Biological Control Programme. During 1987 both degree-training programmes continued, and since ARPPIS began in 1983, a total of 50 students (40 Ph.D. and 10 M.Phil.) have registered for degree studies.

ICIPE believes these degree studies are an extremely important vehicle for training scientists to do independent and original research and to become leaders in science.

In March the fifth ARPPIS Ph.D. class began its studies. The nine scholars came from five countries: Chad, Kenya, Sierra Leone, Uganda and Zambia. The 40 Ph.D. students within ARPPIS have come from a total of 12 African countries (Table 9.1). The third M.Phil. class registered at RSUST in October. The Master's degree students complete

Table 9.1 Ph.D. scholars in the African Regional Postgraduate Programme in Insect Science (ARPPIS)

	1983	1984	1985	1986	1987	Total
Chad	—	—	—	—	1	1
Ethiopia	—	—	1	—	—	1
Ghana	—	1	2	—	—	3
Kenya	3	3	2	4	5	17
Malawi	—	1	—	—	—	1
Nigeria	—	—	1	1	—	2
Sierra Leone	—	—	—	—	1	1
Sudan	2	1	—	—	—	3
Tanzania	1	—	—	2	—	3
Uganda	2	2	—	1	1	6
Zaire	—	—	1	—	—	1
Zambia	—	—	—	—	1	1
Total	8	8	7	8	9	40

two semesters of coursework at RSUST, in Port Harcourt, Nigeria, before travelling to ICIPE to undertake a 12-month research project in the Crop Pests Research Programme.

Thus, as the third class began its coursework, members of the second class arrived at ICIPE to begin their research. The ten M.Phil. students in the programme have come from six countries (Table 9.2).

Table 9.2 M.Phil. scholars in ARPPIS

	1985	1986	1987	Total
Cameroon	—	—	1	1
Kenya	—	2	—	2
Nigeria	2	1	—	3
Sierra Leone	—	—	2	2
Tanzania	—	—	1	1
Uganda	—	1	—	1
Total	2	4	4	10

In 1987, four Ph.D. and two M.Phil. students successfully defended their theses in their registering universities, bringing to a total of eight the number of students who have been awarded degrees through ARPPIS. Details of the students examined during the year are given in Table 9.3.

The 1987 Ph.D. class completed the compulsory and examined ARPPIS semester of six courses. Professor R. Kumar, RSUST, and Dr. R. Bagine, National Museums of Kenya, taught 'Insect Taxonomy'; Professor El Amin El Rayah, University of Khartoum, Sudan, taught 'Insect Functional Morphology'; Dr. J. Allotey, RSUST, together with Dr. S. K. Firempong and Dr. C. M. Mutero, both of ICIPE, taught 'Insect Ecology'; Dr. R. Dransfield, ICIPE, taught 'Biostatistics' and ICIPE scientists with Professor L. Riddiford and Professor J. Truman, all coordinated by Dr. M. F. B. Chaudhury, ICIPE, taught 'Insect Physiology and Biochemistry'.

After the members of the 1987 Ph.D. class completed their coursework, they began their research projects supervised jointly by ICIPE and university scientists. Because the research is undertaken wholly within ICIPE, the ICIPE scientists are responsible for the day-to-day supervision of the students. The 1987 class members joined their ARPPIS colleagues from the 1985 and 1986 classes in the ICIPE research programmes and units (Table 9.4).

ARPPIS encourages and supports visits to ICIPE by the university supervisors of ARPPIS students. In 1987 we welcomed the supervisors of fifteen Ph.D. scholars: Professor C. Baliddawa, Makerere University, supervisor of Mr. M. Ogenga-Latigo (1984 class) and Mr. E. Karamura (1986); Professor W. Z. Coker, University of Ghana, supervisor of Miss D. A. Adabie (1984) and Mr. C. Kyorku (1985); Professor R. Kumar, RSUST, supervisor of Mr. I. Aniedu (1985) and Mr. M. Basimike (1985); Professor J. R. Mainoya, University of Dar es Salaam, supervisor of Mr. M. A. Njau (1986); Professor H. G. Morgan, University of Sierra Leone, supervisor of Mr. J. O. Davies-Cole (1987), Mr. M. Hassane (1987), Mrs. R. Sang (1985) and Mr. G. Tikubet (1985); Dr. J. M. Mueke, Kenyatta University, supervisor of Mr. M. Gethi (1986) and Mrs. M. F. Ndonga (1986); Dr. A. Mutani, University of Dar es Salaam, supervisor of Mr. J. F. Omollo (1984); and Dr. W. R. Phillips, University of Ghana, supervisor of Mr. B. Torto (1985).

Table 9.3 ARPPIS students granted degrees in 1987

Name	Thesis title*	Registering university
<i>Ph.D. Scholars</i>		
Dr. B. C. Njau	Studies on the resistance acquired by rabbits experimentally infested with <i>Rhipicephalus evertsi evertsi</i>	Dar es Salaam
Dr. J. H. P. Nyeko	The influence of mode of transmission of <i>Trypanosoma congolense</i> on the stability and induction of resistance to Samorin	Makerere
Dr. S. H. Okech	Colonizing responses of <i>Maruca testulalis</i> to different cowpea cultivars in relation to their resistance or susceptibility	Rivers State University of Science and Technology
Dr. L. M. Kantiki	Studies on some aspects of the biology and feeding behaviour of <i>Eldana saccharina</i> on one maize and one sorghum cultivar	Malawi
<i>M.Phil. Scholars</i>		
Miss R. B. Bob-Manuel	A morphometric study of the cassava green mite complex, <i>Mononychellus</i> , in Africa	Rivers State University of Science and Technology
Miss E. Nwofor	The biology and behaviour of <i>Neoseiulus ideaus</i> reared on natural and artificial media	Rivers State University of Science and Technology

* Thesis titles are abbreviated.

Table 9.4 1985, 1986 and 1987 ARPPIS Ph.D. scholars attached to ICIPE research programmes and units for degree research projects

	1985	1986	1987	Total
Crop Pests	—	7	2	9
Livestock Ticks	1	—	3	4
Tsetse	3	1	2	6
Medical Vectors	2	—	2	4
Chemistry and Biochemistry	1	—	—	1
Total	7	8	9	24

Mr. B. E. Wishitemi (1985), doing his research in the ICIPE Livestock Ticks Research Programme, worked at the University of Neuchatel, Switzerland, for three months during the year, where he learned new techniques and extended his Ph.D. investigation on the induction of artificial immunity in sheep to the tick *Rhipicephalus appendiculatus*.

One more university joined ARPPIS during 1987: Kenyatta University, Kenya. The ARPPIS network now includes 14 participating universities (Table 9.5).

As is customary, the ARPPIS Academic Board met twice during 1987, both times in Nairobi. Following the very successful combined ARPPIS scientific meeting and Academic Board Meeting held in December 1986, the same arrangement of meetings was adopted for December 1987. On Thursday, 17 December, the Academy Board met to discuss the administration and development of ARPPIS. On

Table 9.5. The participating universities of ARPPIS

Addis Ababa University	Ethiopia
Anambra State University of Technology	Nigeria
University of Dar es Salaam	Tanzania
University of Ghana	Ghana
University of Ibadan	Nigeria
Kenyatta University	Kenya
University of Khartoum	Sudan
Makerere University	Uganda
University of Malawi	Malawi
Moi University	Kenya
Rivers State University of Science and Technology	Nigeria
University of Sierra Leone	Sierra Leone
University of Zambia	Zambia
University of Zimbabwe	Zimbabwe

the preceding day an all-day scientific meeting, during which all second- and third-year Ph.D. students gave an account of their work, was followed by the Second ARPPIS Distinguished Lecture. Dr. W. S. Saint, Representative of the Ford Foundation in eastern and southern Africa, gave a talk entitled, 'Groundnuts, dudus and wananchi', extolling the importance and virtue of the single-minded pursuit of science in Africa appropriate to the needs and conditions of its people.

9.5 POSTDOCTORAL RESEARCH FELLOWS

M. E. Smalley

Four new postdoctoral scientists joined ICIPE in 1987. They were Dr. M. Brownbridge, from the UK, and Dr. V. O. Okoth, from Uganda, both of whom were attached to the Crop Pests Research Programme; Dr. E. I. P. Kamanga-Sollo, from Tanzania, who joined the Livestock Ticks Research Programme; and Dr. R. S. Copeland, from the USA, who joined the Tsetse Research Programme. These four scientists joined eight other postdoctoral scientists already in residence at the start of the year: Dr. S. A. Tarimo, Tanzania, in the Tsetse Research Programme; Dr. S. K. Firemping, Ghana and Dr. J. Bartkowski, Poland, with the Crop Pests Research Programme; Dr. J. Jondiko, Kenya, and Dr. S. Essuman, Ghana, were in the Chemistry and Biochemistry Research Unit; Dr. M. E. Hussein, India, in the Sensory Physiology Research Unit; and Dr. W. Jura, Kenya, in the Histology and Fine Structure Research Unit.

9.6 SPECIAL PROJECT ON FINANCIAL AND ADMINISTRATIVE MANAGEMENT OF RESEARCH PROJECTS IN EASTERN AND SOUTHERN AFRICA (FAMESA)

Z. M. Nyiira

FAMESA's concept of a network of national research and development institutions that would improve the management of scientific research institutions was consolidated during the year by activities that include the identification of research management constraints, field research and a workshop on the FAMESA manual *Project Planning, Monitoring and Evaluation*, regional delivery of training material and interaction with national research institutions to develop and stage in-country training fora.

9.6.1 *Research management constraints*

The FAMESA Coordinator undertook follow-up action to assess the impact of FAMESA missions in member countries and to discuss what elements in national research institutions needed attention or for which training material could usefully be developed. National FAMESA contact persons were helpful in this exercise. At the same time, the training manuals developed by FAMESA were evaluated by scientists and managers of national research institutions.

9.6.2 *Development of training manuals*

The final draft of the second training manual on research and development institutions, *Facilities and Material Management*, was submitted for editing before publication. It was made available to institutions for national training workshops. The contents of this manual cover crucial elements on strategic facilities and materials management, procurement procedures, management of scientific equipment, the keeping of stock records, the physical control of materials and the management of land and physical resources.

The draft text of the training material for the management manual, 'Productive R&D: The R&D Institute/Constituency

Relationship,' went through an initial review workshop. The following topics will comprise most of the manual: the research and development (R&D) institute-constituency relationship, R&D institute-client needs, R&D institute extension services, why an information system?, marketing R&D output, the consequences of an R&D institute neglecting its constituency, contractual agreements and legal requirements from clients, social accountability for R&D efforts, protection of R&D innovations, patent and licensing systems and the formulation of patent policy in R&D institutions.

Field research work was undertaken and the draft for a management manual, 'Project Planning, Monitoring and Evaluation,' was completed during the year and a workshop convened in August to approve the text. This is the fourth manual in the series of FAMESA management manuals.

9.6.3 *Delivery of training materials*

Apart from the workshop held in August, there were no other regional curriculum development meetings. However, continued interest from national research institutions was expressed in the form of requests for national training seminars and workshops. Significant among the requests were those from Tanzania's National Council for Scientific Research, Ethiopia's Institute of Management, Uganda's National Council and FAMESA's National Coordinator in Malawi. The International Development Research Centre supported these requests, and other donor agencies, notably the Ford Foundation and the German Foundation for International Development, expressed interest in FAMESA's relations with national institutions and the training material FAMESA distributes to these institutions.

The FAMESA Coordinator visited Zimbabwe, Malawi, Tanzania and Uganda to discuss the logistics for staging national training workshops.

9.6.4 *Exchange of information*

Production of the quarterly newsletter, *Research Management Review*, was continued to increase communication among the Network R&D institutions and managers.

9.7 INTERNATIONAL PROGRAMME FOR SPECIALIZED SHORT-TERM TRAINING IN INSECT SCIENCE (IPTIS)

J. F. Omange

Four specialist group training courses were conducted during the year, two of them held for the first time.

9.7.1 *The International Group Training Course on Components Essential for Ecologically Sound Pest and Vector Management Systems*

The tenth course in this series was conducted in August. Twenty-six participants from 11 tropical developing countries (Chad, Ghana, India, Kenya, Nigeria, Sierra Leone, Somalia, Sudan, Tanzania, Uganda and Zambia) attended the course. The main sponsor for the course was once again the International Development Research Centre.

The revised syllabus for the course included a one-week

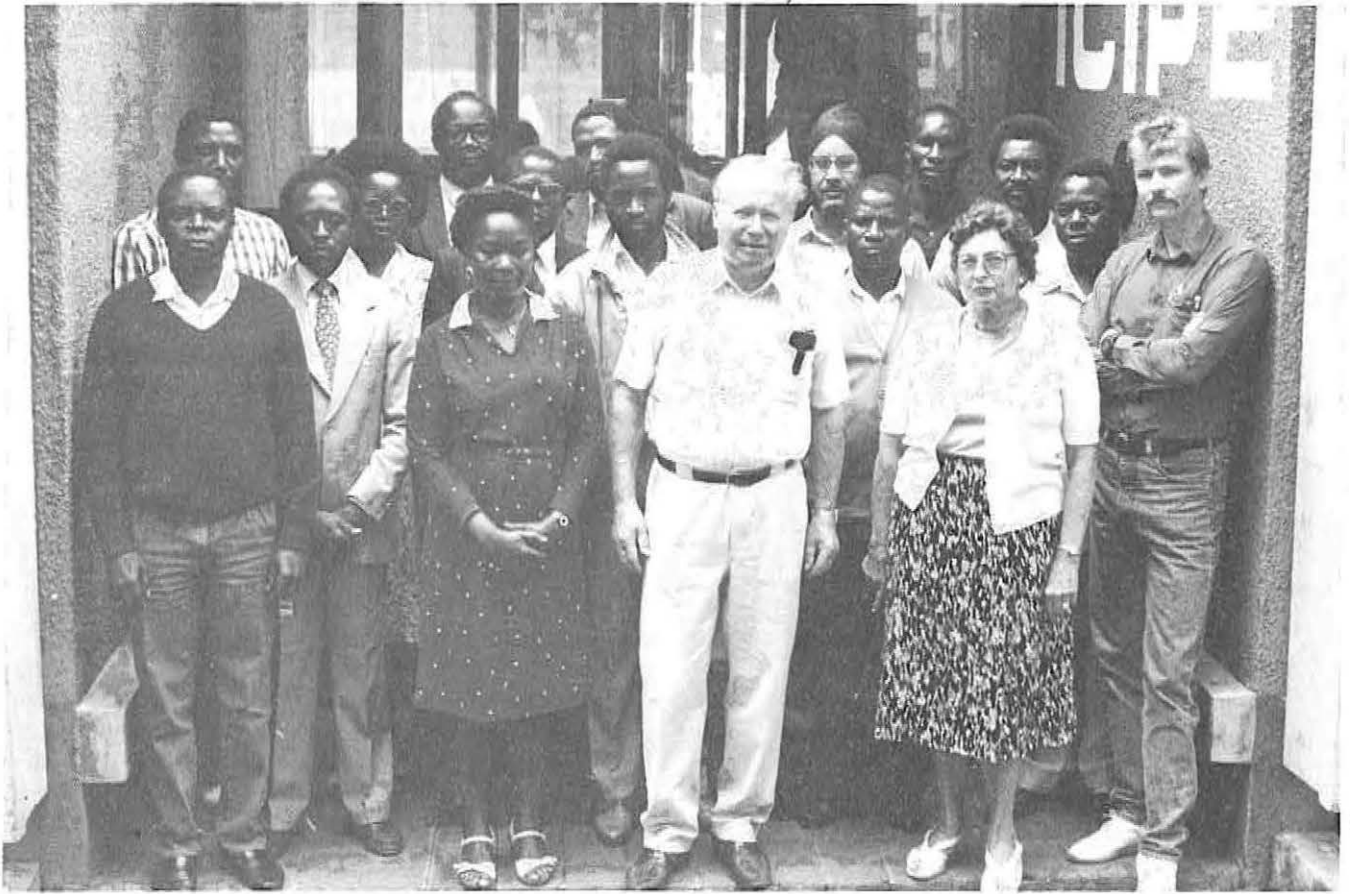


Figure 9.1 Trainees and lecturers for the first ICIPE Radioisotope Course. At front centre is Professor Dr. W. J. Kloft; Mrs. E. S. Kloft is to his left.



Figure 9.2 Participants of the Radioisotope Course during practicals.

session at ICIPE's Mbita Point Field Station, at which lecturers based at Mbita delivered lectures and gave demonstrations and practicals. A new excursion site for the vector management group of trainees was the Small Ruminant Collaborative Research Support Programme and Maseno Veterinary Research Station, both based at Maseno. In their evaluation at the end of the course, all participants expressed satisfaction with the course and their wish that the series be continued.

9.7.2 *The International Course on Insect Growth,*

Development and Behaviour: Insect Endocrinology

The third course in the series on Insect Growth, Development and Behaviour, which was postponed last year, was held in Nairobi in June/July. Nine participants (from Egypt, Ghana, Kenya, Nigeria, Sudan and Tanzania) attended the course.

Of the eight lecturers for the course, five were from ICIPE and three from North America (Professors L. M. Riddiford and J. W. Truman, from the University of Washington, USA, and Professor G. R. Wyatt, from Queen's University, Ontario, Canada).

The course syllabus emphasized practicals on the latest techniques in insect endocrinology and covered the following areas: insect endocrine systems, morphology and ultra-structure; insect hormone, hormone analogues and anti-hormones; biochemistry of hormone, synthesis and metabolic inactivation; neuropeptides, physiology, biochemistry and purification; hormones in growth, diapause and development; hormones in behaviour; hormones in metabolism; and the biochemical and molecular basis of hormone action.

9.7.3 *The African Regional Training Course on the Use and Safe Handling of Radioisotopes in Insect Sciences*

This new course was sponsored by the International Atomic Energy Agency for ten participants (from Kenya, Tanzania, Uganda and Zambia) (Figures 9.1 and 9.2). Professor W. J. Kloft and Mrs E. S. Kloft were the visiting lecturers for this course. It is hoped that this new course series, which the first group of participants found valuable, will be continued.

9.7.4 *The Regional Training Course on the Use of Microbial Pathogens in the Control of Insect Pests and Vectors*

The objective of this course was to introduce young scientists from Africa, particularly from the eastern and northern regions, to the use of microbial insect pathogens in the control of insect pests and vectors and to indicate the potential field application of these microbial pesticides. The course sponsors were the United Nations Environment Programme, the International Cell Research Organisation, the Nairobi Microbiological Resources Centre, the University of Nairobi and the International Centre of Insect Physiology and Ecology.

Twelve trainees were invited to the course in October, of whom eight attended. The countries represented by the participants were Egypt, Ethiopia, Kenya, Sudan and Uganda. The course syllabus consisted of lectures, practicals and field trips. The first part of the course, consisting of lectures, was conducted in Nairobi; the rest of the course, involving lectures, practicals and field trips, was conducted at Mbita Point Field Station. At the end of the course a dinner was held at ICIPE's Duduville International Guest Centre, at which Dr. Genady N. Goulbev, of the United Nations Environment Programme, was the Guest of Honour.

9.8 IN-SERVICE TRAINING

Z. M. Nyiira

Trainees from Mozambique, Uganda and Burundi came to ICIPE during the year to receive in-service training for one to three months. Mr. Muacanhia, from the Universidade Eduardo Mondlane, was financially supported by the International Development Research Centre for three months at Mbita Point Field Station. His training was in field ecology. Two officials from Uganda's Ministry of Agriculture were trained for three months with support from ICIPE's Crop Pests Research Programme and International Fund for Agricultural Development funds. They were exposed to methodologies and techniques used in field investigations of the stem borers of maize and sorghum. Dr. G. Nigarura spent one month with senior scientists in the Livestock Ticks Research Programme, receiving training in several areas, including tick taxonomy and ecology.

Further requests for in-service training were received from Somalia, Tanzania, Zambia and Rwanda.

9.9 STAFF DEVELOPMENT TRAINING

Z. M. Nyiira

The Staff Development Training Programme continues to offer specialized training for ICIPE staff with the aim of upgrading their skills and techniques for the better performance of their duties at the Centre.

During the year a comprehensive survey was conducted starting in April, with the aim of determining the training needs of the Centre for the next three to five years. From this exercise priority areas have been determined, which will form a basis for staff training. One of the priority areas identified by nearly all ICIPE departments was computer use. To fulfil this need, ICIPE's Biostatistics and Computer Services Unit has given staff in-house computer courses since May.

Several members of staff successfully completed their staff development training this year and returned to resume their duties at the Centre. The record of staff development training at ICIPE over the last ten years is shown in Table 9.6.

Table 9.6 Number of ICIPE staff trained under the staff development scheme from 1978 to 1987

Category	Year										Total
	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	
Scientists	19	20	23	20	21	22	20	7	7	11	170
Technicians	16	18	20	20	23	19	18	11	11	7	163
Administrators	2	5	5	5	4	5	6	10	5	5	52
Secretaries	4	4	2	1	—	1	2	1	1	2	18
Total	41	47	50	46	48	47	46	29	24	25	403

Social Science Interface Research

**10.1 The ICIPE/Rockefeller Collaborative Social Science Interface Research
Project 133**

10

Social Science Interface Research

The Social Science Interface Research (SSIR) office activity moved, in 1987, towards incorporation as a core unit of the ICIPE. After an in-depth review at the 1987 Annual Research Conference of the research conducted under the special project initiated in 1985 in collaboration with the Rockefeller Foundation, 'Food Security and Production Constraints at the Household Level,' the ICIPE Governing Council recommended that social science be formally incorporated into the ICIPE's core programme on a long-term basis. In November, the Executive Board of the Council approved the establishment of the Social Science Interface Research Unit (SSIRU) and its initiation in January 1988. The unit will conduct research on the social, economic and environmental constraints to food and animal production at the household level. These studies will generate a database, which will make it possible to predict how farmers will respond to technical recommendations for integrated pest management procedures being developed by the ICIPE's biological research programmes. SSIRU scientists will collaborate with scientists from other core programmes to develop an effective, transdisciplinary methodology and to investigate substantive research questions relevant to each programme.

10.1 THE ICIPE/ROCKEFELLER COLLABORATIVE SOCIAL SCIENCE INTERFACE RESEARCH PROJECT

A. Pala-Okeyo

In 1987 the Social Science Interface Research Project (SSIRP) worked jointly with the Crop Pests Research Programme (CPRP) and the Livestock Ticks Research Programme (LTRP) on four research projects funded by four international donors. The thrust of the research has been to examine the agricultural and livestock management practices of resource-poor farmers to better evaluate the end users' response to ICIPE's packages for integrated pest management.

In association with CPRP, SSIRP completed field work at two sites—Seme (Kisumu) and Awendo (South Nyanza)—where field trials and farmers' surveys were conducted to identify intercropping combinations of food and cash crops that met small farmers' income and food security priorities.

At the Seme site, an anthropologist and an agronomist conducted studies to investigate the effect on pest populations and yields of intercropping cotton with the food

crops of sorghum, maize, cowpea and beans. Results from the field trials show that intercropping maize with cotton protects both crops from their major insect pests: stem borers (*Chilo partellus*) for maize and leaf feeders and cotton boll worms (*Aerias* spp. and *Pectinophora gasciella*) for cotton. The number of squares and bolls damaged by boll worms was reduced from 20% in a pure cotton stand to 17% when cotton was intercropped with beans and to 11% when cotton was intercropped with maize and beans. Similarly, the infestation of stem borers on pure maize (0.10 borer per plant) was reduced to 0.05 borer per plant in cotton intercropped with maize and 0.07 borer per plant in cotton planted together with maize and beans. The percentage of plants damaged in a pure maize stand was reduced from 17% to 12% when maize was intercropped with cotton and to 11% when maize was intercropped with cotton and beans.

Intercropping these crops also reduced the area covered by weeds from 21% in pure maize stands to 9% in maize intercropped with cotton and 6% in maize, beans and cotton interplanted. Finally, it is clearly indicated in Table 10.1 that though intercropping did not have a significant negative effect on the yield of either maize or cotton, it did have an adverse effect on beans. This effect, however, could have been due to shading and waterlogging in that particular year.

Table 10.1 Yield of cotton, maize and beans under different cropping patterns: Seme, western Kenya, long rains, 1987

Cropping pattern	Cotton (kg/ha)	Maize (kg/ha)	Beans (kg/ha)	LER*	LEC†
Cotton	1006.5 ^a			1.0	
Maize		3017.0 ^a			
Beans			951.0 ^a	1.0	
Cotton/maize	850.0 ^b	2817.0 ^b		1.67	0.7
Cotton/beans	1023.0 ^a		400.1 ^b	1.43	0.42
Cotton/maize/beans	891.0 ^b	2646.0 ^c	560.3 ^b	2.35	0.46
Mean	942.6	2826.7	637.1		

Within a column, mean values bearing different superscript letters are significantly different at $P < 0.05$.

* LER: land equivalent ratio.

† LEC: land equivalent coefficient.

In terms of productivity per unit of land area, intercropping cotton with maize, beans, and maize and beans gave intercropping an advantage in the line of 67%, 43% and more than 100% in the case of cotton, maize and beans.

Results of the social survey research revealed insect pests to be a major problem in Seme. Farmers reported losses due to insect pests of up to 50% in the field, with 62% of farmers reporting the worst damage during the pre-flowering stage. Insect pest damage of maize, sorghum, cowpea and other anchor crops in storage was reported by over 43% of the farmers. Crop borers and weevils were the most notorious pests on farms and in storage, respectively. Table 10.2 shows farmers' perceived levels of insect pest damage.

Table 10.2 Perceived levels of insect pest damage: Seme, western Kenya, long rains, 1987

	On farm	In storage
Severe	78.0%	72.3%
Moderate	14.4%	13.5%
Rare	7.6%	14.2%

N: 153.

Source: Food Security and Production Constraints at the Household Level, a project sponsored jointly by ICIPE and the Rockefeller Foundation.

A base map of the study location prepared for the project by a consultant land surveyor shows striking land fragmentation. The majority of surveyed farmers have dispersed land ranging from 2 to 8 separate pieces of land, with an average number of 6 parcels per person. Of the registered land owners whose names appear in the land register, only 50% are women. This confirms an earlier hypothesis about land privatization and gender differentiation—that increasing privatization of land weakens women's legal rights to land. This trend, if allowed to continue, will have important negative implications for the adoption of agricultural and food production technology, an area in which women are the pioneers.

A separate investigation is planned to assess the impact of land ownership on women's agricultural work and discover whether ownership influences the adoption of integrated pest management technology.

Labour problems were found to be another constraint to farming. Bottlenecks were highest in March and in April (Table 10.3). Poor access to farming equipment and lack of

cash for hiring labour contributed to the labour shortage. Consequently, 38% of the farmers relied only on family labour for farm work. Only 2% of the farmers were able to hire labour on a permanent basis.

In Awendo, where ICIPE was collaborating with both the Rockefeller Foundation and the International Food Policy Research Institute, trials were carried out on intercropping sugarcane with maize, beans, cowpeas and groundnuts. Although previous results had shown that intercropping did not significantly reduce the number of cane tillers, number of internodes and cane girth, there was a significant reduction in

Table 10.3 Seasonal labour bottlenecks: Seme, western Kenya, long rains, 1987

Month	No. of farmers	Percentage
March	5	3.3
April	144	94.0
May	2	1.3
July	1	0.7
October	1	0.7

N: 153.

Source: Food Security and Production Constraints at the Household Level, a project sponsored jointly by ICIPE and the Rockefeller Foundation.

cane yield when cane was intercropped with maize, as shown in Table 10.4. Intercropping cane with maize, beans or maize and beans together had no significant effect on the cane quality expressed in such ways as the percentage of soluble solids in the juice, the proportion of fibre to juice content, the percentage of apparent sucrose in the soluble solids and the percentage of apparent sucrose in the juice.

When several crop species are planted on the same field in the same season, these species will compete for the available resources and this competition is usually expressed in a slight yield reduction. But the thrust of our study was not yield increase but rather yield stability; the most important goal of our project was to create a sustainable food supply (food security) at the household level. The reduction in cane and cotton yields was compensated for by the good yields obtained from maize and beans, which are important food crops for the local households.

Sugarcane farmers and nonsugar farmers were found to practise distinctly different cropping patterns on their food plots. Although over 82% of all agricultural households

Table 10.4 Yield of cane, maize and beans under different cropping patterns in Kokuro-Awendo, South Nyanza, long rains, 1987

Cropping	Cane (tons/ha)	(%) Reduction	Maize (kg/ha)	Beans (kg/ha)	LER*	LEC‡
Cane	128.3	100.0			1.0	
Maize			1281.1 ^a		1.0	
Beans				246.7 ^a		
Cane/beans	96.0	74.82		135.3 ^b	1.38	0.410
Cane/maize	57.1	44.51	423.3 ^b		0.78	0.146
Cane/maize/ beans	83.3	64.92	743.7	147.2	1.89	0.222
Mean	91.2		816.0	176.4		

Within a column, mean values bearing different superscript letters are significantly different at $P < 0.05$.

* LER: land equivalent ratio (site A \times site B).

‡ LEC: land equivalent coefficient (site A \times site B).

surveyed had at least one food plot intercropped during the long rains, sugar farmers showed lower rates of intercropping on their food plots than did nonsugar farmers (37% versus 58% in the long rains of 1986; 23% versus 38% in the short rains of 1986/87). In all agricultural households, women were more likely than men to intercrop. Men in sugarcane households controlled only 20% of all food crop plots; men in households raising other crops controlled 35% of all food plots. The division of responsibility between men and women over food plots therefore is seen to be becoming more differentiated as households move more fully into commercialized cane farming.

Heterogeneity within the farming community is reflected in different land and labour uses, specifically in different rates and patterns of intercropping on food plots. This finding makes clear the need for careful targeting of those groups of farmers who will benefit most from using improved intercropping techniques for integrated pest management.

Another SSIR collaborative project, this with the United Nations Economic Commission for Africa (UNECA), Reduction of Food Losses Through Insect Pest Management and Use of Small-Scale Low-Cost Farm Equipment, is testing under field conditions at Oyugis and Kendu Bay two components of a pest management package developed by ICIPE's Crop Pests Research Programme. The tests involve both intercropping and host-plant resistance. Field trials were completed during the long rains of 1987 and the short rains of 1987/88 and the results will be evaluated in 1988.

SSIR staff collaborated also with the Livestock Ticks Research Programme in a socio-economic study on Rusinga Island, in western Kenya. Results from a census of 170 homesteads on the island show that the resident population is dominated by women and children, which is due mainly to a high rate of male migration out of the area. Migrants are usually the most highly educated of the area and in their prime and as a result, it is becoming increasingly difficult to develop the island resources, scarce as they are.

Land tenure is determined by four major variables: poor soils and erratic rainfall, population pressure, commercialization of agriculture and a high rate of polygyny. Farmers often acquire several scattered plots not only to

exploit diverse potential (the range is between 1 and 10 plots), but also to allocate land to each wife. Commercialization and population pressure have led to excessive fragmentation (70% of the plots are under 1 ha), and after the harvest in August, stock animals are usually left to graze unattended, regardless of land ownership.

Seventy-one percent of the homesteads studied raise livestock (cattle, goats and sheep) and 86% of these keep cattle in combination with small stock to achieve several objectives, the most important being the maximization of subsistence production. Concern for maximization is shown by a dominance of female animals (63%–73% of the total herd) and a negligible proportion of castrates (1%–5% of the total herds). Offtake rates reveal a functional complementarity: sales are important for cattle (15%) and goats (11%). Slaughter is significant only for sheep (14%), and cattle alone are given away as bride wealth. Productivity is very low—the mean age at first calving is 5.2 years and the calving interval is 1.5 years. The chief contributory factors for this low productivity are poor management, deficient diet and endemic diseases, chief of which are the tick-borne diseases.

SSIR collaborative work with the United Nations Children's Fund in a time-use study on Rusinga Island confirmed the important role of women in food production, family health and domestic work. Data collection on time-use over a twelve-hour-day covered the peak seasons and the post-harvest period. These data will be analysed and presented in the *1988 Annual Report*.

SSIR collaboration with ICIPE's Tsetse Research Programme is being discussed and should start in 1988. This work will address issues pertaining to community participation in the application of pest and vector management strategies. Specifically, the study will involve testing tsetse control strategies, such as the use of a trap developed by the Tsetse Research Programme, by local people.

Exploratory discussions with the Biological Control section of the Crop Pests Research Programme and the Medical Vectors Research Programme will take place next year.

Administration and Information Division

11.1 Capital development **139**

11.2 Communication services department **140**

11.3 Publishing and documentation **141**

11



Administration and Information Division

1987 was one of consolidation for the Administration and Information Division, following the restructuring reported in the 1986 Annual Report. Dr. Vitalis Musewe, who headed the new Human Resources Department briefly on his return from a management training course in the UK, moved to Mbita Point Field Station to take over from Dr. Z. M. Nyiira as Station Manager, leaving the Human Resources Department in the hands of another experienced administrator, Mr. Julian Evans Okiri, who had himself just returned from an advanced management course in the USA. Mr. Silas M. Kimaita, Senior Administrative Officer, Mbita Point Field Station, continued with his master's degree course in the UK and was expected back in early 1988.

The emphasis on staff development, not only in the Administration and Information Division but also in the Centre as a whole, highlights ICIPE's concern with staff as its single most important resource, and its desire to ensure that its research and training capability is matched by a comparable capability in the support services.

The ICIPE Science Press made a slow but steady start by taking over the publishing of ICIPE's bimonthly journal, *Insect Science and Its Application*, from Pergamon Press, in the UK. Although the journal is still being printed and distributed in the UK, editorial and publishing responsibilities now rest fully with the ICIPE Science Press, which is set to be the Centre's main vehicle for disseminating its research information.

Some basic printing equipment was acquired during the year, in addition to the phototypesetting and word-processing facilities the press already had. It is planned that once the headquarters and the laboratories are transferred to the new premises now under construction at Duduville, ICIPE's Chiromo Campus will be transformed into a Communication and Information Centre and will house the ICIPE Science Press and a permanent insect science exhibition.

The 1987 Annual Research Conference was again a great success and was very well attended, even though it had to be held in the conference facilities of the Nairobi Museum because of the construction work at Duduville. The ICIPE Annual Research Conference has now become a major international scientific event which, in addition to the regular programme of presentations by the Centre's own scientists, features a lecture by a distinguished scientist invited for the occasion. The 1987 lecture was delivered by Professor Alexander Keynan, of the Hebrew University, Jerusalem, on the topic 'Biotechnology: Assessment and Perspective'.

11.1 CAPITAL DEVELOPMENT

Construction for Contract I of the Duduville Capital Development Programme Phase II made good progress during the year, and the management was satisfied with both the pace and the quality of work demonstrated so far. Nevertheless, actual completion and handing over may be

delayed by four to six weeks due to unexpected technical and administrative problems encountered by the contractor. In spite of these minor setbacks, the new facilities are expected to be ready for occupation by June 1988.

At the end of the year it was confirmed that the World Bank would provide an additional \$1.2 million for Contract II, and as a result preliminary planning and design for the

Administration Block was started. After the usual World Bank tendering procedures, construction is scheduled to commence in June or July 1988 and will take approximately 12 months.

With the Administration Block now in hand, the only major segments still to be funded and constructed will be the Library, the Outreach and Training Unit and Communications (including the Biostatistics and Computer Services Unit) and the Conference Centre.

11.2 COMMUNICATION SERVICES DEPARTMENT

11.2.1 *The 17th Annual Research Conference*

The Louis Leakey Memorial Institute Auditorium, at the Nairobi Museum, was a hub of activity as the venue for ICIPE's 17th Annual Research Conference. This year the conference was not only bigger than usual, but was also of additional interest. Two new and innovative components of ICIPE's research programme were reviewed in depth: the Social Science Interface Research Project and the Outreach and Training Unit, incorporating the African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests (PESTNET).

Also reviewed in depth were the biological control component of the Crop Pests Research Programme and the Medical Vectors Research Programme, including leishmaniasis epidemiology and laboratory investigations on leishmaniasis, as well as malaria epidemiology. A PESTNET workshop, held concurrently with the conference, was attended by participants representing member countries from eastern and southern Africa.

The now traditional Special Guest Lecture was delivered by Professor Alex Keynan, of the Hebrew University, Jerusalem, Israel, on 'Biotechnology: Assessment and Perspective'. The highlight of the conference was the presentation of the annual ICIPE Award for Innovative Research to Dr. Eliud Omolo for his research on multi-line intercropping in the control of crop pests.

11.2.2 *Study workshops*

ECA/ICIPE Workshop on Cereal Crop Losses in Africa. In October the United Nations Economic Commission for Africa (ECA) and ICIPE organized a workshop to review the current cereal crop losses in Africa and the methodologies used to assess and monitor these losses, to plan strategies that will assess and monitor cereal losses and to encourage agricultural development programmes to use these strategies. Although laudable efforts have been made to control crop pests, food losses in Africa are still unacceptably high. Scarce reliable data exist on crop losses in cereal grains and only a few methodologies have been developed to determine the magnitude of the losses in relation to farming systems research. Moreover, most of the methodologies that have been developed have been tested insufficiently.

Twenty-nine people attended the workshop, including national representatives from eastern and southern Africa and agricultural experts from research institutes and organizations. Participants presented country reports and technical papers on a wide range of invertebrate, vertebrate

and pathogenic pests that attack seeds, seedlings, seed heads, roots, stems, foliage, panicles and stored grain. Various kinds of crop loss assessments were discussed.

The workshop participants requested the ECA and FAO to seek funds with which to prepare a manual on assessing crop losses and to continue to impress governments with the importance of reducing food losses. ICIPE was requested to strengthen the activities of its PESTNET (African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests) and to distribute the PESTNET bulletin widely.

A patent system for Africa. The topic of technology between developed and developing countries has generated considerable international debate in the last two decades. Two major issues have emerged from this debate. The first one concerns how countries may identify and gain access to appropriate technologies from developed countries on terms that are reasonable. The second one concerns ways of stimulating innovations and of protecting indigenous inventions and resources in developing countries. Of prime concern to the governments of developing countries is what kind of patent policy would be most conducive to both technology transfer and the growth of indigenous inventions and industries.

The national patent systems in Africa today are largely legacies from the colonial powers. Since independence, two major continental patent groups have emerged, one for English-speaking countries and one for French-speaking countries. But a viable patent policy depends on vigorous and comprehensive legal and documentation systems, which have yet to be established in Africa. For this reason a roundtable discussion, 'Toward a More Relevant Patent System for Africa', sponsored by the Third World Academy of Sciences, the African Academy of Sciences and ICIPE, was held at ICIPE in January to act as a catalyst in forming a more beneficial patent policy and legal patent system. Participants addressed one fundamental question: what kind of patent policy and system make most sense under the socio-economic conditions of African states? Participants described and appraised systems of several industrialized and recently industrialized countries and compared these with the patent systems of African countries. The discussions focussed on the philosophical bases and socio-economic rationale for different patent systems and how patent systems have affected and been affected by overall economic considerations and industrial development. It is hoped that these discussions will lay the groundwork for a more comprehensive workshop in the future, which should embrace all the main elements of a fully functioning patent system, including legal and technical infrastructure, industrial and manpower needs, documentation resources, information retrieval and dissemination matters, regional networking and international cooperation.

The International Development Research Centre/ICIPE Regional Training Workshop on Modalities and Techniques for Research Dissemination. This workshop was conducted by Dr. and Mrs. Ian Montagnes, visiting editors from the International Rice Research Institute, in Manila, the Philippines, in November. Twenty participants from institutions in eastern and southern Africa took part in

this course and learned techniques for disseminating information.

Beyond Hunger: Africa's Future 1957-2057. This workshop was held in Kericho, Kenya, in June, and was co-sponsored by the Council for the Development of Economic and Social Research in Africa; the Alan Shawn Feinstein World Hunger Programme, of Brown University; the African Academy of Sciences; and ICIPE.

The workshop brought together twenty prominent African scholars drawn from ten sub-Saharan countries and various academic backgrounds. They came together to consider the conventional wisdom about Africa's future and, through the use of a newly developed methodology, to create alternative and surprise-rich future scenarios for Africa.

11.2.3 Visitors

A wide variety of visitors came to ICIPE in 1987, including representatives from the World Bank, the European Economic Commission, the United Nations Food and Agriculture Organization, the United States Agency for International Development, the International Service for National Agricultural Research, the United Nations Economic Commission for Africa, the United Nations Development Programme and many educational institutions worldwide.

Of special mention are:

- Professor Walter E. Massey, President-elect of the American Association of the Advancement of Science, USA
- Dr. S. C. Nana Sinkam, Director of the Joint Economic Commission for Africa and the Food and Agriculture Organization's Agriculture Division, Addis Ababa, Ethiopia
- Mr. Timothy Rothermel, Director of the Division of Global and Inter-regional Projects, the United Nations Development Program, New York
- Dr. Robert Grunder, Financial Controller of Rural Development and Inter-regional Agricultural Research, West Germany
- Dr. E. V. Leigh, Chief of the Research Development Centre, the Food and Agriculture Organization, Rome
- Dr. Richard Manning, Deputy Director General of the Australian Development Assistance Bureau
- Dr. Clive James, Director General of Centro Internacional de Mejoramiento de Maiz y Trigo

11.2.4 ICIPE wins the Alan Shawn Feinstein World Hunger Award

On 16 October 1986 at Brown University, in Rhode Island, USA, Dr. Achola Pala-Okeyo, Scientist-in-Residence at ICIPE, accepted the Alan Shawn Feinstein World Hunger Award for Research on behalf of the ICIPE.

ICIPE was selected for this award in recognition of the substantial contribution the Centre has made to the understanding of the biology and ecology of major insect pests in tropical Africa. It was also recognized that ICIPE, staffed predominantly by African scientists, has become a highly respected international scientific institution. The Centre has addressed itself to such problems as crop damage by insects and the devastating effects of diseases carried by such vectors as mosquitoes and sandflies. The award givers

applauded the Centre's major goal: to develop various pest management strategies that can be used by African farmers with limited resources to improve the production of their crops and livestock and to improve their quality of life.

The award is a function of the Alan Shawn Feinstein World Hunger Programme, which addresses the long-term persistence of hunger in a world of plenty through research, the development of resources and raising public awareness by conferring the annual Feinstein award.

This programme addresses questions about how the hunger problem will be affected by the world population expanding to a predicted level of ten billion by the middle of the next century. To explore ways of ending hunger, it focuses on long-term trends in climatology, demographics, economics, sustainable resources, technology and values. The programme concerns itself with hunger in both rich and poor countries but places special emphasis on Africa and South Asia, where most of the world's hungry live and die.

11.3 PUBLISHING AND DOCUMENTATION

In January 1987 ICIPE took over the publication and distribution of its bimonthly journal, Insect Science and Its Application, from Pergamon Press, in the UK. All work on the journal is now done in Nairobi except the typesetting, printing and mailing, which are still handled in the UK.

The year also saw the launching of ICIPE's own imprint, ICIPE Science Press, and with it the beginning of a varied but modest science publishing programme which will consolidate the professional editing, design, production and distribution of all ICIPE's publications. These include the ICIPE Annual Report; the quarterly newsletter, Dudu; the Guest Lecture Series, conference proceedings, various bulletins and occasional monographs. In the past these have not been properly coordinated, and some of the critical documentation needed by the Centre has not been produced on schedule or on a professional level equal that of other international research institutions. The Science Press will strengthen ICIPE's capability in this area.

Initially, Insect Science and Its Application will be the major publishing activity of the Press, and any new volumes in the book series, 'Current Themes in Tropical Science', will also be published under the new imprint. Other carefully selected publishing projects relevant to ICIPE's mandate will also be undertaken, but the emphasis will be not so much on expansion as on consolidating and improving what already exists and rationalizing the use of resources that ICIPE is already devoting to the dissemination of its research results.

Collaborative links are being negotiated with the Communication and Publication Department of the International Rice Research Institute, in the Philippines, which has an extensive publishing programme, and with ICSU Press, the publishing arm of the International Council of Scientific Unions. These links will include reciprocal agency arrangements and occasional staff exchanges.

11.3.1 Scientific Editorial Unit

The ICIPE Science Press has been in operation since January 1987, since which we have published and distributed the bimonthly journal *Insect Science and Its Application* through the auspices of the Press. (Pergamon Press published the first seven volumes of this journal.)

A special effort was mounted in mid-1986 to improve substantially the quality and quantity of the journal's submissions. This year we published 1022 pages in Volume 8, which is well over the 1986 page budget of 840 per volume. A special issue, comprising proceedings of the 1986 International Conference on Tropical Entomology, edited by Dr. M. F. B. Chaudhury, of ICIPE, made up pages 387-1022 of this volume.

We have promoted the journal by on-going direct-mail campaigns. Brochures for both the journal and a book series, 'Current Themes in Tropical Science,' have been sent to potential subscribers all over the world and the journal has continued to be widely indexed and abstracted. As a result, the subscription list inherited from Pergamon Press has tripled over the year.

Volumes 1 to 3 of the book series are still on sale in the ICIPE Library and Pergamon Press has reprinted Volume 3: *Natural Products for Innovative Pest Management*. Volume 4 of the series is being prepared for publication in 1989.

11.3.2 ICIPE Library and Documentation Service

The ICIPE Library continued to improve its service through the use of computer applications and to strengthen its periodicals and reference collections. New subject areas were covered in our acquisitions and interlibrary cooperation was strengthened in computerization and acquisitions.

Computer application. Until the third quarter the Library continued to share time on a Wang PC with the Administration and Information Division. Our computer time was thus scarce until October, when a new computer was bought and dedicated fully to the library. The new machine, an AST industry compatible, enabled the Library to computerize 90% of its catalogue.

Acquisition. This year we strengthened ICIPE's African Regional Postgraduate Programme in Insect Science by providing students in the programme with publications. We also acquired publications in new subject areas that have become relevant to ICIPE, including molecular biology,

biotechnology, population ecology, ecological modelling and social science. Altogether, 373 books were acquired, about 25% of which were multiple copies of text books bought through donations. The Library's journal subscriptions increased by about 10%, bringing the total, which includes donations and exchanges, to 145 titles.

Archives. An archives project was moved from the Chiromo campus to the new ICIPE headquarters at Duduville. The processing of archival material continued on a department-by-department basis. The increased physical distance between the Library and the archives somewhat reduced the archive activity but this will change when the main Library moves to Duduville.

Services. Keeping ICIPE staff up to date on the library's resources continued to be a major concern. The *Library and Documentation Bulletin* was issued four times on schedule and in-house SDI profiles as well as regular subscriptions to SDI from the CABI database were maintained. In addition, four retrospective computer searches were done. From the library collections and through interlibrary cooperation, 1404 document requests were satisfied.

Cooperation. Interlibrary partners supplied about 25% of the documents delivered to our readers. The Senior Librarian attended meetings of librarians and documentalists in Nairobi on the subject of collaboration, particularly in the application of information technology. An organization was formed called the Nairobi Information Group, which includes information specialists from the International Laboratory for Research on Animal Diseases, the International Council for Research in Agroforestry, the United Nations Environment Programme, the United Nations Centre for Human Settlement, the Library of Congress Nairobi Field Office, the African Regional Standards Organisation and ICIPE. This group was useful for exchanging ideas on the Library's ventures in mechanized information handling.

Such cooperation was further strengthened and widened by the participation of the Senior Librarian in a Documentation and Information Services Meeting of the Consultative Group on International Agricultural Research in Lima, Peru, in May. The ICIPE Library benefited from recommendations by staff from other international agricultural research centres who attended this meeting.

1987 Seminars Hosted by ICIPE

SPEAKER

Professor Olusegun O. Dipeolu,
School of Veterinary Medicine,
Tuskegee University, Alabama, USA

Professor Michael Bentley,
University of Maine, Maine, USA

Professor Yosef Mizrahi,
Ben Gurion University of
the Negev Beer-Sheva, Israel

Dr. M. J. Rice, University of
Queensland, Department of Entomology,
Queensland, Australia

Dr. R. K. Saini, ICIPE, Nairobi, Kenya

Dr. J. M. Gopo, Department of
Biological Sciences, University of
Zimbabwe, Zimbabwe

Professor L. M. Riddiford, Department
of Zoology, University of Washington,
Seattle, Washington, USA

Professor G. R. Wyatt, Department
of Biology, Queens University,
Kingston, Ontario, Canada

Professor J. W. Truman, Department
of Zoology, University of Washington,
Seattle, Washington, USA

Dr. Hiroshi Takeuchi, Department of
Physiology, Gifu University,
School of Medicine, Trukasa-Machi,
Gifu 500, Japan

Dr. Gaeme Hamilton, Department of
Entomology, Michigan State University,
Michigan, USA

Dr. Steve Mihok, Whiteshell Research
Atomic Energy of Canada, Pinawa,
Manitoba, Canada

TITLE

Application of Integrated Pest Management to the Control of Ticks in Nigeria

Studies of Triterpenoid Insect Antifeedants

Introduction and Domestication of Wild Fruit and Nut Trees in Arid and Semi-Arid Areas

(1) Behavioral and Electrophysiological Effects

(2) To lay or not to lay: Sensory Model of Sheep Blowfly Oviposition

(1) Pheromone Mediated Behaviour of Male Cabbage Looper *Trichoplusia ni* in a Wind Tunnel

(2) Responses of Male *T. ni* to a Combination of Pheromone and High Frequency Sound Similar to the Sonar of Bats

The Development of a Salmonella-Specific Probe for the Routine Diagnosis of Salmonella in Foods

Hormonal Control of Sequential Polymorphism in Insect Epidermis

Juvenile Hormone-Controlled Vitellogenin Synthesis in Locust Fat Body

Ecdysis Hormone Release and Acation in the Tobacco Hornworm, *Manduca sexta*

Sensitivities of Identifiable Giant Neurones of an African Giant Snail, *Achatina fulica* Ferussac, to Glutamate Analogues

Spatial Dynamics of Apple Maggot in Michigan

Stress, Immunity and Population Cycles in Small Mammals

1987 Conferences Attended by ICIPE Staff

World Health Organization (WHO) Steering Committee on Epidemiology, Vector Biology and Control of African Trypanosomiasis: Geneva, Switzerland, January.

R. D. Dransfield

7th Annual Medical Research Conference of the Kenya Medical Research Institute (KEMRI) and Kenya Trypanosomiasis Research Institute (KETRI): Nairobi, Kenya, 2-6 February.

J. B. Kadu

Regional Development and Implementation of Tsetse Control Strategies in Eastern Africa with Emphasis on Targets and Traps: Kenya Trypanosomiasis Research Institute (KETRI), Muguga, Kenya, 7-8 March.

L. H. Otieno

2nd Eastern, Central and Southern Africa Regional Maize Workshop: Harare, Zimbabwe, 15-20 March.

J. K. O. Ampofo, S. K. Firempong, K. N. Saxena

19th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC): Lomé, Togo, 30 March-3 April.

W. G. Z. O. Jura

Consultative Group for International Agricultural Research (CGIAR) Documentation and Information Services Meeting: Lima, Peru, 4-8 May.

N. N. Nsubuga

18th Annual Convention of the Pest Control Council of the Philippines: Davao City, Philippines, 5-8 May.

Z. R. Khan

Training Workshop for Authors and Editors of Science Publications for Children in Africa: Nairobi, Kenya, 18-21 May.

S. W. Mwanjycky

Seminar on Irrigation Policies in Kenya and Zimbabwe: Harare, Zimbabwe, 25-29 May.

J. W. Ssenyonga

International Workshop on Research on Control Strategies for Leishmaniasis: Ottawa, Canada, 1-4 June.

M. J. Mutinga

Beyond Hunger: Africa's Future 1957-2057, Workshop: African Academy of Sciences, Council for the Development of Economic and Social Research in Africa, Alan Shawn Feinstein World Hunger Program, Kericho, Kenya, 1-5 June.

A. Pala-Okeyo

Management of Agricultural Systems Research: Njoro, Kenya, 1-6 June.

D. S. Rubin

5th International Conference, International Federation of Scientific Editors' Associations (IFSEA): Hamburg, West Germany, 14-19 June.

S. W. Mwanjycky, L. Okola

Meeting of the African Academy of Sciences: Rabat, Morocco, 17-19 June.

N. N. Massamba

Workshop on Population Dynamics of Spider Mites and Predatory Mites: Amsterdam, Netherlands, 5-10 July.

L. M. Rogo

4th Annual Meeting of the International Society of Chemical Ecology: Hull, UK, 13-17 July.

A. Hassanali, W. Lwande, P. G. McDowell

28th Annual Meeting of the American Society of Pharmacognosy: Kingston, Rhode Island, USA, 19-22 July.

W. Lwande

Annual Conference of the Society of Invertebrate Pathology: Gainesville, Florida, USA, 20-24 July.

G. P. Kaaya, M. O. Odindo

Workshop on Training Needs for Agricultural Research in Eastern and Southern Africa: Arusha, Tanzania, 20-24 July.

M. E. Smalley

International Commission on Protozoology Meeting: Tsukuba, Japan, 28-30 July.

L. H. Otieno

62nd Annual Meeting of the American Society of Parasitologists (Joint Meeting with the Wildlife Disease Association): Lincoln, Nebraska, USA, 2-6 August.

M. J. Mutinga, D. K. Punyua

12th Conference of the World Association for the Advancement of Veterinary Parasitology: Montreal, Canada, 12-15 August.

P. B. Capstick, J. J. De Castro, A. A. Latif, A. O. Mongi, R. M. Newson

National Symposium on Malaria in Kenya: Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, 15-16 August.

C. M. Mutero

World Veterinary Congress: Montreal, Canada, 16-21 August.

P. B. Capstick, J. J. De Castro, A. A. Latif

3rd Mediterranean Conference of Parasitology: Jerusalem, Israel, 23-27 August.

J. B. Kaddu, C. M. Mutero

International Symposium on Maize Arthropods: Budapest, Hungary, 24-29 August.

J. K. O. Ampofo

International Conference on the Regulation of Insect Reproduction: Zinkovy, Czechoslovakia, 31 August-5 September.

M. F. B. Chaudhury

International Conference on Africa: The Challenge of Economic Recovery and Development: Abuja, Nigeria, August.

A. Pala-Okeyo

Seed Production Workshop: Organization of African Unity (OAU), Semi-Arid Food Grain Research and Development Consultative Advisory Committee (SAFGRAD), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi, Kenya, 13-18 September.

M. O. Odindo, E. O. Omolo, S. K. V. Reddy, K. N. Saxena

24th Trypanosomiasis Seminar: British Society for Parasitology, Bristol, UK, 17-18 September.

L. H. Otieno

Workshop on Characterization of the MHC Class II Region and Products and Their Association with Disease Resistance/Susceptibility: International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya, 28 September-1 October.

A. O. Mongi

11th International Congress of Plant Protection: Manila, Philippines, 5-9 October.

Z. R. Khan

Regional Study Workshop on On-Farm and Post-Harvest Losses of Cereal Crops in Africa Due to Pests and Diseases: ICIPE, Nairobi, Kenya, 11-16 October.

J. K. O. Ampofo, S. K. V. Reddy

Workshop on Integrated Tsetse and Trypanosomiasis Control in Uganda: Mukono, Uganda, October.

R. D. Dransfield

International Development Research Centre (IDRC)/ICIPE Course on Modalities and Techniques for Research Dissemination: Nairobi, Kenya, 15-18 November.

S. W. Mwanjycky

International Workshop on Sorghum Stem Borers: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India, 17-20 November.

J. G. Kibuka, J. D. Onyango, S. K. V. Reddy, K. N. Saxena

National Meeting of Entomological Society of America: Boston, Massachusetts, USA, 29 November-3 December.

Z. R. Khan

International Conference on Medical and Veterinary Dipterology: Ceske, Czechoslovakia, 30 November-4 December.

R. K. Saini

Tanzania Veterinary Association Meeting: Arusha, Tanzania, 1-3 December.

A. O. Mongi

7th Annual Meeting and Scientific Conference: African Association of Insect Scientists (AAIS), Dakar, Senegal, 4-12 December.

J. W. Chiera, J. A. Kongoro, C. M. Mutero, M. O. Odindo, L. M. Rogo

1987 Publications by ICIPE Staff

- Ampofo, J. K. O.; Kidiavai, E. L. *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) larval movement and growth on maize plants in relation to plant age and resistance or susceptibility. *J. Appl. Entomol.* 103: 483-488.
- Arshad, M. A.; Schnitzer, M. The chemistry of a termite fungus comb. *Plant Soil* 93: 247-256.
- Awiti, L. R. S.; Nyindo, M.; Dhadialla, T. S.; Chintawi, M. Ultrastructural characterization of cultured embryonic cells of *Rhipicephalus appendiculatus* Neumann (Acarina: Ixodidae). *Int. J. Insect Morphol. & Embryol.* 16 (5/6): 369-377.
- Barrion, A. A.; Saxena, R. C. Inheritance of body colour in the brown planthopper *Nilaparvata lugens*. *Entomol. exp. appl.* 43: 267-270.
- Bentley, M. D.; Hassanali, A.; Lwande, W.; Njoroge, P. E. W.; Ole Sitayo, E. N.; Yatagai, M. Insect antifeedants from *Tephrosia elata* Defflers. *Insect Sci. Applic.* 8 (1): 85-88.
- Birley, M. H.; Mutero, C. M.; Turner, I. F.; Chadwick, P. R. The effectiveness of mosquito coils containing esbiothrin under laboratory and field conditions. *Ann. Trop. Med. Parasitol.* 81 (2): 163-171.
- Brightwell, R.; Dransfield, R. D.; Kyorku, C.; Golder, T. K.; Tarimo, S. A.; Mungai, D. A new trap for *Glossina pallidipes*. *Trop. Pest Management* 33 (2): 151-159.
- Capstick, P. B. Tick control: the need for a reappraisal. *Kenya Vet.* 10: 30-31.
- Darlington, J. P. E. C. Primary reproductives and royal cells of the termite *Macrotermes michaelseni*. *Insect Sci. Applic.* 8 (1): 121-128.
- De Castro, J. J. Effects of artificial and natural tick infestations on cattle. In: Sutherst, R. W., ed. *Ticks and Tick-Borne Diseases. Proceedings of an international workshop on the ecology of ticks and epidemiology of tick-borne diseases, held in Nyanga, Zimbabwe, 17-21 February 1986.* Australian Centre for International Agricultural Research, ACIAR Proceedings No. 17, pp. 113-115.
- . Effects of field tick infestation on Boran (*Bos indicus*) cattle with differing previous exposures to ticks. In: Sutherst, R. W., ed. *Ticks and Tick-Borne Diseases. Proceedings of an international workshop on the ecology of ticks and epidemiology of tick-borne diseases, held in Nyanga, Zimbabwe, 17-21 February 1986.* Australian Centre for International Agricultural Research, ACIAR Proceedings No. 17, p. 159.
- . Host resistance to ticks. *Kenya Vet.* 10: 32.
- De Lima, C. P. F. Insect pests and postharvest problems in the tropics. *Insect Sci. Applic.* 8 (4/5/6): 673-676.
- Golder, T. K.; Patel, N. Y.; Darji, N. The effect of *Trypanosoma brucei* infection on the localization of salivary gland cholinesterase in *Glossina morsitans morsitans*. *Acta Trop.* 44: 325-331.
- Hassanali, A.; Bentley, M. D. Comparison of the insect antifeedant activities of some limonoids. In: Schmutterer, Ascher, K. R. S.; eds. *Proceedings of the 3rd International Neem Conference.* G.T.Z. Publications, Eschborn. pp. 683-689.
- Hassanali, A.; Bentley, M. D.; Slawin, A. M. Z.; Williams, D. J.; Shephard, R. N.; Chapya, A. W. Pedonin, a spiro tetranortri-terpenoid insect antifeedant from *Harrisonia abyssinica*. *Phytochemistry* 26 (2): 573-575.
- Irungu, L. W. Studies on the *in vitro* exsheathment of *Brugia pahangi*-2. The *in vitro* exsheathment of *B. pahangi* microfilariae incubated with mosquito tissues and cells. *Insect Sci. Applic.* 8 (1): 49-51.
- . Studies on the *in vitro* exsheathment of *Brugia pahangi*-1. The effects of abdomen and midgut homogenates of susceptible *Aedes aegypti* compared with refractory *Anopheles stephensi* and *Culex quinquefasciatus* on endopeptidase induced *in vitro* exsheathment of *B. pahangi*. *Insect Sci. Applic.* 8 (2): 229-234.
- Kaaya, G. P. Future prospects of biological control of tsetse. *Kenya Vet.* 10: 5.
- Kaaya, G. P.; Darji, N.; Otieno, L. H. Effects of bacteria, antibacterial compounds and trypanosomes on tsetse reproduction and longevity. *Insect Sci. Applic.* 8 (2): 217-220.
- Kaaya, G. P.; Flyg, C.; Boman, H. G. Insect immunity: induction of cecropin and attacin-like antibacterial factors in the haemolymph of *Glossina morsitans morsitans*. *Insect Biochem.* 17 (2): 309-315.
- Kokwaro, E. D.; Okot-Kotber, B. M.; Odhiambo, T. R.; Murithi, J. K. Biochemical and immunochemical evidence for the origin of the spermatophore material in *Glossina morsitans morsitans* Westwood. *Experientia* 43: 448-451.
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- Lwande, W.; Bentley, M. D.; MacFoy, C.; Lagemwa, F. N.; Hassanali, A.; Nyandat, E. A new pterocarpin from the roots of *Tephrosia hildebrandtii*. *Phytochemistry* 26 (8): 2425-2426.
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- Mutero, C. M.; Birley, M. H. Estimation of the survival rate and oviposition cycle of field populations of malaria vectors in Kenya. *J. Appl. Ecol.* 24: 858-863.
- Mutinga, M. J.; Kamau, C. C.; Mwandandu, J. Laboratory investigations on the survival and fecundity of *Phlebotomus duboscqi* (Diptera: Psychodidae), a vector of *Leishmania major* in Kenya. *Trop. Med. Parasitol.* 38: 86-88.
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- . Current observations on development and survival of *Rhipicephalus appendiculatus* in Kenya. In: Sutherst, R. W., ed. *Ticks and Tick-Borne Diseases. Proceedings of an international workshop on the ecology of ticks and epidemiology of tick-borne diseases, held in Nyanga, Zimbabwe, 17-21 February 1986.* Australian Centre for International Agricultural Research, ACIAR Proceedings No. 17, pp. 44-46.
- Newson, R. M.; Chiera, J. W. Some effects of acquired resistance in cattle on populations of *Rhipicephalus appendiculatus* in the

- field. In: Sutherst, R. W., ed. Ticks and Tick-Borne Diseases. Proceedings of an international workshop on ecology of ticks and epidemiology of tick-borne diseases, held in Nyanga, Zimbabwe, 17-21 February 1986. Australian Centre for International Agricultural Research, ACIAR Proceedings No. 17, p. 60.
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1987 Personnel*

* as at 31 December 1987

OFFICE OF THE DIRECTOR

Professor T. R. Odhiambo, *director*
 Dr. D. L. Haynes, *deputy director*
 Mrs. G. M. Ochola, *personal assistant to the director*
 Miss R. N. Runo, *senior administrative secretary*
 Mrs. R. J. Kemei, *senior secretary*
 Miss S. M. Kagonda, *senior secretary*
 Miss S. A. Omondi, *secretary*
 Mr. D. A. Odhiambo, *clerical assistant/telex operator*
 Mr. A. O. Were, *clerical assistant*
 Mr. J. K. Kibor, *senior driver*
 Mr. J. M. Mwangangi, *driver*
 Mr. O. E. Ogallo, *driver*
 Mr. J. O. Otunge, *driver*
 Mr. J. L. Mwangai, *messenger*

Planning and Development Unit

Mrs. R. A. Odingo, *chief planning officer*
 Mr. V. S. Mutisya, *principal internal auditor*
 Miss M. H. Bugembe, *senior planning officer*
 Mrs. C. D. Clarke, *planning officer*
 Mrs. M. H. Cashin, *planning and finance development officer*
 Miss D. W. Mwangi, *audit assistant*
 Miss B. A. Muganda, *secretary*

CROP PESTS RESEARCH PROGRAMME

Plant Resistance to Insect Pests

Professor K. N. Saxena, *senior principal research scientist/programme leader*
 Dr. R. S. Pathak, *senior research scientist*
 Dr. J. K. O. Ampofo, *senior research scientist*
 Dr. S. K. Firempong, *postdoctoral research fellow*
 Dr. P. G. Tokro, *research associate*
 Dr. T. O. Tayo, *research associate*
 Dr. S. H. O. Okech, *senior scientific officer*
 Miss E. Bonitatibus, *scientific officer*
 Mr. J. D. Onyango, *research assistant*
 Mr. J. C. Olela, *chief technician*
 Mr. S. M. Othieno, *principal technician*
 Mr. E. O. Nyangiri, *principal technician*
 Mr. J. G. Kibuka, *technician*
 Mr. F. D. Odawa, *technician*
 Mr. E. L. Kidiavai, *technician*
 Mr. M. O. Arwa, *technical assistant*
 Mr. S. M. Otieno, *field assistant*
 Mr. J. O. Ogoro, *field assistant*
 Mr. P. O. Omolo, *field assistant*
 Mr. P. O. Akello, *field assistant*
 Mr. J. O. Ngare, *field assistant*
 Mr. P. O. Okello, *field assistant*

Mr. J. O. Adero, *field assistant*
 Mr. J. O. Ondijo, *field assistant*
 Miss J. A. Oduol, *field assistant/copy typist*
 Mrs. R. A. Okoth, *senior secretary*
 Mrs. H. A. Abade, *secretary*
 Mr. W. Jayatileka, *driver*
 Mr. S. G. Ogechi, *driver*
 Mr. J. K. Mokaya, *driver*

Bionomics and Applied Ecology

Dr. K. V. Seshu Reddy, *senior research scientist/section head*
 Dr. E. O. Omolo, *senior research scientist**
 Dr. G. C. Unnithan, *senior research scientist*
 Dr. A. M. Alghali, *research scientist†*
 Dr. T. Okuda, *research scientist*
 Dr. V. A. Okoth, *postdoctoral research fellow*
 Mr. F. O. Oduol, *postgraduate research fellow§*
 Miss R. A. Nyangor, *senior research assistant*
 Mr. K. O. Sum, *research assistant*
 Mr. C. O. Simbi, *senior technician*
 Mr. M. C. Lubega, *senior technician*
 Mr. P. O. Ollimo, *senior technician*
 Mr. S. O. Paye, *technician*
 Mr. G. O. Amala, *junior technician*
 Mr. D. O. Nyagol, *field assistant*
 Mr. I. O. Odhul, *field assistant*
 Mr. J. A. Onyango, *field assistant*
 Mr. D. A. Atieno, *field assistant*
 Mr. P. A. Orenge, *field assistant*
 Mr. G. S. Odhiambo, *field assistant*
 Mr. W. O. Owuor, *field assistant*

* Seconded from PESTNET

† Based at IITA

§ Study leave

Biological Control

Dr. G. W. Oloo, *senior research scientist/section head*
 Dr. M. O. Odindo, *senior research scientist*
 Dr. W. A. Otieno, *research scientist*
 Dr. M. Brownbridge, *postdoctoral research fellow*
 Dr. L. M. Rogo, *senior scientific officer*
 Mr. J. B. Okeyo-Owuor, *scientific officer*
 Mr. L. Quin-Guang, *postgraduate research fellow*
 Mr. K. Ogedah, *research assistant*
 Mr. K. K. Oyugi, *research assistant**
 Mr. C. O. Ogol, *research assistant*
 Mr. Z. O. Ngalo, *research assistant*
 Mr. J. T. Kilori, *senior technician*
 Mr. O. Mbai, *senior technician*

Mr. R. C. Okello, *technician*
 Mr. R. C. Odhiambo, *technician*
 Mr. P. A. Amutalla, *junior technician*
 Mr. P. O. Agwaro, *junior technician*
 Mr. M. T. Lusele, *junior technician*
 Mr. L. O. Were, *junior technician*
 Mr. J. O. Ochieng[†], *field assistant*
 Mr. M. O. Odoyo, *field assistant*
 Mr. J. O. Awendo, *field assistant*
 Mr. R. O. Okange, *field assistant*
 Mr. J. A. Nyawach, *field assistant*
 Mrs. J. A. Okello, *field assistant*
 Mr. M. Y. Oriwo, *field assistant*
 Miss T. A. Odero, *field assistant*
 Mrs. D. T. Ongondo, *field assistant/copytypist*

* Study leave

Insect Mass-Rearing Technology Mbita Point Field Station

Dr. R. S. Ochieng[†], *research scientist/section head*
 Mr. J. P. Odero, *research assistant**
 Mr. H. K. Banda, *senior technician*
 Mr. F. O. Onyango, *senior technician*
 Mr. M. D. Bungu, *technician*
 Mr. P. E. Njoroge, *technician*†
 Mr. E. O. Amboga, *junior technician*
 Mr. B. O. Ogal, *technical assistant*
 Mr. J. O. Osuri, *field assistant*
 Mrs. J. N. Kunyu, *field assistant*
 Mr. P. A. Nyakwamba, *field assistant*
 Mr. S. O. Okoth, *field assistant*

* Study leave

† Based in Nairobi

Nairobi

Mr. J. Wanyonje, *chief technician/controller for insectary services*
 Mr. J. M. Kagoyia, *principal technician*
 Mr. A. S. Ikhunyalo, *technician*
 Mr. J. H. Ongudha, *technician*
 Mr. E. O. Awuoche, *technician*
 Mr. G. M. Birir, *junior technician*
 Mrs. R. G. Kariuki, *junior technician*
 Miss M. G. Wanjiru, *junior technician*
 Mr. J. K. Gitegi, *junior technician*
 Mr. G. M. Ng'ang'a, *junior technician*
 Mr. S. M. Mbugua, *technical assistant*
 Mr. J. O. Omolo, *technical assistant*
 Mr. N. M. Mwikya, *technical assistant*

United Nations Economic Commission for Africa/ ICIPE/Kenya Government Special Project

Professor K. N. Saxena, *project coordinator*
 Dr. K. V. Seshu Reddy, *entomologist*
 Dr. E. O. Omolo, *agronomist*
 Dr. A. Pala-Okeyo, *social anthropologist*
 Mr. L. Ngode, *national project officer**
 Mr. C. O. Okoth, *field technician*
 Mr. R. O. Mbuga, *field technician*
 Mrs. M. O. Olweny, *field technician*
 Mr. D. O. Ombuoro, *field technician**
 Mr. T. O. Oyoyo, *field technician**
 Mr. P. A. K'Odondi, *field technician**
 Mr. M. O. Owino, *field assistant*
 Mr. F. S. Odawo, *driver*

* Seconded from the Kenya Government Ministry of Agriculture

ICIPE/IITA Cassava Green Spider Mite Special Project

Dr. J. Bartkowski, *postdoctoral research fellow*

Mr. C. K. Ogol, *research assistant*
 Mr. J. O. Obilo, *field assistant*
 Miss J. A. Ong'oma, *field assistant*
 Mr. J. Obara, *field assistant*
 Mr. A. G. Oduol, *field assistant*
 Mr. P. O. Wagara, *field assistant*
 Miss B. Mbati, *copytypist*

ICIPE/IITA Cowpea Special Project

Dr. J. D. Ehlers, *research scientist**
 Mr. J. O. Okumu, *field assistant*
 Mr. W. J. Odhiambo, *field assistant*
 Mr. G. O. Asino, *field assistant*
 Mr. S. P. Ojwang, *field assistant*

* Seconded from IITA

ICIPE/IRRI Special Project on the Rice Brown Planthopper

Dr. Z. R. Khan, *research scientist/team leader**

* Based at IRRI

LIVESTOCK TICKS RESEARCH PROGRAMME

Dr. P. B. Capstick, *principal research scientist/programme leader*
 Dr. R. M. Newson, *senior research scientist*
 Dr. K. S. Nokoe, *senior research scientist*
 Dr. M. A. Nyindo, *senior research scientist*
 Dr. J. J. de Castro, *research scientist*
 Dr. A. O. Mongi, *research scientist*
 Dr. J. Ssenyonga, *scientist-in-residence**
 Dr. R. Knechtli, *research associate*
 Dr. F. Gigon, *research associate*
 Dr. A. A. Latif, *postdoctoral research fellow*†
 Dr. E. I. Kamanga-Sollo, *postdoctoral research fellow*
 Mr. D. K. Punyua, *senior scientific officer*†
 Mr. J. W. Chiera, *research assistant*
 Mr. C. A. Aganyo, *chief technician*
 Mr. M. M. Malonza, *senior technician*
 Mr. R. Ojowa, *senior technician*
 Miss R. Chesang, *senior technician*
 Mr. P. O. Ngoko, *technician*†
 Mr. J. G. Mugane, *junior technician*
 Mr. P. Muteria, *junior technician*
 Mr. F. M. Thuo, *junior technician*
 Mr. M. G. Kimondo, *junior technician*
 Mr. J. N. Ndungu, *junior technician*
 Mr. G. M. Hindi, *technical assistant*
 Mr. M. Gitau, *field assistant*
 Mr. M. K. Jamia, *field assistant*
 Mr. J. Opere, *field assistant*
 Mr. K. O. Ochung, *field assistant*
 Mr. A. O. Sanga, *field assistant*†
 Mr. J. N. Odhiambo, *field assistant*†
 Miss V. M. Nderitu, *secretary*
 Mr. G. M. Kinyanjui, *driver*

* Seconded from the Social Science Interface Research Project

† Based at MPFS

MEDICAL VECTORS RESEARCH PROGRAMME

Dr. M. J. Mutinga, *principal research scientist/programme leader*
 Dr. J. B. Kaddu, *research scientist*
 Dr. N. N. Massamba, *research scientist*
 Dr. C. M. Muteru, *research scientist*
 Mr. C. C. Kamau, *research assistant*
 Mr. F. A. Amimo, *research assistant*
 Mr. B. N. Odero, *chief technician*
 Mr. M. P. Nyamori, *senior technician*
 Mr. F. M. Kyai, *junior technician*
 Mr. D. M. Omogo, *junior technician*
 Mr. J. Mwandandu, *junior technician*
 Mr. R. M. Musyoki, *junior technician*

Mr. P. K. Munguti, *technical assistant*
 Mr. S. Mutua, *technical assistant*
 Mr. D. M. Mativo, *technical assistant*
 Miss R. O. Omeno, *laboratory assistant*
 Mr. J. Ndambuki, *laboratory assistant*
 Mr. R. K. Rotich, *laboratory assistant*
 Mr. P. B. Chepkoimet, *field assistant*
 Mr. W. Mulwa, *field assistant*
 Mr. D. Mbavu, *field assistant*
 Mr. S. Singi, *field assistant*
 Mr. P. Munywoki, *field assistant*
 Mr. R. Muoki, *field assistant*
 Mr. P. Wandei, *field assistant*
 Mr. M. Miti, *field assistant*
 Mr. B. M. Muia, *field assistant*
 Mr. P. O. Manyuanda, *field assistant*
 Miss D. T. Adhiambo, *senior administrative secretary*
 Mr. R. M. Mogaka, *driver/mechanic*
 Mr. G. K. Too, *driver*
 Miss C. Cheboiwa, *cleaner*
 Mr. J. Mgorma, *watchman*
 Mr. P. Lokwawi, *gardener*

TSETSE RESEARCH PROGRAMME

Dr. L. H. Otieno, *principal research scientist/programme leader*
 Dr. M. F. B. Chaudhury, *senior research scientist*
 Dr. R. D. Dransfield, *senior research scientist*
 Dr. G. P. Kaaya, *senior research scientist*
 Dr. S. R. Tarimo, *research scientist*
 Dr. R. S. Copeland, *postdoctoral research fellow*
 Dr. L. W. Irungu, *postdoctoral research fellow*
 Mrs. M. L. Owaga, *senior scientific officer*
 Miss N. F. Darji, *principal research assistant*
 Mr. R. Brightwell, *research assistant*
 Mr. P. O. Agutu, *principal technician*
 Mr. E. Mpanga, *senior technician*
 Mr. S. S. Wakape, *technician*
 Mr. C. O. Machika, *technician*
 Mr. D. P. Uvyu, *technician*
 Mr. M. O. Kotengo, *technician*
 Mr. M. Reuben, *technician*
 Mr. P. M. Mwamisi, *junior technician*
 Mr. J. K. Kiulu, *junior technician*
 Miss E. M. Mwangi, *junior technician/driver*
 Mr. D. K. Mungai, *technical assistant/driver*
 Mr. J. Likhanga, *technical assistant/driver*
 Mr. J. M. Muchiri, *technical assistant*
 Miss E. Afandi, *secretary*
 Mr. Z. M. Muriuki, *driver*

CHEMISTRY AND BIOCHEMISTRY RESEARCH UNIT

Dr. A. Hassanali, *senior research scientist/unit head*
 Dr. P. G. McDowell, *research scientist*
 Dr. T. S. Dhadialla, *research scientist*
 Dr. W. Lwande, *research scientist*
 Dr. S. Essuman, *postdoctoral research fellow*
 Dr. E. O. Osir, *postdoctoral research fellow*
 Dr. J. I. Jondiko, *postdoctoral research fellow*
 Mrs. R. M. Vundla, *senior scientific officer*
 Mrs. M. A. Okech, *scientific officer*
 Mr. W. P. Ouma, *research assistant*
 Mr. A. W. Chapya, *chief technician*
 Mr. E. N. Ole Sitayo, *senior technician*
 Mr. E. Nyandat, *senior technician*
 Mr. L. V. Labongo, *technician*
 Mr. L. M. Moreka, *junior technician*
 Mr. P. O. Amoke, *junior technician*
 Mr. G. V. Achieng', *junior technician*
 Mr. H. Amiani, *field assistant*
 Mrs. S. M. Otieno, *secretary*

HISTOLOGY AND FINE STRUCTURE RESEARCH UNIT

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 Dr. W. G. Jura, *postdoctoral research fellow*
 Mrs. J. A. Kongoro, *senior research assistant*
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 Mr. M. M. Chimtawi, *chief technician*
 Mrs. J. K. Murithi, *principal technician*
 Mr. N. T. Ogoma, *junior technician*
 Mrs. M. M. Olutatwa, *secretary*

SENSORY PHYSIOLOGY RESEARCH UNIT

Dr. S. M. Waladde, *research scientist/acting unit head*
 Dr. R. K. Saini, *research scientist*
 Dr. M. E. Hussein, *postdoctoral research fellow*
 Mr. H. M. Kahoro, *principal technician*
 Mr. S. A. Ochieng', *technician*
 Mrs. M. N. Baraza, *technician*
 Mr. P. O. Ahuya, *junior technician*

BIOSTATISTICS AND COMPUTER SERVICES UNIT

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 Dr. H. F. Magalit, *senior research scientist**
 Dr. K. S. Nokoe, *senior research scientist*
 Dr. B. G. Williams, *senior research scientist*
 Mrs. W. N. Ssebunnya, *senior systems analyst*
 Mr. J. K. Maina, *senior computer programmer*
 Mr. S. O. Lota, *computer engineer*
 Mr. O. O. Okello, *assistant statistician*
 Mrs. P. M. Alila, *office systems supervisor†*
 Miss B. A. Nanga, *senior secretary*

* Based at MPFS

† On leave of absence

OUTREACH AND TRAINING UNIT

Dr. Z. M. Nyiira, *senior principal research scientist/unit head*
 Mrs. J. K. Eyobo, *senior secretary*
 Mr. J. Isedia, *messenger*

ARPPIS: African Regional Postgraduate Programme in Insect Science

Dr. M. E. Smalley, *ARPPIS academic coordinator*
 Mr. I. G. Aniedu, *Ph.D. student 1985 class*
 Mr. M. Basimike, *Ph.D. student 1985 class*
 Mr. C. A. Kyorku, *Ph.D. student 1985 class*
 Mrs. R. C. Sang, *Ph.D. student 1985 class*
 Mr. G. Tikubet, *Ph.D. student 1985 class*
 Mr. B. Torto, *Ph.D. student 1985 class*
 Mr. B. E. Wishitemi, *Ph.D. student 1985 class*
 Miss G. O. Akpokodje, *Ph.D. student 1986 class*
 Mr. M. Gethi, *Ph.D. student 1986 class*
 Mr. E. B. Karamura, *Ph.D. student 1986 class*
 Mr. M. Wa Macharia, *Ph.D. student 1986 class*
 Miss E. M. Minja, *Ph.D. student 1986 class*
 Mr. P. K. Muange, *Ph.D. student 1986 class*
 Mrs. M. F. Ndonga, *Ph.D. student 1986 class*
 Mr. M. A. Njau, *Ph.D. student 1986 class*
 Mr. J. O. Davies-Cole, *Ph.D. student 1987 class*
 Mr. H. Mahamat, *Ph.D. student 1987 class*
 Mr. S. K. Mbogo, *Ph.D. student 1987 class*
 Mr. T. N. Murega, *Ph.D. student 1987 class*
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 Mrs. E. N. Mwangi, *Ph.D. student 1987 class*
 Mrs. V. S. Nyambati, *Ph.D. student 1987 class*
 Mr. J. Ogwang', *Ph.D. student 1987 class*
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 Miss R. Bob-Manuel, *M.Sc. student 1985 class*
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 Mr. K. O. Kambona, *M.Sc. student 1986 class*

Mr. G. R. Ochiel, *M.Sc. student 1986 class*
 Mr. B. Odongo, *M.Sc. student 1986 class*
 Mr. A. B. Fonti Kanu, *M.Sc. student 1987 class*
 Mr. S. I. Kamara, *M.Sc. student 1987 class*
 Mr. J. Mbapila, *M.Sc. student 1987 class*
 Miss A. Ngi-Song, *M.Sc. student 1987 class*
 Mrs. A. A. Okumali, *senior secretary*
 Mrs. M. S. Myendo, *secretary*
 Mr. D. I. Isoso, *driver*

Training

Mr. J. F. Omenge, *senior training officer*
 Mrs. M. Antao, *senior secretary*
 Mr. L. Maina, *driver*

FAMESA: Financial and Administrative Management of Research Projects in Eastern and Southern Africa

Dr. Z. M. Nyiira, *coordinator*
 Mrs. M. U. Arara, *senior secretary*

PESTNET: African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests

Dr. E. O. Omolo, *scientific coordinator*
 Mrs. J. K. Eyobo, *senior secretary*

SOCIAL SCIENCE INTERFACE RESEARCH PROJECT

Dr. A. Pala-Okeyo, *senior research scientist/project leader*
 Dr. E. O. Omolo, *senior research scientist**
 Dr. D. S. Rubin, *scientist-in-residence†*
 Dr. J. W. Ssenyonga, *scientist-in-residence*
 Mrs. E. Nzioki, *graduate research assistant*
 Mr. B. A. Omolo, *technician*
 Mr. S. Ambogo, *technical assistant*
 Mrs. M. A. Warrakah, *secretary*
 Mr. E. Kongere, *driver*

* Seconded from PESTNET

† Rockefeller Foundation postdoctoral fellow

ADMINISTRATION AND INFORMATION DIVISION

Mr. L. Okola, *manager for administration and information*
 Mrs. M. R. Opande, *senior administrative secretary*

Human Resources Department

Mr. J. E. Okiri, *principal administrative officer*
 Miss M. W. Wafula, *senior secretary*
 Mrs. J. J. Gombe, *senior secretary*
 Mrs. P. N. Owitti, *senior secretary*
 Mr. J. M. Mwendar, *clerical assistant*

Administrative Services Department

Mr. H. G. Awich, *chief administrative officer*
 Mr. M. M. Moinde, *principal administrative officer*
 Mrs. P. A. Oriwa, *transport/security officer*
 Miss G. M. Wachuru, *senior secretary*
 Mrs. G. M. Weya, *senior telephonist/receptionist*
 Mrs. M. M. Mohochi, *receptionist/telephonist*
 Mr. R. M. Ng'ang'a, *automobile foreman*
 Mr. F. O. Hamala, *senior mechanic*
 Mr. J. O. Oduol, *senior mechanic*
 Mr. A. J. Ombija, *senior mechanic*
 Mr. S. A. Akhaya, *janitor*
 Mr. J. Elegwa, *assistant mail clerk*
 Mr. P. N. Mahugu, *senior driver*
 Mr. S. N. Achochi, *driver*
 Mr. R. K. Gathu, *junior technician/driver*
 Mr. C. N. Kageche, *driver*
 Mr. E. N. Kiio, *driver*
 Mr. P. Otiende, *driver*
 Mr. P. O. Owuor, *driver*

Mr. S. N. Rukungu, *driver*
 Mr. L. W. Kisutia, *machine operator*
 Mr. E. Asami, *cleaner*
 Mr. A. M. Bubusi, *cleaner*
 Mr. D. K. Chege, *cleaner*
 Mr. L. L. Ayekha, *gardening assistant*
 Mr. S. A. Abdalla, *security guard*
 Mr. W. Achiroma, *security guard*
 Mr. T. S. Ekisa, *security guard*
 Mr. A. J. Laban, *security guard*
 Mr. A. N. Makori, *security guard*
 Mr. W. W. Mayiinga, *security guard*
 Mr. A. M. Muhindi, *security guard*
 Mr. F. M. Muindi, *security guard*
 Mr. C. K. Mulela, *security guard*
 Mr. A. A. Muyanda, *security guard*
 Mr. D. M. Mwilu, *security guard*
 Mr. J. D. Nyawalo, *security guard*
 Mr. J. N. Nzioka, *security guard*
 Mr. J. O. Odero, *security guard*
 Mr. A. A. Ogaja, *security guard*
 Mr. J. A. Ole Kobaai, *security guard*
 Mr. E. H. Otieno, *security guard*
 Mr. M. A. Ouma, *security guard*
 Mr. D. O. Singa, *security guard*
 Mr. M. O. Songa, *security guard*
 Mr. M. O. Yongason, *security guard*

Publishing and Documentation Department

Mr. L. Okola, *manager*
 Miss S. A. MacMillan, *managing editor*
 Mrs. S. W. Mwanjyck, *science editor*
 Mr. N. S. Nsubuga, *senior librarian*
 Miss M. W. Mathai, *librarian**
 Mrs. W. A. Oyuko, *production officer (graphics)*
 Miss D. Munene, *typesetter/secretary*
 Mrs. R. P. Ortega, *documentalist*
 Mr. N. M. Komeri, *scientific illustrator*
 Mr. E. W. Mwangi, *paste-up artist*
 Miss E. N. Kahuhu, *library assistant*
 Miss A. W. Muhato, *senior secretary*
 Mrs. Y. Obiero, *secretary*
 Mrs. D. O. Odhiambo, *proofreader*
 Mr. Z. B. Ooko, *library assistant**
 Mr. A. Shisoka, *clerical assistant*

* Based at Mbita Point Field Station

Communication Services Department

Miss R. A. Washika, *principal communication officer*
 Mr. M. P. Arrumm, *senior communication and protocol officer*
 Mrs. J. A. Ojuka, *secretary*

Capital Development (Duduville Phase II) Project

Mr. R. Jackson, *project manager*
 Mrs. G. N. Gathura, *secretary*

FINANCE DIVISION

Mr. E. J. English, *financial manager*
 Mr. R. M. Okura, *principal accountant*
 Mr. G. W. Kanza, *senior accountant*
 Mr. A. A. Oguda, *accountant*
 Mr. R. Otieno, *accountant*
 Mr. V. M. Kamanyi, *assistant accountant*
 Mrs. L. W. Kimani, *assistant accountant*
 Mr. P. O. Ngugi, *assistant accountant*
 Miss R. A. Nyamunga, *assistant accountant*
 Mr. F. K. Ongola, *assistant accountant*
 Mr. P. Bosire, *accounts assistant*
 Mr. E. Mulwa, *accounts assistant*
 Mr. L. J. Ondieki, *accounts assistant*

Miss L. E. Wanjiku, *accounts assistant*
 Mr. C. M. Oloo, *supplies officer*
 Mr. F. K. Cheserek, *assistant supplies officer*
 Mr. D. M. Olalo, *storekeeper*
 Miss F. Ojode, *senior secretary*
 Mrs. M. Butali, *secretary*
 Mr. J. B. Oyondi, *driver*

INTERNATIONAL GUEST CENTRE SYSTEM

Duduville International Guest Centre

Mr. J. A. Achilla, *senior business and catering controller*
 Mr. S. Aritho, *assistant accountant*
 Mrs. J. R. Musiga, *housekeeper*
 Mr. J. E. Mwangi, *head cook*
 Mr. A. Lweya, *assistant head cook*
 Mr. A. I. Okapesi, *assistant head cook*
 Miss H. N. Githinji, *secretary*
 Mrs. R. M. Wekesa, *telephonist/receptionist*
 Mrs. M. A. Aseto, *telephonist/receptionist*
 Mr. G. Gichuru, *cook*
 Mr. J. M. Mwakisha, *cook*
 Mr. D. K. Yaem, *stores assistant*
 Mr. J. W. Gadonya, *junior technician*
 Mr. P. A. Omollo, *barman/waiter*
 Mr. H. Owilli, *barman/waiter*
 Mr. G. Odero, *barman/waiter*
 Mr. J. Omondi, *barman/waiter*
 Mrs. P. A. Ocholla, *senior room steward*
 Mr. L. M. Mulae, *room steward*
 Mr. A. M. Mutwoli, *room steward*
 Mr. O. Wara, *room steward*
 Mrs. T. Ogongo, *room steward*
 Mr. H. M. Kibisu, *senior launder*
 Mr. C. M. Lumati, *assistant launder*
 Mr. P. Mungithya, *messenger/machine operator*
 Mr. J. O. Mukhobi, *janitorial assistant*
 Mr. S. O. Araka, *driver*
 Mr. L. Ombech, *driver*
 Mr. D. Ojowi, *front office*
 Mr. W. Otieno, *kitchen assistant*
 Mr. W. Mahindu, *kitchen assistant*
 Mr. O. Mbuya, *stores*
 Mr. O. Okech, *cleaner*
 Mr. S. Obondo, *gardener*

Mbita Point Guest Centre

Mr. C. B. Oyieyo, *supervisor*
 Mr. P. Okeyo, *head cook*
 Mrs. H. Ouma, *receptionist/shop assistant*
 Miss J. Weru, *receptionist/shop assistant*
 Mr. F. Okebe, *kitchen assistant*
 Mr. G. Onyango, *kitchen assistant*
 Mr. V. Musoga, *assistant storekeeper*
 Mr. C. Oyata, *barman/waiter*
 Mr. E. Juma, *barman/waiter*
 Mr. C. Odera, *room steward*
 Mr. H. Omala, *assistant room steward*
 Mr. P. Omusembi, *launder*
 Miss M. Awino, *assistant launder*
 Mr. L. A. Nyolo, *junior attendant*
 Mr. P. Ajwala, *general assistant*

WORKSHOPS AND LABORATORY SERVICE UNIT

Nairobi

Mr. J. A. Mando, *principal controller for technical services*
 Mr. R. C. Joshi, *maintenance engineer*
 Mr. A. R. Bhaloo, *electronics and instrument engineer*
 Mr. J. O. Konyino, *electronics and instrument engineer*
 Mr. H. N. Rai, *refrigeration technologist*
 Mr. P. O. Nyachio, *principal technician*
 Mr. J. O. Onyango, *principal technician*
 Mr. J. M. Maina, *principal technician*

Mr. S. O. Obiero, *principal technician*
 Mr. P. O. Auma, *senior technician*
 Mr. T. O. Ocholloh, *senior technician*
 Mr. J. O. Ogalo, *technician*
 Mr. J. B. Omullo, *technician*
 Mr. P. A. Oluya, *technician*
 Mr. J. N. Nyoike, *technician*
 Mr. K. Kinuthia, *technician*
 Mr. J. O. Omondi, *junior technician*
 Mr. C. Kageche, *technical assistant/driver*
 Mr. T. Okal, *technical assistant*

Mbita Point Field Station

Mr. J. A. Mtei, *controller for technical services*
 Mr. P. M. Alianda, *senior technician*
 Mr. T. O. Bwana, *senior technician*
 Mr. P. M. Okwanyo, *senior technician*
 Mr. J. Asanyo, *senior technician*
 Mr. T. L. Ngutu, *technician*
 Mr. R. M. Nzioka, *technician*
 Mr. E. E. Okello, *technician*
 Mr. S. M. Karanja, *junior technician*
 Mr. J. M. Ogare, *junior technician*
 Mr. J. Ogone, *junior technician*
 Mr. O. Ohato, *junior technician*

MBITA POINT FIELD STATION

Dr. V. O. Musewe, *station manager*
 Mr. S. M. Kimaita, *senior administrative officer*
 Mr. W. O. Ogallo, *planning and administrative officer*
 Mr. M. A. Kawaka, *accountant*
 Mr. M. E. Asudi, *accounts assistant*
 Mr. C. N. Keli, *supplies assistant*
 Mr. S. O. Juma, *security officer*
 Mr. S. S. Pertet, *senior janitorial assistant*
 Mrs. G. A. Kwanya, *senior secretary*
 Mrs. M. N. Okach, *assistant secretary*
 Miss D. A. Apondi, *receptionist/telephonist/typist*
 Miss A. W. Makoko, *typist/telephonist*
 Mr. S. L. Andembe, *receptionist/telephonist*
 Mr. J. O. Madiwia, *clerical assistant*
 Mr. R. Nyaridi, *clerical assistant*
 Mr. T. O. Kokelo, *clerical assistant*
 Mr. E. O. Jasor, *clerical assistant*
 Mr. J. A. Kisero, *assistant boat master*
 Mr. C. O. Ojoo, *transport assistant*
 Mr. P. O. Mboya, *senior driver*
 Mr. J. Mokaya, *driver*
 Mr. W. Jayatileka, *driver*
 Mr. S. G. Ogechi, *driver*
 Mr. L. O. Otieno, *driver*
 Mrs. J. A. Ogutu, *office cleaner*
 Mr. S. M. Mkamba, *workshop cleaner*
 Mr. Z. O. Nyandere, *cleaner/messenger*
 Mr. E. A. Sonye, *cleaner/messenger*
 Mr. B. S. Masyanga, *farm controller*
 Mr. E. G. Kabiru, *farm foreman*
 Mr. P. L. Rakwach, *tractor driver/mechanic*
 Mr. J. W. Achola, *farm assistant*
 Mr. F. O. Arum, *farm assistant*
 Mr. P. O. Auta, *farm assistant*
 Mrs. P. Ogito, *farm assistant*
 Mr. J. M. Sagini, *farm assistant*
 Mr. S. O. Odero, *farm assistant*
 Mr. E. K. Ongonge, *farm assistant*
 Mr. J. O. Osumba, *farm assistant*
 Mr. P. O. Ouma, *farm assistant*
 Mr. J. O. Ojunga, *field assistant*
 Mr. D. O. Oyoto, *security guard*
 Mr. D. L. Debe, *security guard*
 Mr. A. Agoro, *security guard*
 Mr. J. J. Okach, *security guard*

Mr. B. W. Okello, *security guard*
 Mr. J. K. Opere, *security guard*
 Mr. D. O. Otuoma, *security guard*
 Mr. B. M. Mogendi, *security guard*
 Mr. H. A. Ngaji, *security guard*
 Mr. W. K. Makori, *security guard*
 Mr. S. Abdalla, *security guard*
 Mr. Z. O. Marenje, *groundsman*
 Mr. V. O. Nyangute, *groundsman*
 Mr. T. A. Owiti, *groundsman*
 Mr. T. K. Adwar, *groundsman*
 Mr. G. O. Ogero, *groundsman*
 Mr. A. W. Not, *groundsman*
 Mrs. P. A. Ochieng', *groundsman*
 Mr. C. O. Adinda, *groundsman*
 Mrs. M. O. Walter, *groundsman*
 Mr. L. O. Okello, *garbage collector*

MBITA POINT INTERNATIONAL SCHOOL

Mrs. P. A. Ogada, *principal*
 Mr. B. C. Ojil, *deputy principal*

Mrs. C. O. Ndiege, *teacher*
 Mr. F. O. Owino, *teacher*
 Mr. D. P. Makachola, *teacher*
 Mr. A. M. Sentamu, *teacher*
 Mr. H. M. Mulwa, *teacher*
 Mr. N. H. Ebrahim, *teacher*
 Mr. Y. M. Koko, *teacher*
 Mr. D. B. Okong'o, *teacher*
 Miss F. B. Esalako, *secretary*
 Miss S. A. Omune, *janitorial assistant*
 Mrs. O. A. Ojwang', *janitorial assistant*

ST. JUDE'S CLINIC

Dr. J. B. Odhiambo, *institutional doctor*
 Mr. J. H. Odoyo, *clinical officer*
 Mrs. S. A. Chybire, *public health nurse*
 Mr. P. M. Kaliech, *laboratory technologist*
 Mr. E. O. Kirowo, *pharmaceutical technologist*
 Mrs. F. K. Mukoko, *clerical assistant*
 Mrs. L. A. Abuya, *janitorial assistant*
 Mr. A. A. Oluoko, *senior driver*

