SIXTEENTH ANNUAL REPORT 1988

The International Centre of Insect Physiology and Ecology

Cover Picture

Maps of *Glossina pallidipes* tsetse fly population density (males left, females right) at the Nguruman study site, produced by the ICIPE's GIS systems. Coloured polygons represent increasing fly density, progressing from grey to purple; fine red lines are contours at 100-metre intervals; broad red lines are roads; white lines are streams. (See also chapters 4.1 and 9.5 of this report.)

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The International Centre of Insect Physiology and Ecology

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Foreword

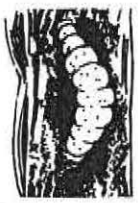
The most difficult task that the International Centre of Insect Physiology and Ecology (ICIPE) ever set itself was, as stated in the International Charter of the ICIPE, "to undertake research on selected insects and arthropods, to study their identity, abundance, distribution, ecology, behaviour, physiology, pathology, genetics, and the application of this knowledge to the problems of integrated pest and vector management problems, as well as the beneficial use of insects" with special reference to Africa and other tropical regions of the world. The crux of the problem is the linkage between advanced mission-oriented research and the technology development for the management of tropical insects within the context of the developing tropical world where the traditional policy is to give exclusive emphasis to applied research.

It has always been the philosophy of the ICIPE to push the frontiers of insect science, not simply for its own sake, but because of its direct relevance to the major tropical pest problems that still defy effective management. It is the scientific and technological strategy of the ICIPE to focus on those basic facets of the biology of these target pests which contain the most prospects for technological management content for special research concentration. This strategy is beginning to pay handsome dividends as can be gleaned from this Annual Report. For instance, the ICIPE project at Nguruman Escarpment in Masailand of south-western Kenya is beginning to highlight the technical efficacy, ecological sustainability, and social acceptability of the visually potent, odour-baited NGU2B tsetse trap as the technique of choice for managing tsetse populations in suppressing trypanosomiasis to negligible levels. Much research and development work is still being undertaken to perfect this tactic, by for example increasing its multi-valent control potentials, but the basic foundation for community-based, long-range control of *Glossina pallidipes* has surely been laid by ICIPE's 15 years of dedicated mission-oriented basic research on tsetse behaviour, population ecology, sensory physiology, reproductive biology, and trypanosomiasis epidemiology. It is this self-same strategy that is being implemented on each of ICIPE's target pests.

The year under review has been a rewarding one; and we hope to develop some ideas on the closing years of the current decade as to how to measure the success of a pest management research institute — which challenges the conventional approaches that commodity-oriented research institutions so easily adopt as yardsticks (such as the rate of spread of a new crop hybrid, or the adoption of a particular cropping system). Such new yardsticks are vitally important for an advanced research institute geared towards solving tropical science-based problems in the context of limited resources.

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

CROP PESTS RESEARCH PROGRAMME



1 Crop Pests Research Programme

The primary goal of the Crop Pests Research Programme (CPRP) is to contribute to a sustainable increase in food production, through reduction of crop losses by insect pests, in developing tropical countries including Africa. To achieve this goal, the CPRP is engaged in research on, and development of, strategies of integrated pest management (IPM) of key insect pests of major food crops by resource-poor small-scale farmers. These strategies must be environmentally safe and economically, as well as technically, feasible for these farmers. The work is therefore being carried out in 4 sections:

- *Bionomics and Applied Ecology (BAE)*
- *Plant Resistance to Insect Pests (PRIP)*
- *Biological Control (BC)*
- *Insect Mass-Rearing Technology (IMRT).*

The following insect pests and the crops which they attack are under study: sorghum and maize stem-borers Chilo partellus, Busseola fusca, Eldana saccharina, Sesamia calamistis *(with emphasis on* C. partellus *as a model); sorghum shootfly* Atherigona soccata *(for ovipositional kairomones); cowpea pod-borer* Maruca testulalis *and aphid* Aphis craccivora; *banana weevil* Cosmopolites sordidus; *cassava green spider mite* Mononychellus spp.; *rice leaffolders* Cnaphalocrocis medinalis *and* Marasmia patnalis, *under the International Rice Research Institute (IRRI)-ICIPE Project based at Los Baños, Philippines) and other pests of upland rice at Mbita Point Field Station (MPFS).*

Development of the IPM components involves basic as well as applied research and is being undertaken in 3 stages: stage 1 involves investigative experiments at the field station and elsewhere; stage 2 involves testing the promising IPM elements on farmers' fields under our management; stage 3 involves pilot trials with the most promising IPM elements on farmers' fields under their management but our supervision. Collaboration with social scientists and national programmes has been initiated for our work in each of the above 3 stages, more particularly for interfacing with farmers in stage 3.

There has also been collaboration with other research units of the ICIPE, particularly Chemistry and Biochemistry, Sensory Physiology, Cell Biology and Biomathematics. Outside the ICIPE, collaboration with social scientists and ICIPE-IRRI Project and with the International Institute for Tropical Agriculture (IITA) under a joint ICIPE-IITA Project has involved basing our teams at each of these institutes. Collaboration with international agricultural centres like the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Hyderabad, India) and the International Maize and Wheat Improvement Centre (CIMMYT) (Mexico) has involved exchange of sorghum and maize germplasm, respectively, and information on these crops on their resistance to stem-borers. Collaboration with national programmes of Kenya, Zambia and Somalia is being strengthened through the African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests (PESTNET) and involves collaborative experiments, exchange of plant materials and information, and training.

BIONOMICS AND APPLIED ECOLOGY

The major research activities of the section are to: (1) undertake studies on biology, behaviour and ecology of the target pests; (2) develop and standardize methodologies for assessment of on-farm yield losses caused by the borers and for monitoring and surveillance of pest populations; (3) develop strategies for manipulating different cultural practices including intercropping, behaviour, and genetic methods through induction of hybrid sterility for IPM.

1.1 STUDIES ON BANANA WEEVIL

K. V. Seshu Reddy

1.1.1 Biology

The weevil, *Cosmopolites sordidus* has been studied in the laboratory. The white egg, 2 mm long and oval in shape, is laid singly in cut pieces of rhizome or pseudostem. It hatches in 5–7 days and immediately the grub starts to bore into the rhizome or other tissue. There are 6 larval instars over a period of 30–40 days. The full-grown grub is about 12 mm long, white with a conspicuous reddish brown head, and is smooth, fleshy and legless. Pupation takes place in the rhizome or pseudostem and the pupa is initially white, later changing to brown. On emergence, the adult weevil is soft and brown, becoming almost black in about 5–7 days.

1.1.2 Damage

At the Ungoye field site, on the shores of Lake Victoria, weevil damage was assessed using the percentage coefficient of infestation (PCI). This measures the area of the outside of the corm damaged by weevil larvae, using a template. PCI values ranged from 3% to 70% among different banana cultivars studied. In general, cooking types were found to be more damaged by the weevils than sweet bananas.

1.1.3 Trapping

In order to estimate the adult weevil population at Ungoye split-pseudostem traps and disc-on-stump traps, were used. The disc-on-stump traps were more attractive, with maxima of 50 adult *C. sordidus* and 134 adult *Temnoschoita nigroplagiata* (another weevil damaging the bananas) caught per week, as against 42 and 82, respectively, in split-pseudostem traps.

1.1.4 Banana weevils, root lesion nematodes and agronomic practices

A survey was conducted in the major banana growing areas of Kagera, Arusha and Kilimanjaro Districts in northern Tanzania to evaluate the interrelationships between the occurrence of insect damage, plant parasitic nematodes and agronomic factors as they relate to the current decline in banana production. The results showed that this decline is caused in order of importance by: (a) the banana weevil *C. sordidus*, (b) the nematodes *Pratylenchus goodeyi, Helicotylenchus multicinctus* and *Radopholus similis* and (c) poor agronomic practices.

The most obvious symptoms of damage observed were toppling, snapping, leaning, stunting and reduced fruit size (in that order). The damage caused by the weevil to the corm was quantified using the PCI, with values from 52–95% between different banana cultivars, indicating differences in resistance or susceptibility to the weevil. Observed weevil damage was snapping (in 27% of plants) and stunting (in 20%). The degree of root necrosis due to nematode attack ranged from 50% to over 75% between the banana varieties examined; population densities of the major nematode *P. goodeyi* exceeded 2000/10g root. The high prevalence of toppling, snapping and root necrosis due to weevil and nematode damage was found to be due, ultimately, to poor agronomic practices such as inadequate sanitation of planting material, lack of mulching, insufficient propping, severe wind exposure, improper destruction of old pseudostems and low inputs of fertilizer.

1.2 GRAIN YIELD LOSSES IN THE MAIZE DUE TO *C. PARTELLUS*

K. V. Seshu Reddy

An experiment was conducted on the effect of varying densities of larval *Chilo partellus* on plant damage and yield of maize in relation to plant age at infestation. A randomized complete block design (factorial) was used with 3 replications. The plants were artificially infested with newly hatched larvae at 0, 2, 4, 6, 8, 10 and 12 per plant at 20, 40 and 60 days after plant emergence (DAE). There were 30 maize plants (cultivar Katumani) in each treatment, grown in cages (2 × 4 m) and covered with nylon mosquito net immediately after emergence to prevent infestation from outside.

The results showed that there was an increase in leaf injury as the larval density increased. The effect was more pronounced at 20 DAE than later. With regard to the effect of larvae on stalk damage, as the number of larvae infested per plant increased, the stem-tunnelling and the number of nodes bored also increased. More tunnelling, though not significantly different between densities, was observed at 8, 10 and 12 larvae/plant. There was no interaction between DAE and the number of larvae in influencing the tunnel lengths. Larval densities affected grain yields significantly when applied 20 DAE and 40 DAE, but there was no effect at 60 DAE (Table 1.1). At 20 DAE and 40 DAE, there was a significant negative correlation between tunnelling and grain yield ($r = -0.926$ and $r = -0.630$, respectively). Information generated from these studies shows that plant age and larval densities are significant factors and sources of variation in damage and in yields of maize. Also, stem-tunnelling is found to be one of the most important parameters for assessing the intensity of damage caused by the borers.

Table 1.1 Mean grain yield (g/plot) after artificial infestation of maize plants with *C. partellus* larvae. Each plot (8m²) containing 30 plants was replicated 3 times

No. of larvae	Days after plant emergence		
	20	40	60
0	2802.4a**	2802.4a	2802.4a
2	2727.0a	2732.9a	2769.8a
4	1980.0b	2227.8b	2780.3a
6	1631.9c	1878.7c	2776.7a
8	1170.8d	1227.3d	2728.1a
10	714.4e	1186.8d	2707.1a
12	574.0e	882.2e	2668.5a

* Within-column means followed by the same letter do not differ significantly ($P > 0.05$) by Duncan's Multiple Range Test.

1.3 YIELD LOSSES IN MAIZE DUE TO *B. FUSCA* AND ITS SURVIVAL IN CROP RESIDUES

M. Macharia

1.3.1 Grain yield losses in relation to crop phenology and larval infestation level

Field experiments were conducted at Njoro (altitude 2165 m) in the Nakuru District of Kenya to ascertain yield losses due to *Busseola fusca* at different borer densities and at different stages in the growth of maize hybrid 625. Newly hatched larvae were applied artificially at densities of 0, 2, 4, 8 and 16 per plant at 3 crop growth stages: 30, 45 and 60 days after emergence (DAE), equivalent to the 6, 8 and 10-leaf stages, respectively.

Significant ($P < 0.05$) differences were observed in grain yield and percent yield loss for the different levels of borer density and at different growth stages. Plants infested at the 6-leaf stage incurred yield losses of 18%, 49%, 78% and 99%, respectively, whereas at the 8-leaf stage the yield losses were 17%, 34%, 63% and 96%, and at the 10-leaf stage they were 14%, 27%, 39% and 51%, respectively. Adverse effects due to *B. fusca* larvae were therefore greatest in the early growth stages and as the crop grew older, irrespective of larval density, grain yield losses were less. In each case the percent grain yield loss was proportional to the numbers of borer larvae present.

1.3.2 Survival of B. fusca *and crop residue disposal practices*

After the maize crop is harvested, a large amount of crop residue remains in the fields that would impede preparation of the next seed bed. Post-harvest practices of the farmers include the following: (a) partial burning, (b) deep ploughing and (c) harrowing. Studies were therefore conducted to compare the effect of these practices on the survival of *B. fusca* larvae diapausing in the standing stalks. Borer populations were monitored by removing samples of stubble and stalks and recording all larvae and pupae within them.

The results showed that the practice of leaving the standing stalks in the field allows high borer populations to survive (13.4 larvae and 4.6 pupae/m²). All the other disposal practices significantly reduced the mean number of live *B. fusca* larvae found relative to that observed in the standing stalks, by the following percentages: cut stumps (64%), partial burning (65%), deep ploughing (67%) and harrowing (89%). Significant reductions in the mean number of *B. fusca* pupae were also observed in all cases, compared to that observed in the standing stalks, i.e. reductions of 14%, 71%, 91% and 97%, respectively. These studies indicate that disposal of the maize crop waste by deep ploughing and harrowing holds great promise for reducing *B. fusca*, besides the considerable agronomic potential for conservation of soil moisture and humus.

1.4 ENDOCRINOLOGY OF DEVELOPMENT IN THE LAST LARVAL INSTAR OF *B. FUSCA*

M. A. Njau

Busseola fusca completes one generation in the cropping season, but mature larvae undergo a facultative diapause during the second generation. Studies on the development of the last larval instar of *B. fusca* were undertaken with the main objective of finding out the role of the neuroendocrine system in regulating development with and without diapause.

Histological and ultrastructural methods were used to study the morphology of the brain neurosecretory cells, corpora allata and prothoracic glands in early, mid, and late phases of non-diapausing last larval instars, and in early, mid, and late phases of diapausing larvae. Extracts of juvenile hormone (JH) and moulting hormone (ecdysone) were obtained from the haemolymph using n-hexane and methanol as solvents. Extracts containing JH were bioassayed on newly-moulted last-instar nymphs of *Dysdercus nigrofasciatus*. Titres of ecdysone were determined by radioimmunoassay. The effects of injecting ecdysone into both intact and ligated diapausing larvae were also investigated.

The results showed that the A-type neurosecretory cells in the pars intercerebralis contained material which stained with Paraldehyde Fuchsin in both types of larvae. There was more stainable material in these cells at the beginning of the prepupal phase in non-diapausing larvae than in diapausing larvae. The size of the nuclei of the A cells at early, mid and late phases did not differ significantly.

The corpora allata of non-diapausing larvae were significantly smaller ($P < 0.05$) than those of diapausing larvae. The prothoracic glands increased in size during the prepupal stage of the non-diapausing larvae, indicating activity during this period, but remained small during diapause. Juvenile hormone titre was very low in non-diapausing larvae, but higher in early and late diapause.

Moulting hormone titres of non-diapausing last-instar larvae were 10, 170 and 500 ng/ml on days 1, 4 and 7

respectively, but were generally low with a maximum of 290 ng/ml in the diapausing larvae.

Ecdysone injections induced larval-larval moulting in intact diapausing larvae and larval-larval, as well as larval-pupal, moults in abdomens of ligatured diapausing larvae. These results suggest that diapausing larvae contain a high titre of JH. In view of the above observations, a high JH titre appears to be responsible for the induction and maintenance of diapause in *B. fusca*.

1.5 REPRODUCTIVE BIOLOGY, PHEROMONAL TRAPPING AND MONITORING OF *C. PARTELLUS*

G. C. Unnithan and K. N. Saxena

Sex pheromones of insects including Lepidoptera play an important role in mating and thereby in the reproductive processes. Efforts therefore are being made to manipulate these chemical signals in such a way as to monitor and/or suppress stem borer populations. It is essential to understand the reproductive behaviour and biology of the pest and the factors determining emission and perception of the pheromone, and what happens in the pheromonal trapping of the males. Previous investigations of some of these aspects were covered in the *ICIPE Annual Reports* for 1986 and 1987.

1.5.1 Diel periodicity of eclosion and mating

The diel periodicity of pupal eclosion and adult mating in *Chilo partellus* were investigated under controlled conditions of 12 h:12 h light:dark cycles at 26 ± 1°C and about 60% r.h., in order to monitor pheromone production by the female and pheromone perception by the male.

Eclosion of the male pupae started one hour before the onset of darkness (scotophase) and reached a peak (34% emergence) 2 h into scotophase; more than 70% of the male pupae eclosed within 4 h of darkness. Eclosion of the female pupae started 2 h into scotophase and reached a peak (42% emergence) 4 h later. Thus, the peak eclosion periods of males and females were 4 h apart.

Both sexes were capable of mating on the night of eclosion (scotophase 1), when mating started 6 h into scotophase, with a peak (26%) 3 h later, and continued upto 1 h before the beginning of the light period (photophase). However, on the night following eclosion (scotophase 2), mating only occurred 6–8 h after the onset of darkness, with a peak at 7 h. If males were paired on the night of eclosion with females that had emerged on the previous night, mating occurred over a 4-h period, starting 6 h into scotophase. The mating pairs remained *in copula* for more than 3 h, sometimes extending into the early hours of daylight. These observations will be useful for monitoring the emission and chemical identification, and in making behavioural assays, of the female pheromones.

1.5.2 Effects of age and mating status on the attraction of males to pheromone traps

Mark-release-recapture methods were used in studying the attraction of male *C. partellus* to traps with virgin females as lures. The attraction of virgin males to "calling" females (in pheromone traps) did not decline for the first 4 days. The proportions of 1, 2, 3, 4 and 5-day-old released virgin males that were recaptured were 28%, 19%, 30%, 21% and 9%, respectively. The attraction of the males to the females declined slightly, but not significantly, after mating and remained at the same level for 3 days. Previous studies have shown that *C. partellus* males mate several times with virgin females.

1.5.3 Competition between females in the field: communication disruption

Earlier studies *(ICIPE 1986 Annual Report)* revealed that the presence of a *C. partellus* female population in the field reduced the effectiveness of pheromone traps in catching males. Further studies were conducted to investigate the competition between females in the field and in the pheromone trap. The results revealed that a low density of virgin females did not affect either male-female communication or pheromone trap catches. But a high density of virgin females resulted in a significant disruption (95–100%), as measured by pheromone trap catches of males, although mated females surrounding the pheromone trap did not affect the catch significantly. When groups of 10 tethered females were placed in areas of 2500 m^2, 20% of them mated, whereas when the females were increased to groups of 50 only 1% of them, on average, were mated. These preliminary observations suggest that permeating the field with female sex pheromone could disrupt the reproductive process.

1.5.4 Pheromone trap designs

In earlier studies intended to standardize the pheromonal trapping of *C. partellus* it was shown that the water trap with virgin females as the lure performed better than several of the commonly used sticky traps *(ICIPE 1986 Annual Report)*. The funnel-type "Unitrap" was also not as effective as the water trap. However, due to difficulties in servicing the water trap in farmers' fields it was decided to develop a more efficient sticky trap. Several designs of sticky traps were tried. Of these a modified "Delta"-type trap performed as efficiently as the water trap. Mean daily catches of *C. partellus* males in water, Delta and modified Delta-type traps were 3.9, 2.6 and 5.3, respectively, and the latter is now being used routinely for monitoring male *C. partellus* populations.

1.5.5 Chilo partellus *population monitoring with pheromonal traps*

Populations of *C. partellus* on sorghum were monitored at Mbita Point Field Station (MPFS) and in farmers' fields using traps baited with virgin females. The objective was to understand the population fluctuations and to relate these to the timing and intensity of infestation of the immature stages of the borer, and to cropping seasons, crop cultivars and environmental factors.

Fluctuations of *C. partellus* populations showed the same trends as in the previous year *(ICIPE 1987 Annual Report)*. Major events in population development were reflected in the catches of trapped males. There was no clear quantitative relationship between the incidence of immature stages of the borer and the catches of males. However, the catches of males showed a close relationship to the temporal pattern of distribution of the borer larvae and pupae on the crop.

The relationship between the pheromonal trap catches and varietal differences in the host plant sorghum was also studied in the farmers' fields in Ogongo in the long rains of 1987 and at MPFS in 1988. Populations of *C. partellus* males were monitored weekly on plots planted with a tolerant sorghum (Serena) and 16 other cultivars showing different levels of resistance to *C. partellus*. Catches of males were significantly lower for only one cultivar during the first 2–6 weeks after plant emergence. After this, the catches of none of the cultivars differed significantly from one another.

1.6 COMMUNICATION DISRUPTION IN *B. FUSCA*

G.C. Unnithan

The efficiency of *Busseola fusca* synthetic sex pheromone in attracting males, and the fluctuations of male populations on sorghum monitored by pheromone traps, were reported in the *ICIPE 1987 Annual Report*. During the 1988 long rains preliminary studies were conducted on Rusinga Island and at Mbita to see if permeating a field with synthetic pheromone can disrupt male-female communication as measured by pheromone trap catches. Although *B. fusca* populations were extremely low the results clearly indicated disruption of male flights in plots permeated with the synthetic pheromone of *B. fusca* which suggests that it holds potential for disrupting male-female communication and thereby the reproductive processes of this moth.

1.7 EFFECT OF INTERCROPPING SORGHUM AND COWPEA ON *C. PARTELLUS* OVIPOSITION

E. Minja

In previous experiments *Chilo partellus* larval populations were observed to be lower in diversified crop stands. An intercropping field experiment was therefore set up to look at *C. partellus* oviposition, using the sorghum cultivar Serena and a locally selected semi-erect cowpea cultivar ICV 6. Plots were established at Mbita Point Field Station (MPFS), and in a farmer's field on Rusinga Island during the 1987 long rainy season. Five treatment combinations were adopted:

Sorghum monocrop
Cowpea monocrop
Sorghum and cowpea intercrop planted simultaneously
Sorghum planted 2 weeks before cowpea on the same plot
Cowpea planted 2 weeks before sorghum on the same plot.

The 5 treatments were randomly assigned to plots (14 × 10 m) in one block which was replicated thrice at both sites.

Monocrop plant spacings were 90 × 15 cm and 75 × 30 cm for sorghum and cowpea respectively. Sorghum spacing in the intercrops was similar to that in the monocrop but cowpea was added to the sorghum at a spacing of 90 × 40 cm, giving a small population that would not over-burden the plots. After plant establishment the plots were subdivided into 2 × 2 m experimental sampling units (cells). All border cells acted as plot guard rows.

Observations on the oviposition of the natural *C. partellus* populations were made in the field, starting 3 weeks after plant emergence. Twenty sorghum plants, 10 each in each of 2 inner cells in every treatment plot, were randomly selected and used for egg counts. This was done every 4 days at MPFS and once per week on the farmer's field. Each sorghum plant was searched carefully and the number of egg batches together with their size (and estimated number of eggs per batch) were recorded.

Table 1.2 Mean numbers of *C. partellus* egg batches and eggs in 4 cropping patterns at MPFS and on Rusinga Island in 1987

	MPFS		Rusinga	
Cropping pattern	Egg batches	Eggs	Egg batches	Eggs
Sorghum monocrop	0.32[a]*	1.40[a]	0.12[a]	0.51[a]
Sorghum and cowpea sown simultaneously	0.08[b]	0.37[b]	0.12[a]	0.45[a]
Sorghum sown 2 weeks before cowpea	0.13[b]	0.56[b]	0.05[a]	0.31[a]
Sorghum sown 2 weeks after cowpea	0.27[a]	1.30[a]	0.13[a]	0.49[a]

* Within-column means followed by the same letter do not differ significantly ($P > 0.05$) by Duncan's Multiple Range Test.

The total number of *C. partellus* egg batches and eggs recorded on sorghum plants in the farmer's field on Rusinga Island were small (25 batches with 543 eggs) compared to MPFS (112 batches with 3250 eggs). The results are summarised in Table 1.2. At MPFS, both the sorghum monocrop and sorghum sown after cowpea had significantly more egg batches and eggs compared to the other 2 intercrops. On Rusinga Island the 4 cropping patterns were not significantly different, probably due to the small size of the *C. partellus* population. The open cropping season facilitated by irrigation at the field station might explain the higher egg population, compared to the farmer's field where there is only one cropping season per year.

Eggs were also occasionally noted on cowpea leaves in the intercrops and on common local weed plants found near the experimental fields, particularly *Setaria verticillata, Corchorus olitorius* and *Flaveria australasica*. The presence of cowpea in intercrops could therefore be important by providing an alternative, though unsuitable, site which attracts some of the oviposition. This

might explain the lower egg counts on some of the intercropped sorghum compared to the monocrop, leading to lower stem-borer larval populations as observed previously in some of intercrops (see the *ICIPE 1987 Annual Report).*

1.8 INTERCROPPING RESISTANT AND SUSCEPTIBLE COWPEA CULTIVARS WITH MAIZE: INFESTATION AND DAMAGE BY *M. TESTULALIS*

M. Gethi

Previous reports have indicated that certain crop combinations in an intercropping system (e.g. sorghum/cowpea or maize/cowpea) reduce infestation and damage by stem- and pod-borers. Field studies were therefore initiated on (a) the effect on *Maruca testulalis* populations of intercropping resistant (TVu 946) and susceptible (ICV 2) cowpea cultivars with maize, (b) the effect of intercropping on resistance and susceptibility of cowpea cultivars to *M. testulalis* and (c) the influence of maize on the colonisation of resistant and susceptible cowpea cultivars by *M. testulalis.*

The results are given in Table 1.3, from which it can be seen that larval population density on both cultivars in monocrops was identical statistically to the density in ICV 2/maize. But the intercrop of resistant cowpea with maize significantly reduced larval density. The pooled means of infestation for each cultivar showed that TVu 946 was slightly (though not significantly) more resistant to *M. testulalis* than ICV 2. Resistance was improved when this cultivar was under maize though again the difference was not significant.

Table 1.3 Mean numbers (± s.e.) of *M. testulalis* larvae/plant when 2 cowpea cultivars, TVu 946 (resistant) and ICV 2 (susceptible), were monocropped and intercropped with maize

Cropping system	No. of larvae	Pooled means
TVu 946/maize	$2.1 \pm 0.4^{b*}$	
		2.2 ± 0.2^{a}
TVu 946 mono	2.4 ± 0.5^{ab}	
ICV 2 mono	2.7 ± 0.2^{a}	
		2.5 ± 0.1^{a}
ICV 2/maize	2.5 ± 0.2^{a}	

* Within-column means followed by the same letter do not differ significantly ($P > 0.05$) by Student-Newman-Keuls Test; mean coefficient of variation 11.0%.

Regression analysis showed that the linear relationship between the percentage of pods with *M. testulalis* damage and the number of *M. testulalis* larvae/plant during the short rains was significant ($r = 0.60$; $P < 0.05$), but not in the long rains.

The results further indicated that initially there was no appreciable difference in the number of eggs laid on the plants by *M. testulalis* in all 4 treatments. It was concluded that it was the establishment of the newly hatched larvae that was affected by both intercropping and resistance. Also, as the maize over-grew the cowpea crop, its canopy acted as a further barrier to ovipositing females.

1.9 INDUCTION OF INHERITED OR F_1 STERILITY IN *C. PARTELLUS*

V. A. O. Okoth

An investigation was started into the basic genetics of *Chilo partellus* and the identification of gene markers in normal and in gamma-irradiated populations. These studies were to enable us to develop autocidal techniques for the control of *C. partellus.*

Various developmental stages of *C. partellus* were subjected to a range of doses of gamma radiation (0–15 krad). Only the non-irradiated eggs hatched, or those irradiated with 2.5 krad, indicating their sensitivity to radiation.

Mortality of fifth-instar larvae and pupae both increased with increasing radiation doses. The percentage of successful pupations also decreased with increase in radiation dose, and in addition a number of the males and females which emerged from these pupae were grossly deformed.

Table 1.4 Mean total number of eggs laid by untreated *C. partellus* females mated with P_1 males (treated as pupae with various doses of gamma radiation), compared with the number of eggs laid by females mated with the F_1 male progeny of these crosses

Dose (krad)	Generation P_1	Generation F_1	% egg reduction
Control	386.2^{a*}	196.0^{b}	49.2
2.5	163.7^{a}	119.7^{a}	26.9
5.0	190.2^{a}	70.6^{b}	62.9
7.5	210.9^{a}	108.3^{b}	48.6
10.0	211.4^{a}	190.7^{a}	9.8
15.0	158.6^{a}	122.6^{a}	22.7

* Means in each row followed by the same letter do not differ significantly ($P > 0.05$) by *t*-test.

Females mated with irradiated males oviposited substantially fewer eggs than normal and this phenomenon was magnified in the F_1 progeny (Table 1.4). The radiation study itself demonstrated that the progeny of crosses in which the male parent had received a sub-sterilizing dose (2.5–15.0 krad) as a pupa were often semi-sterile when mated to non-irradiated females. This was exemplified by the 10–63% reduction in oviposition and 60–95% reduction in egg hatchability recorded in the F_1 generations compared to the parents. There was also a predominance of males, leading to a reduction in adult populations in subsequent generations. This semi-sterility might be utilized for field suppression of *C. partellus,* especially since the male progeny of treated males crossed with normal females were found to be fully competitive and also to respond normally to pheromone calls.

PLANT RESISTANCE TO INSECT PESTS

The work in this section is concerned with strategies for utilizing plant resistance to target insect pests. Studies on resistance in sorghum and maize to stem-borers, and in cowpea to the pod-borer and an aphid pest, are being carried out at Mbita Point Field Station (MPFS). Resistance in rice to leaffolders is being studied in the Philippines under the International Rice Research Institute (IRRI)-ICIPE Project whilst, under the same project, the performance and adaptability of upland rice varieties from different sources are being studied by the IRRI's Rice Breeder at MPFS (see separate report). Two major activities are being undertaken.

1. Evaluation of levels of resistance and its components in genetic material of ICIPE's target crops, obtained from various sources. The aim is to distinguish those cultivars that show the required characters, including good yield, making them suitable for cultivation; and those that show resistance or tolerance to the target pests but are poor agronomically so that they can serve as sources of resistance only. The emphasis in such evaluations is on comparing the components of borer resistance among different cultivars. This would identify lines with high resistance in particular components, thus providing guidelines for designing crossing strategies. Knowledge of these components would also indicate factors that might impart resistance or susceptibility to a cultivar and, thus, provide a base for elucidation of mechanisms of pest resistance.

2. Elucidation of the mechanisms and genetics of resistance in the target crops to the target insect pests, to assist plant breeders in producing crop varieties that would combine pest-resistance, good yield and other desirable characters.

1.10 SORGHUM RESISTANCE TO STEM-BORERS

K.N. Saxena

1.10.1 Evaluation of sorghum cultivars for resistance to stem-borers

The acquisition and testing of newer germplasm/cultivars/breeding lines is a continuous process and is important for identifying more and more lines with adequate but diverse resistance components for direct cultivation or use in breeding programmes.

Each evaluation is done in 2 stages. The first stage is in single-row plots under natural, followed by artificial, infestation with the pest. The second stage is evaluation in multi-row plots of lines that are found promising in the first stage.

1.10.2 Single-row evaluation of additional cultivars

Evaluation is for overall resistance and also for the following components: (a) infestation levels (egg population density and larval plus pupal population density), (b) damage levels (foliar lesions, "dead-heart", and stem-tunnelling). "Dead-heart" is drying of the central leaves due to larval feeding activity. The methods for measuring these were described in previous *ICIPE Annual Reports*. The ratios of the value of each of the 5 components for a tested line to that for a reference line (IS 18520, Serena) — which rates as 1.0 for each component — are calculated in order to give the relative values. The 5 ratios derived from each test line are then averaged to express its overall resistance/susceptibility index (ORSI). The lower the value of this index, the greater is the resistance. Tested cultivars are then graded by ORSI values into highly susceptible (>1.6), susceptible (>1.2 ≤ 1.6), intermediate or tolerant (>0.8 ≤ 1.2), resistant (> 0.4 ≤ 0.8) and highly resistant (≤ 0.4). During 1988, 31 sorghum lines received from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and other sources were evaluated.

Evaluation under natural infestation. On the basis of the ORSI values obtained, only one of the lines (ICSV 1 from ICRISAT) had a value as low as 0.4, rendering it highly resistant to stem-borers. Such a high resistance is due to 4 components being very low, though one is at the same level as in Serena. Four other lines, also from ICRISAT, were rated resistant with ORSI values of 0.4–0.8. These lines are IS 23411, IS 13100, IS 4751, and IS 2267. One other cultivar (IS 5619) had a high larval plus pupal infestation but a low level of damage in respect of dead-heart and stem-tunnelling, meaning that it could tolerate high infestation without suffering much injury and can thus be regarded as tolerant to stem-borers. Only two cultivars (IS 18333 and IS 23411) were found to be susceptible and the remainder were similar to Serena.

Evaluation under artificial infestation. The same 31 cultivars were evaluated after infesting them with about 30 newly hatched larvae per plant. Ten cultivars gave ORSI values of 0.8 or less and could be regarded as resistant. Of these, 3 also showed similar levels of resistance under natural infestation, as described above. One, however, which showed high resistance under natural infestation was found to have an ORSI value of 1.0 and was therefore intermediate or tolerant, like Serena, under artificial infestation.

These single-row evaluations thus identified promising borer-resistant lines that can be taken for the next stage tests.

1.10.3 Multi-row evaluation in selected lines

Ten promising sorghum lines were selected from those previously evaluated in single-row plots for multi-row evaluation during the 1988 long rainy season (Table 1.5). IS 1044 continued to show resistance which was also observed in IS 5619 and IS 1748. The remaining cultivars had high levels of infestation, but medium or low levels of damage, particularly in respect of dead-heart and stem-tunnelling. These cultivars would thus be regarded as tolerant to stem-borers.

The agronomic quality and mean grain yield per plant of all the tested cultivars were good. The resistant IS 1044 yielded 102.6 g grain per plant (g/plant), which was higher than Serena which gave 92.3 g/plant. LRB 6 gave the highest grain yield of 154.6 g/plant. This cultivar has a large semi-compact head with creamy white grains, but LRB 6 and IS 1044 are both prone to bird attack. Another very promising tolerant cultivar LRB 8 also

Table 1.5 Relative values of the different components of the overall resistance/susceptibility index (ORSI) for 10 selected sorghum cultivars in multi-row plots at MPFS during the long rainy season 1988

Cultivar	Infestation levels		Damage levels			ORSI value
	Eggs	Larvae + pupae	Foliar damage	Dead-heart	Stem-tunnelling	
IS 18520 (Serena)	1.0	1.0	1.0	1.0	1.0	1.0
IS 1044	1.6	1.0	0.4	0.1	0.4	0.7
LRB 6	2.5	1.5	1.3	0.5	0.9	1.3
LRB 7	2.1	1.8	0.9	0.5	0.9	1.2
LRB 8	1.3	1.5	1.1	0.6	0.4	1.0
DKV 2	1.4	1.5	1.0	0.6	1.3	1.2
IS 5619	0.9	1.3	1.1	0.3	0.5	0.8
IS 17948	0.9	0.6	0.6	0.2	0.7	0.6
ICSV 1	1.4	1.8	0.9	0.6	0.8	1.1
LRB 13	1.6	1.2	0.9	0.4	0.5	0.9

gave a fairly good yield of 131.0 g/plant. This line has a large semi-loose head with drooping panicles and chalky white grains. Its main advantage is that it is not attacked by birds and is therefore very suitable for cultivation.

1.10.4 Levels of infestation by C. partellus *larvae versus plant damage and mortality*

Using the methods and criteria already described, it was shown by field evaluation that IS 1044 shows a high level of resistance, IS 18520 (Serena) tolerance, and IS 18363 high susceptibility to *C. partellus*. Next it was important to examine the relationship between the levels of infestation of these cultivars by the stem-borer larvae and the levels of damage to the plants. This was studied during 1988 in the screenhouse to prevent contamination from natural populations of insect pests.

The results (Table 1.6) show the following: (a) The number of leaves per plant that, in general, developed lesions due to larval feeding increased gradually with the number of larvae present. (b) The total score of foliar lesions of the damaged leaves per plant was also slightly higher at higher levels of infestation. Among the lines tested, the total foliar damage score for the resistant line was less than that for the tolerant and susceptible lines.

Table 1.6 Effects of infesting 3-week-old plants of 3 sorghum lines with newly hatched *C. partellus* larvae: foliar damage and mortality of the plants

Sorghum line (IS no.)	Foliar lesions			% plant mortality**
	No. larvae applied per plant	No. damaged leaves/plant (mean ± s.e.)	Total score* (mean ±s.e.)	
18520 (Serena)	0	0	0	4.3***
(tolerant)	5	5.0 ± 0.7	14.0 ± 2.5	21.7
	15	4.7 ± 0.2	16.0 ± 0.9	54.2
	30	5.2 ± 0.9	20.5 ± 3.1	70.8
	45	6.0 ± 0.4	20.4 ± 1.7	65.2
	60	6.8 ± 0.7	22.6 ± 2.7	75.0
18363	0	0	0	4.3***
(susceptible)	5	5.2 ± 0.5	16.8 ± 1.1	8.3
	15	7.2 ± 0.3	26.1 ± 0.9	29.2
	30	7.2 ± 0.8	25.0 ± 3.1	50.0
	45	7.0 ± 0.7	21.8 ± 2.7	69.6
	60	5.6 ± 0.9	19.4 ± 5.9	78.3
1044	0	0.3 ± 0.3***	0.8 ± 0.8***	0.0
(resistant)	5	4.2 ± 0.2	12.5 ± 0.6	0.0
	15	3.8 ± 0.4	12.7 ± 1.4	0.0
	30	4.7 ± 0.3	17.7 ± 1.3	4.2
	45	4.5 ± 0.3	14.0 ± 0.4	4.2
	60	5.0 ± 0.2	16.0 ± 1.0	4.2

* Sum of visual scores on 1–9 scale for each of the damaged leaves per plant.
** Mortality due to dead-heart and foliar damage as a result of larval feeding.
*** Due to causes other than borer damage.

(c) Infested plants died in the vegetative phase of development, without flowering, due either to dead-heart or excessive feeding on the leaves by the larvae, although dead-heart was not produced. The percentage of plants that died increased markedly with the larval density for the tolerant and the susceptible lines but not for the resistant line. In fact, the mortality of plants infested with even 45–60 larvae per plant was very low for the resistant line, whereas that for the other two sorghum lines was as high as 75–78%.

A model was developed relating the percentage mortality of plants (in probits) with the logarithm of the larval infestation level.

1.10.5 Mechanisms of resistance to stem-borers

The investigations involve: (a) comparison of the pest's colonising responses to susceptible and resistant sorghum cultivars (studied in previous years) and (b) the role of the physical and chemical characters of these cultivars in determining the above responses, which was initiated during 1988.

The role of plant chemicals in oviposition and larval orientation was investigated using the same 3 cultivars — susceptible IS 18363, tolerant IS 18520 (Serena) and resistant IS 1044. The top leaves of 3-week-old plants were extracted first with n-hexane and then with acetone. These extracts were concentrated under vacuum and then tested for eliciting oviposition and larval orientation responses in *C. partellus*.

Oviposition responses to sorghum extracts on contact. These tests were conducted in a circular chamber (4.5 cm diam.) The extract was impregnated on a semicircular piece of filter paper covering half the area. A similar paper without extract covered the other half and served as the control. A single ovipositing female was released within the chamber overnight. Next morning, the number of eggs laid on each filter paper was recorded. Differences in the percentages of eggs laid on the treated and control semicircles could reflect the effect of the contact chemosensory stimuli contained in the plant extracts.

Table 1.7 Oviposition response of *C. partellus* on contact with n-hexane and acetone extracts of three sorghum cultivars. Each test was a choice between equal-sized semicircles of treated and untreated filter paper (4.5 cm radius)

Test materials and source	Total eggs laid (mean ± s.e.)	% eggs laid on treated paper (mean ±s.e.)
*n-Hexane extract**		
IS 18363 (susceptible)	135.6 ± 22.3	50.3 ± 7.3
IS 18520 (tolerant)	107.5 ± 20.4	45.7 ± 7.2
IS 1044 (resistant)	97.4 ± 20.0	34.0 ± 11.8
*Acetone extract***		
IS 18363 (susceptible)	145.7 ± 18.8	53.0 ± 7.3
IS 18520 (tolerant)	174.1 ± 23.6	36.1 ± 5.4
IS 1044 (resistant)	78.3 ± 32.6	62.3 ± 14.2

* Paper impregnated with 0.5 ml solution containing 0.025 mg of extractibles.

** Paper impregnated with 0.5 ml solution containing 0.5 mg of extractibles.

The results (Table 1.7) show that for the n-hexane extracts of IS 18363 and IS 18520 the percentages of eggs laid on the control papers and on the treated paper were similar. But the mean percentage of eggs laid on the paper treated with IS 1044 extract was significantly less than on the control. These observations suggest: (a) that n-hexane extractibles from IS 1044 inhibit or deter oviposition and could contribute to this cultivar's resistance to *C. partellus* through avoidance and (b) the extractibles from the other two cultivars have no effect on the oviposition behaviour of the insect.

The acetone extractibles (Table 1.7), from the susceptible IS 18363 appear not to be involved in stimulating oviposition. Those from the tolerant IS 18520, however, elicited increased oviposition compared to the control, suggesting that they are stimulatory. On the other hand, the acetone extractibles from IS 1044 were as inhibitory as the n-hexane extractibles and would also contribute to the resistance of this cultivar.

Role of plant chemicals in larval orientation. Previous studies have shown that the larvae of *C. partellus* in the first and later instars are attracted (from a distance, prior to contact) to the leaves of the tolerant IS 18520 (Serena) and susceptible IS 18363 sorghum cultivars. It has also been reported that this attraction is partly to visual stimuli (green colour) from the leaves but mostly in response to volatiles giving olfactory stimuli. It was therefore necessary to see what plant chemicals might be involved in attracting the larvae.

The first step was to extract and test the whole juice of sorghum leaves. The tests were conducted in a glass tunnel as described in previous reports. A freshly excised segment of sorghum leaf (5.0 × 1.5 cm) was pressed and squeezed onto an equal-sized piece of muslin cloth which had previously been impregnated with 1% liquid paraffin in n-hexane. After evaporating the n-hexane, the liquid paraffin would serve to slow down the evaporation of the volatiles from the juice squeezed onto the muslin. The latter was then introduced as a roll into a glass plunger, occupying 5 cm of its length from the inner end. The plunger was then introduced into the tunnel so as to keep its inner end with the muslin at a distance of 1 cm from the centre. On the other side of the tunnel another plunger was introduced bearing a similar roll of muslin with liquid paraffin, but no leaf juice, to serve as the control. Ten first-instar larvae were introduced into the tunnel through a central hole. The percentages of those that turned and moved towards the juice-treated muslin and towards the control were recorded.

The results show that 74.3% larvae moved towards the muslin treated with leave juice compared to 25.7% towards the control, indicating attraction by volatiles in the juice. Further experiments with other cultivars are in progress.

1.11 MAIZE RESISTANCE TO STEM- BORERS

J. K. O. Ampofo and K. N. Saxena

The work on assessment of resistance in maize to stem-borers has been proceeding along exactly the same lines

Table 1.8 Relative levels of components of stem-borer resistance, and overall resistance/susceptibility indices (ORSI), for 7 maize lines in multi-row plots under natural infestation at MPFS during the 1988 long rainy season, compared to line V 37

		Relative values					
Maize line	Source	Eggs	Larvae + pupae	Foliar damage	Dead-heart	Stem-tunnelling	ORSI
V 37	CIMMYT	1.00	1.00	1.00	1.00	1.00	1.00*
V 50	"	0.74	1.40	1.20	0.68	1.96	1.20*
V 68	"	0.74	2.45	0.98	2.00	2.10	1.65
KRNI	ICIPE	0.86	1.15	0.99	1.68	1.08	1.15*
Bulk CG 4141	Zambia	0.31	1.50	1.05	2.99	1.64	1.50
MM 752	"	0.86	1.95	1.20	6.84	1.90	2.55
MM 400	"	0.51	1.50	1.06	1.72	1.11	1.18*
Poza Rica 7832	"	0.48	0.65	0.94	4.75	1.17	1.60

* These lines can be regarded as tolerant and are promising for further tests and selection for resistance.

as in sorghum (see section 1.10). The progress made during 1988 on the evaluation of mechanisms of resistance in diverse maize cultivars to stem-borers is highlighted below.

1.11.1 Evaluation of stem-borer resistance

Evaluation in single-row plots. The new maize accessions that were received from Zambia (24), Mozambique (6), Kenya (5) and ICIPE (7) were subjected to first stage evaluation in single-row plots under artificial infestation with *Chilo partellus* and *Eldana saccharina* larvae. One line from Zambia (Bulk CG 4141) showed high resistance since the larval plus pupal density of *C. partellus* plus *E. saccharina,* foliar damage and stem-tunnelling by these larvae were all very low. Among the lines from Mozambique, "Matuba" had low levels of infestation and damage relative to the others. This line was similar to ICIPE's ICZ2-CM and V 37 from the International Maize and Wheat Improvement Centre but not as resistant as the Zambian line.

Evaluation in multi-row plots. Eight maize lines, selected on the basis of single-row tests were evaluated for the same 5 components as sorghum and overall resistance/susceptibility indices (ORSI) were calculated (Table 1.8). The line used as the reference check was V 37.

It is evident that although certain maize lines showed good resistance in respect of individual components compared to check V 37, in total performance none was better. For example, Bulk CG 4141 which was the most resistant in single-row evaluation, had a very low egg population density but quite high larval plus pupal density, dead-heart and stem-tunnelling. Consequently, the ORSI value was 1.50, reflecting the higher susceptibility of this line relative to V 37. Another line from Zambia (MM 752) had the highest ORSI value (2.55) and would thus be the most susceptible among the lines tested.

1.12 COMPONENTS OF RESISTANCE TO *A. CRACCIVORA* IN SOME COWPEA CULTIVARS

S. F. Firempong

Following the screening of some cowpea cultivars for resistance to the cowpea aphid *(Aphis craccivora),* the mechanisms governing the observed resistance were investigated in 6 cultivars (see Table 1.9). The ICV group includes cultivars developed by the ICIPE while the IT group includes advanced lines developed at the International Institute for Tropical Agriculture (IITA).

Table 1.9 Colonizing ability of alate cowpea aphids *(A. craccivora)* on 6 cowpea cultivars, including susceptible (S) and resistant (R) lines

		No-choice situation		Choice situation
Cultivar	Status	No. settling (mean ± s.e.)	No. of births (mean ± s.e.)	Total settling score
ICV 2	S	14.3 ± 1.4^{a}**	1.8 ± 0.2^{ab}*	19.5^{c}**
ICV 12	R†	13.3 ± 2.4^{a}	2.6 ± 0.2^{bc}	8.5^{a}
IT81/D-1137	S†	27.0 ± 2.0^{b}	1.7 ± 0.2^{ab}	13.5^{b}
IT82D-812	R†	20.5 ± 3.6^{ab}	1.2 ± 0.3^{a}	4.5^{a}
HB35/4/ID	S	22.8 ± 6.4^{b}	1.7 ± 0.5^{ab}	19.5^{c}
TVu 946	S	46.8 ± 11.3^{c}	2.8 ± 0.3^{c}	18.5^{c}

* Within-column means followed by the same letter do not differ significantly ($P > 0.05$) by Duncan's Multiple Range Test.

** Within-column means followed by the same letter do not differ significantly ($F_{5,20} = 15.20 > 5.49$; $P = 0.05$).

† Check (reference) strain.

TVu 946 is an IITA germplasm accession and is a semi-wild type, early-maturing cultivar with small seeds. It is resistant to attack by the pod-borer *Maruca testulalis* but is susceptible to aphids. HB35/4/ID is an early-maturing, high-yielding line developed at the National Farming Research Station, Katumani, Kenya. The role of antixenosis (behavioural non-preference) and antibiosis (metabolic inhibition of growth) were determined separately.

1.12.1 Antixenosis

This was investigated under both no-choice and choice situations. The no-choice situation was studied in a screenhouse of wirenetting containing 5 pots each with 4 cowpea seedlings at the 3-leaf stage. In each pot, at the base of the seedlings, 20 cultured alatae of *A. craccivora* (age not determined) were liberated and left overnight. The following day the seedlings were examined and all the alatae that settled and reproduced were counted along with the number of young born. The results were subjected to analysis of variance and Duncan's Multiple Range Test and are given in Table 1.9, with the cultivars placed in an increasing hierarchy of colonization. There were some significant differences in both numbers settling and numbers of young produced.

The choice experiment was conducted in the field in larger cages screened with wirenetting. The cowpea seeds were planted in a latin-block design and spaced 1 m apart within and between rows. From the 2-leaf stage and subsequently at 3-day intervals, the seedlings were examined and records taken of any aphids that settled and reproduced. A non-parametric test (analysis of variance by ranks) was used to analyse the results.

The results are also given in Table 1.9. The hierarchy of colonization was rather different with HB35/4/ID as the most susceptible and IT82D-812 the least with, again, some significant differences.

1.12.2 Antibiosis

Aphids were reared on young, tender, leaf petioles of potted plants enclosed in special clip-on cages constructed from plastic pill boxes, with ventilation holes protected by fine nylon gauze. A snug fit to the leaf surface was ensured by a cushion of foam rubber.

Two adult apterae were put in the cage overnight. The next day the cages were opened, the adults removed, and the number of young counted, labelled and retained on the plant. The next count was taken 4 days later, when the surviving aphids were separated and caged individually. No census was taken between days 0 and 4 because nymphal aphids are fragile and tend to wander. From day 4 onwards, a daily census was taken of the aphids and their progeny till the cohort became extinct. All newly-born aphids were removed at each counting.

On the basis of these observations the demographic and biological statistics given in Table 1.10 were computed, including age-specific survival (l_x), age-specific fecundity (m_x) and intrinsic rate of natural increase (r_m).

(a) *Percent surviving to reproduce* (l_x). Survival ranged from 20–64%; most mortality occurred during the nymphal stage, and on most cultivars half of the initial population died before the onset of reproduction. Survival was highest on ICV 1 and IT82D-812.

(a) *Percent surviving to reproduce* (l_x). Survival ranged from 20–64%; most mortality occurred during the nymphal stage, and on most cultivars half of the initial population died before the onset of reproduction. Survival was highest on ICV 1 and IT82D-812.

(b) *Pre-productive period.* This is a measure of the rate of aphid development. The aphids took 4–5 days to mature and reproduction occurred one day later.

(c) *Reproductive period.* The mean duration of reproduction was shortest on ICV 12 (4.0 days) and longest on ICV 1 (6.8 days).

Table 1.10 Demographic and biological statistics of *A. craccivora* reared on 6 cowpea cultivars

Statistic	ICV 12	ICV 1	IT82D-812	HB35/4/ID	IT8ID-1137	TVu 946
Survival rate percent (l_x)	21.5	57.4	64.2	19.8	26.8	39.6
Pre-reproductive period (days)*	5.0 ± 0.2	4.0 ± 0.1	4.3 ± 0.1	4.9 ± 0.2	4.7 ± 0.1	5.1 ± 0.8
Reproductive period (days)*	4.0 ± 0.5	6.8 ± 0.4	5.2 ± 0.4	5.0 ± 0.6	4.8 ± 1.2	5.5 ± 0.6
Fecundity*	16.6 ± 2.1	30.6 ± 0.9	15.2 ± 1.5	23.8 ± 3.7	24.0 ± 6.6	27.6 ± 3.7
Specific fertility/ day (m_x)*	4.1 ± 0.4	4.6 ± 0.2	2.8 ± 0.2	4.4 ± 0.4	5.5 ± 0.9	4.9 ± 0.3
Gross reproductive rate (Σm_x)	29.5	41.0	27.9	45.8	58.6	30.0
Net reproductive rate (R_0)	1.2	17.6	10.1	4.5	3.8	5.5
Finite rate of natural increase	1.013	1.363	1.405	1.214	1.190	1.239
Generation time (days) (T)	11.4	9.3	6.8	7.8	7.6	7.9
Doubling time (days)	53.7	2.2	2.0	3.6	4.0	3.2
Intrinsic rate of natural increase (r_m)	0.013	0.310	0.340	0.194	0.174	0.214

*Mean ± s.e.

(d) *Gross Reproductive Rate (m_x)* This summation of the age-specific fecundity rates *(m_x)* is akin to the mean fecundity. Aphids reared on ICV 1 and TVu 946 were the most fecund and those on ICV 12 and IT82D-812 were the least. In most cases, the pattern of reproduction showed a first peak on the seventh day, a middle peak (when present) on the eleventh day and a final peak on the last day of the cohort's life. Aphids reared on ICV 1 had only one peak.

(e) *Net reproductive rate (R_o).* This is defined as the ratio of the total number of female births in one generation to the number in the previous generation. It was highest on ICV 1 and least on ICV 12.

(f) *Intrinsic rate of natural increase (r_m)* This statistic sums up the data in a demographic table. It is a measure of the fitness of an organism under a given set of conditions. Thus, aphids on ICV 12 had the lowest value, indicating that it is the least favourable cultivar for development under the conditions of the experiment. On the other hand, aphids on IT82D-812 and ICV 1, had similar high r_m values, meaning that they are the most suitable cultivars for aphid development.

1.12.3 Conclusions

(a) The hierarchy of the 6 cowpea cultivars for antibiosis *(r_m)* values is different from that for antixenotic resistance. Thus, stimuli from the host plant that deter colonisation by alatae (antixenosis) are different from those that inhibit the development of apterae and population build-up (antibiosis). (b) Both IT82D-812 and ICV 12 are resistant to aphids, but their mechanisms of resistance are different. Resistance in IT812D-812 is due to antixenosis alone, whilst that in ICV 12 is caused by both antixenosis and antibiosis.

1.13 GENETICS OF PLANT RESISTANCE TO INSECTS

R. S. Pathak

The objectives of these studies remain the same, that is to determine the inheritance of resistance in maize, sorghum and cowpea and to suggest appropriate breeding methods and strategies to be adopted by plant breeders in the national and international research centres to develop insect-resistant cultivars. The plant materials used are the sources of resistance confirmed at ICIPE. Part of the results were reported last year *(ICIPE 1987 Annual Report)*. The results obtained during the 1987/88 short rains and the 1988 long rains are given in this report.

1.13.1 Inheritance of maize resistance to C. partellus

The F_1, F_2, B_1, and B_2 generations of crosses between 3 resistant parents (P_1) and a susceptible inbred line were evaluated along with the parents. Each cross was planted in a separate field experiment in a randomised complete block design with 4 replications. Each plant was artificially infested in the fourth week after emergence with egg batches containing 40 blackhead-stage eggs of *Chilo partellus*. Resistance was evaluated on 3 damage parameters: leaf lesions (scale 1–9), dead-heart (%) and stem-tunnel length (%). Leaf lesion and per cent dead-heart were evaluated at 4 weeks after artificial infestation; percentage stem-tunnel length was measured at harvest. Statistical analysis was performed on the percentage data after arcsine transformation. Mean values of each damage parameter of 6 populations (P_1, P_2, F_1, F_2, B_1 and B_2) were used to estimate gene effects for each cross using the joint-scaling test.

Significant differences were found between susceptible and resistant parents for all the damage scores. Percent dead-heart was found to be the most discriminatory of the 3 damage parameters used to estimate the level of resistance, and leaf lesion scores the least. The data from F_1 of all crosses showed the highest degree of dominance for resistance with respect to dead-heart. The distributions in F_2 were continuous and unimodal.

Joint-scaling tests were applied to determine the presence of non-allelic gene interactions. Non-significant chi-squared values of the joint-scaling test suggested that the additive-dominance genetic model involving three parameters — mean effect *(m)*, additive gene effect *(d)* and dominance effect *(h)* — was sufficient to explain the inheritance of the resistance measurements for leaf lesions and percent stem-tunnel length in all crosses. Contrary to this, the significant chi-squared values of the joint-scaling tests suggested the presence of gene interactions for dead-heart where a 6-parameter genetic model involving *m, d, h,* plus three epistatic gene effects, additive × additive *(i)*, additive × dominance *(j)* and dominance × dominance *(l)* was required to explain the inheritance. The effect of *d* was significant for all 3 types of damage scored, although an *h* effect of high magnitude appeared to be of greater importance in the inheritance of dead-heart. Among the epistatic gene effects, *j* and *l* appeared more important than *i* in the inheritance of dead-heart. These results suggest that both additive and non-additive dominance and epistasis gene effects are important in the inheritance of dead-hearts while only additive gene effects are important in the inheritance of leaf lesions and stem-tunnelling. These observations confirm the results already obtained.

1.13.2 Inheritance of sorghum resistance to C. partellus

Preliminary results of studies on the genetics of resistance to *C. partellus* in a cross of IS 1044 (resistant) and IS 18363 (susceptible) have been reported *(ICIPE 1987 Annual Report)*. Three crosses were studied during the 1988 long rainy season: IS 1044 × IS 18363, IS 1044 × Serena (tolerant) and IS 18363 × Serena.

The parents, F_1, F_2, B_1 and B_2 of each of these 3 crosses were planted in separate experiments in a randomized complete block design with 4 replications. In the fourth week after emergence each plant was infested with egg batches containing 40 blackhead-stage eggs of *C. partellus*. The same 3 damage parameters as in maize (leaf lesion, dead-heart and stem-tunnel length) were measured in exactly the same way (see above).

Significant differences were found between the progenies evaluated, which provided the basis for further

Table 1.11 Seedling reaction to *A. craccivora* of F_1 and back-cross populations from crosses between susceptible ICV 1 with 4 resistant cowpea cultivars

Cross	No. of F_2 plants				No. of back-cross			
	R*	S**	Chi-squared (3:1)	*P*	R*	S**	Chi-squared (1:1)	*P*
ICV 1 × ICV 10	181	57	0.14	0.70–0.80	24	27	0.18	0.50–0.70
ICV 1 × ICV 11	152	49	0.04	0.80–0.90	25	27	0.08	0.70–0.80
ICV 1 × ICV 12	166	43	2.18	0.10–0.20	29	23	0.69	0.80–0.90
ICV 1 × TVu 310	187	55	0.67	0.30–0.50	30	29	0.02	0.80–0.90

* Resistant.
** Susceptible.

analysis to estimate the gene effects for the damage parameters.

Joint-scaling tests revealed that an additive-dominance model involving three parameters *(m, d* and *h)* was sufficient to explain the inheritance of the resistance measured in the 3 crosses. These results suggest that epistatic gene effects have a minimal effect on the expression of the traits studied. Only additive gene effects were significant for all 3 damage parameters. Dominance gene effects also appeared to play little part in the inheritance of resistance to *C. partellus* in sorghum. These results suggest that transgressive segregation for higher resistance levels may result from crosses among sorghum lines containing different levels of resistance.

1.13.3 Inheritance of cowpea resistance to A. craccivora

Previous studies on several cultivars of the cowpea on the inheritance of resistance to the aphid *Aphis craccivora* indicated that it was governed by a single dominant gene *(ICIPE 1985 Annual Report)*, and that the resistance gene in ICV 10 and TVu 310 was different from the resistance gene in ICV 11 *(ICIPE 1986 Annual Report)*. Further studies were undertaken to confirm the mode of inheritance in a new resistant cultivar ICV 12. The cultivar ICV 1 was used as the susceptible parent in crosses with these 4 resistant cultivars. The F_1 and F_2 and back-cross (ICV 1 × F_1) progenies of these crosses were used to determine the mode of inheritance. The F_1 and F_2 progeny arising from the crosses between resistant cultivars were used to determine the allelic relationships of the resistance genes.

Three-day-old seedlings were each infested with 10 apterous adult aphids and the seedling reaction was scored 10 days after infestation. The parental, F_1, F_2 and back-cross populations were rated on a single plant basis and were classified as resistant (surviving) or susceptible (killed). The parental and F_1 populations of each cross contained 30 plants and the number of plants in the F_2 and back-cross populations varied depending upon the number of crossed seeds obtained.

All plants of the resistant parental populations tested as resistant and all ICV 1 seedlings as susceptible. The F_1 populations were resistant, indicating the dominant nature of the resistance gene in all 4 resistant cultivars. The F_2 showed a segregation of 3 resistant : 1 susceptible seedling (Table 1.11), indicating that resistance to the aphid was governed by a single dominant gene in each case. These conclusions were confirmed from the reaction of back-cross progenies, i.e. all segregated in a ratio of 1 resistant : 1 susceptible (Table 1.11).

Allelic relationships were determined from the seedling reactions of F_1 and F_2 populations arising from crosses between resistant cultivars. As expected, the F_2 plants of all crosses were resistant (Table 1.12). The F_2 populations from the cross ICV 10 × TVu 310 showed no segregation for susceptibility, indicating that the dominant genes for resistance in ICV 10 and TVu 310 are allelic. Similarly, the F_2 populations from the cross ICV 11 × ICV 12 were resistant, indicating that the dominant resistance genes in these cultivars are also allelic. The segregation data of F_2 populations from crosses ICV 10 × ICV 11, ICV 10 × ICV 12, TVu 310 × ICV 11 and Tvu 310 × ICV 12 were tested against a 15 resistant : 1 susceptible ratio based on the expectation of independent segregation in these populations. It is obvious that the dominant resistance gene of ICV 10 and TVu 310 is non-allelic to, and independent of, the resis-

Table 1.12 Seedling reaction to *A. craccivora* of F_1 and F_2 populations from crosses between 4 resistant cowpea cultivars

Cross	F_1 populations		F_2 populations			
	Resistant	Susceptible	Resistant	Susceptible	Chi-squared (15:1)	*P*
ICV 10 × TVu 310	20	0	175	0	–	–
ICV 10 × ICV 11	27	0	179	12	0.0004	0.98–0.99
ICV 10 × ICV 12	30	0	229	13	0.3184	0.50–0.70
TVu 310 × ICV 11	21	0	197	15	0.2465	0.50–0.70
TVu 310 × ICV 12	28	0	161	8	0.6631	0.30–0.50
ICV 11 × ICV 12	29	0	213	0	–	–

tance gene of ICV 11 and ICV 12. It may be mentioned that the ICV 11 and ICV 12 were isolated from the gamma-irradiated population and have a different resistance gene. The gene symbols Rac_1 and Rac_2 (resistance to *A. craccivora)* were assigned for the two non-allelic and independent loci controlling this trait in the cultivars ICV 10 and TVu 310, and ICV 11 and ICV 12, respectively. The results provided the first evidence ever of the existence of a second, independent dominant gene controlling resistance to *A. craccivora* in cowpea, which has enhanced the prospect of controlling aphid in cowpea genetically.

BIOLOGICAL CONTROL

The Biological Control (BC) Sub-programme aims at studying, evaluating and developing strategies for utilising the natural enemies of target pests for their management.

Since January 1988 the sub-programme has therefore taken up studies on the target crop pests, as well as livestock ticks, tsetse and medically important vectors like sandflies and mosquitoes. Work done in collaboration with the respective research programmes is included in their reports.

Several parasitoids and pathogens of crop pests have been identified and tested, and a few have been shown to hold promise for the control of cereal stem-borers, the legume pod-borer, and the cassava green spider mite (CGSM). In addition, work has continued on the taxonomy and biology of CGSM to enable better utilization of natural enemies for its control. Parasitoids which have undergone further evaluation are Pediobius furvus, Dentichasmias busseolae, Trichogramma mwanzai *and* Tetrastichus *sp. Among the pathogens* Nosema sp., Vairimorpha *sp.*, Beauveria bassiana, Hirsutella *sp., and* Bacillus thuringiensis *were selected for further studies.*

1.14 *TRICHOGRAMMA MWANZAI* FOR BIO-CONTROL OF CROP-BORERS

Lu Quing Guang

These studies concentrated upon the evaluation of *T. mwanzai* for control of stem-borers, especially *Chilo partellus*. The dispersal capacity is an important ecological characteristic in relation to effective use of *Trichogramma* in biological control programmes. To examine the dispersal capacity of the Kenyan indigenous species *T. mwanzai*, a field study was conducted during the long rainy season of 1988 at Mbita Point Field Station (MPFS). An experimental plot measuring 10 × 10 m was planted with sorghum. *Chilo partellus* eggs were artificially distributed on plants across the plot and *T. mwanzai* were released in the centre of the field. Four such releases were made and percentages of parasitism on the *C. partellus* eggs were recorded.

The results (Table 1.13) show that, generally, the parasitism by *T. mwanzai* on *C. partellus* eggs decreased as the distance from the release point increased. For example, the parasitism at 1 m (82%) was significantly higher than that at 5 m (51%). However, the average parasitism was about 62%. This observation is very important since it demonstrates that an indigenous parasite can disperse in the field after its release and can cause a high degree of parasitization of *C. partellus* eggs.

These results also show that under certain conditions *T. mwanzai* may be successfully used for increased parasitization of *C. partellus* eggs in the field by inundative release. Studies on this *Trichogramma* species for the management of stem-borers are continuing.

Table 1.13 Mean percent parasitism of *C. partellus* eggs exposed at different distances from the point of release of *T. mwanzai* adults

Distance (m)					
1	2	3	4	5	Average (± s.e.)
82.3^{a}*	65.4^{b}	55.2^{c}	57.4^{bc}	51.1^{c}	62.3 ± 4.6

* Means followed by the same letter do not differ significantly ($P > 0.05$).

1.15 CROP PHENOLOGY AND THE EFFECTIVENESS OF *NOSEMA* SP. TO CONTROL *C. PARTELLUS*

M. O. Odindo

It has already been shown that the microsporidian pathogen *Nosema* species is an effective control agent against the cereal stem-borer *Chilo partellus*. Field tests were conducted to determine the effect of the stage of growth of sorghum plants infested with *C. partellus* on the level of control that is achieved by foliar application of *Nosema*. Sorghum was planted in 4 blocks of 12 plots each and treated according to standard agronomic practice. Three weeks after plant emergence (WAE), and every alternate week thereafter, 2 plots in each block were infested with newly hatched *C. partellus* larvae. One of the infested plots was then sprayed with an aqueous suspension of *Nosema* spores. This procedure was repeated on other plots at 5, 7, and 9 WAE.

Damage to the plants by borers was assessed on foliar damage, larval plus pupal numbers, proportion of plants with dead-hearts, and dissection of plants in order to quantify stem-tunnelling and borer numbers. These depended on several factors including the age at which the plants were infested, the source of the infesting larvae (artificially introduced into leaf-whorls or infested by the naturally-occurring borer population) and the application of foliar sprays of *Nosema* spores.

The level of damage was found to be most severe on plants that had been infested at 3 WAE. With increasing age at infestation, the amount of damage was observed to decrease gradually, and was least in plants infested at 9 WAE. The influence of crop phenology on the protection given by *Nosema* was also demonstrated by the yields from the various plots (Table 1.14). The highest increase in yields of clean seed (120% and 100%) were achieved by plants treated at 3 and 5 WAE, respectively. Plots treated at 7 and 9 WAE showed a lower increase in

yield (56% and 26%, respectively). It is clear that treatment with *Nosema* was most effective if it was applied 3–5 WAE. The diminishing margin of benefit on sprayed plots as the plants matured is an important factor in deciding when to apply *Nosema* in the field for borer management.

Table 1.14 Mean yields (± s.e.) of clean sorghum seed per plot, from plots infested with *C. partellus* and treated with *Nosema* at various ages

Age at infestation (weeks)	Treatment	Seed yield (g)	Percent yield increase
Control*	none	1967.8 ± 165.1	–
Control**	none	2752.5 ± 367.9	–
3	none	1391.8 ± 115.7	–
3	sprayed	3055.0 ± 324.7	119.6
5	none	1574.5 ± 116.4	
5	sprayed	3147.8 ± 562.2	99.9
7	none	1667.5 ± 545.8	
7	sprayed	2594.3 ± 200.2	55.6
9	none	2048.5 ± 400.0	
9	sprayed	2565.8 ± 235.6	25.3

* Non-infested control.
** Naturally-infested control.

1.16 FACTORS AFFECTING INTERSEASONAL TRANSMISSION AND SURVIVAL OF *NOSEMA* SP. IN *C. PARTELLUS*

J. Ogwang

A species of *Nosema* can protect sorghum plants against stem-borers, especially *Chilo partellus*. A knowledge of the survival period of the pathogen on the crop, and the effects of an infection on the biology of the pest are therefore important. This enables us to identify the developmental stage of the pest that is most vulnerable to the pathogen for purposes of integrated pest management, and for timing the application of the pathogen. The mode of transmission of the pathogen in *C. partellus* was studied and also the effects of infection on fecundity.

Fifth-instar larvae of *C. partellus* were inoculated with a sublethal dose of *Nosema* suspension (4×10^3) spores/ml). Of the moths which emerged after treatment 98% were infected with *Nosema*. Infected females (IF) were paired with healthy males (HM) to see whether IF can transmit the infection to their offspring.

The reverse pairing was done to check if infected males (IM) crossed with healthy females (HF) can venereally transmit the infection to their progeny. The results indicated that only IF can transmit infection to their offspring. The effects of infection on fecundity were studied by pairing IF with IM, while HF were paired with HM as a control. To investigate if infection affects the ability of the males to fertilize the females, IM were paired with HF, while HM were paired with IF as control. The results showed that infected females laid significantly fewer eggs than healthy ones, while infection seemed not to affect the ability of males to fertilize females.

Infection also caused some adult deformities. There were moths with deformed wings while some pharate adults failed to free themselves from the pupal case.

These observations indicate that in addition to causing heavy mortality *Nosema* sp. can be an important factor in depressing the population of *C. partellus* since it lowers female fecundity. Furthermore, infected, deformed adults are unable to fly, so they cannot disperse, and they are also more vulnerable to natural enemies.

1.17 EVALUATION OF *B. THURINGIENSIS* FOR CONTROL OF *C. PARTELLUS*

M. Brownbridge

Following the advances made in the evaluation of *Bacillus thuringiensis* for the control of *Chilo partellus* and *Spodoptera exempta (ICIPE 1987 Annual Report)*, in-depth laboratory evaluation of a number of promising *B. thuringiensis* strains was carried out during 1988, and also a number of field trials.

1.17.1 Chilo partellus

Several of the identified *B. thuringiensis* strains were shown to be toxic to *C. partellus* larvae. The active strains have been bio-assayed to elucidate the most toxic varieties, which are now under consideration for field trial evaluation.

Some 62 new bacterial isolates, provisionally identified as *B. thuringiensis* strains owing to their growth characteristics, microscopic appearance and crystal-forming capacity, have been recovered from the material collected in 1987 and 1988. Isolates active against *C. partellus* have been recovered. These have been bio-assayed against the borer larvae.

A number of strains were isolated which, on analysis of the bio-assay data, appeared to be more pathogenic to *C. partellus* larvae than the identified *B. thuringiensis* strains obtained from overseas collections. Four of the most toxic strains were selected and cultured on a large scale to enable powders to be prepared for field evaluation of the bacteria.

Formulations of the bacteria were sprayed in a screenhouse on sorghum plants (var. Serena) which had been artificially infested 24 h previously with 20 newly hatched *C. partellus* larvae. The protective effects of the preparations were then monitored over a 10-week period by recording data every second day on: (a) plant height (growth), (b) leaf damage, (c) formation of dead-hearts, (d) date and rate of head formation and (e) rate of head emergence. Data recorded from all plants at harvest included: (a) plant height, (b) presence, number and position of entry/exit holes in the stems, (c) presence, number and developmental stages of *C. partellus* in the stems, (d) extent of tunnelling in the stems and (e) fresh grain yield.

All of these data remain to be statistically analyzed, but examination of the raw data clearly shows that all the

strains tested provided some protection compared to the unprotected, infested controls. Two of the strains, recovered from material collected in Mombasa and Busia, appeared to provide better levels of protection, with minimal plant damage evident.

The effects of such materials as molasses and sugar on the feeding activity of *C. partellus* larvae were also determined, with a view to incorporating phagostimulants in a *B. thuringiensis* formulation in order to increase the efficacy of treatment. Sugar solution (1%) appeared to stimulate the greatest feeding response in the larvae, followed by 10% molasses and 0.1% sugar. Greater efficacy was obtained in treatments against larvae that included sugar, compared with those without added sugar indicating, therefore, that the inclusion of sugar in a treatment can promote the efficacy of the *B. thuringiensis* preparation.

1.17.2 Spodoptera exempta

Two *B. thuringiensis* powders, based on *B. t. aizawai* and *B. t. entomocidus,* have been tested in the field against outbreaks of *S. exempta,* the African armyworm. Very good levels of control were obtained with both strains at several rates of application. The highest concentration used, a 2% w/v suspension, caused over 95% larval mortality within 48 hours. Lower concentrations (1% and 0.5% w/v suspensions) gave over 60% larval mortality and also reduced larval feeding and activity within the treated plots.

A healthy armyworm colony has finally been established at Mbita, enabling a number of *B. thuringiensis* strains isolated in both Israel and Kenya to be screened and then bio-assayed against armyworm larvae. The data will be used to select several candidate strains for large scale batch production and powder preparation for field trials against 1989 armyworm outbreaks.

Work is progressing in all areas, and the experiments to be undertaken in 1989 should provide further field evidence on the potential of *B. thuringiensis* for incorporation into a pest management programme.

1.18 THE GENETIC TAXONOMY OF THE CASSAVA GREEN SPIDER MITE USING HYBRIDIZATION AND REPRODUCTIVE ISOLATION STUDIES

T. N. Murega

The taxonomy of the cassava green spider mite (CGSM), *Mononychellus* sp. (Acari: Tetranychidae), attacking cassava in Africa is controversial. It was identified as *Tetranychus tanajoa* in 1938 and, after several name changes, that of *Mononychellus tanajoa,* proposed by Flechtmann and Baker in 1970, is now generally accepted.

Two species, however, *M. tanajoa* and *Mononychellus progresivus* have been reported from Africa, of which the latter is thought to be more widespread. It has also been suggested that *M. tanajoa* really consists of 4 species, including *M. progresivus.* The exact taxonomic identity of CGSM is therefore still open to debate. There is, however, an urgent need to control CGSM in Africa using predators from the original South American habitats, as the latter tend to be adapted specifically to their prey.

Hybridization and reproductive isolation studies were, therefore, initiated to resolve this taxonomic problem. The objective was to establish the capacity for gene exchange among 6 Kenyan strains by measuring the relative degree of reproductive isolation among them, and hence establish whether one, two or several species of CGSM attack cassava in Africa.

Hybridization tests involving crosses using the cassava leaf disc technique revealed various levels of reproductive isolation among 6 populations of CGSM from Nyanza and Western Provinces over 3 subsequent filial generations (F_1–F_3). Evidence was adduced showing that this reproductive separation is not complete since gene exchange occurred. Mean egg lethality was highest in the F_2 and lower in the F_1 and F_3 ($F_1 < F_3$; $P < 0.001$). All 36 possible cross-combinations, including reciprocal crosses, were examined and egg lethality values scored. These values varied from 0–11%, indicating an interaction of genes and extra-chromosomal factors giving partial sterility. The intrastrain egg lethality showed that the strains tested were heterogeneous, mortality being highest in generation 2 and lower in generations 1 and 3 ($G_1 < G_2 > G_2$; $P < 0.001$).

Table 1.15 Egg lethality and sex ratio values of 6 CGSM populations as measures of intrastrain heterogeneity over 3 generations (G_1–G_3); ($G_1 < G_2 > G_3$; $P < 0.001$)

Intrastrain population hybridized	% egg lethality (eggs laid)			Sex ratios*		
	G_1	G_2	G_3	G_1	G_2	G_3
Rusinga	6.9 (101)	10.5 (268)	0.0 (190)	3:1	3:1	5:1
Rongo	6.4 (141)	7.2 (135)	0.7 (152)	2:1	2:1	5:1
Kibos	7.4 (230)	8.4 (332)	1.2 (243)	2:1	3:1	4:1
Kakamega	3.8 (395)	1.1 (286)	0.0 (399)	4:1	3:1	2:1
Siaya	6.3 (159)	10.6 (227)	3.5 (173)	3:1	4:1	3:1
Busia	0.4 (289)	5.3 (170)	1.3 (299)	4:1	3:1	2:1

* Females per male.

Hybrid success of the resultant progeny of interpopulation and intrapopulation hybridization was indicated by the absence of hybrid sterility, hybrid inviability or hybrid breakdown, as well as the absence of distorted sex ratios. The sex ratio values (Table 1.15) varied from 2:1 to 6:1 in favour of females which are close to the conventional 3:1 ratio established by other workers for spider mites of the *Tetranychus urticae* group. The observed hybrid success also indicated that copulation, insemination and fertilization were successful among all the populations hybridized.

The terminalia of the haploid progenies conformed to the shape of the type species *M. progresivus*. In haplodiploid organisms the sons inherit half the genome of their diploid mothers; the males are therefore of the same species as their mothers, and are indispensable diagnostic tools in spider mite taxonomy. The evidence at hand favours the conclusion that *M. tanajoa* and *M. progresivus* are one and the same. However, the species seems to be a heterogeneous one constituting a cluster of forms sharing a common gene pool.

1.19 POPULATION FLUCTUATIONS OF *M. TANAJOA* IN RELATION TO ITS INDIGENOUS PREDATORS

M. F. O. Ndonga

In order to monitor the temporal distribution of the cassava green spider mite *Mononychellus tanajoa* and its indigenous predators, a pure stand of cassava (variety Kibandameno) was planted on Rusinga Island in Lake Victoria in April 1987. The cassava cuttings were planted at a spacing of 1 × 1 m on a plot of 650 m^2.

Natural infestation by *M. tanajoa* occurred 8 weeks after planting. Population density of the developmental stages of *M. tanajoa* was sampled by plucking leaves 1 to 5 from 10 randomly selected plants. Concurrently, the indigenous predators were counted on each of the 10 randomly selected bushes, identified and recorded. The leaves were examined under a binocular dissecting microscope and the following developmental stages of *M. tanajoa* were counted: eggs, larvae, nymphs (protonymph, deutonymph, protochrysalis, deutochrysalis and teliochrysalis), males and females. Eggs showed the highest density among the stages examined.

The population of *M. tanajoa* fluctuated and attained peak numbers during the months of June–July 1987 and December 1987–January 1988. All stages attained their maximum densities during these peak periods. The adult population was dominated by females at a ratio of about 3 females per male.

The predators which were collected from the field were identified as *Holobus fageli* (Coleoptera:Staphylinidae), *Schymus morelleti* (Coleoptera:Coccinellidae), *Phiseius degenerans* and *Neoseiulus teke* (Acarina:Phytoseiidae) and several species of spiders. The peak numbers of the indigenous predators followed those of *M. tanajoa* about 2 weeks later. *Phiseius degenerans* (33%) and *H. fageli* (35%) dominated the predator population during the first peak in July–August 1987, while spider species (40%) and *H. fageli* (25%) dominated the second peak during the months of January–March 1988.

Therefore, it is postulated that indigenous predators contributed significantly to the reduction in population density of *M. tanajoa* and that *H. fageli* and *P. degenerans* being dominant in this system played an important role in the suppression of this pest.

1.20 MATING BEHAVIOUR OF THE CASSAVA GREEN SPIDER MITE

E. B. Karamura

Among the Tetranychidae, most of the work on mating behaviour has been done on the two-spotted spider mite *(Tetranychus urticae)*. A 3-stage mating behavioural sequence of "hovering", "guarding" and "mating" has been described. In the case of the cassava green spider mite (CGSM) *Mononychellus tanajoa* there are no reports about the mating behaviour. Such information would be useful in identifying aspects of CGSM behaviour that could be exploited for its control. Thus observations were made to describe the mating behaviour of the deutonymph and the adult to identify signals and factors that may be involved in that behaviour.

From these studies 7 consecutive acts were identified and described: forelimb flapping, web-spinning, arrest, forelimb flapping, clasping of the female, aedeagus extension and interlocking of genitalia.

At 26°C and 50% r.h. the mean duration of the female quiescent stage was 23.4 h; during this period males were able to locate and sit on quiescent females within a mean time of 1.3 h. Under the same conditions, the mean arrest duration of the male by the quiescent female was 22.0 h. During the arrest period, males were seen to walk off and feed in the vicinity of their quiescent mate. Occasionally some males were observed to walk randomly around on the substrate, but such departures were thought to be largely associated with hunger rather than loss of the arrest signal. However towards the end of the quiescence, particularly during the silvery stage, male departure ceased, implying an increase in the magnitude of the arrest signal from the quiescent female.

In addition to responding to quiescent female deutonymphs, the males were also observed to be arrested by both quiescent pre-adult stages (protochrysalis and deutochrysalis) and by quiescent male deutonymphs. However, the duration of arrest by these stages was 12 times shorter than that imparted by the quiescent female deutonymph. This implied that males responded to a signal that drew them as close to the quiescent female stage as possible. After arriving beside the quiescent stage, the male would depart again if it failed to perceive an arrest signal (in the case of the quiescent male and pre-adult stages) or might stay if the arrestant signal was perceived (in the case of the quiescent deutonymph).

Studies on the signals involved in the mating behaviour of CGSM revealed that the quiescent web was the main tactile signal that drew the male near to the quiescent female. Upon arrival, a chemical arrestant

kept the male sitting on the female until the final moult. If the quiescent female was killed by dry heat, the first 2 acts of pre-copulatory behaviour were not affected, but the copulatory acts themselves were disrupted, implying that the former were controlled by physical (tactile) signals while the latter were prompted by the chemical (olfactory) arrestants that could be destroyed by heat. Conversely, removing the web with a fine pin, washing the quiescent female in a torrent of distilled water, or removing the quiescent female from its nest, all disrupted the pre-copulatory behaviour, but had minimal effects on copulatory behaviour. Under field conditions, torrential rain and wind would also wash away the quiescent webs, thereby disrupting the mating behavioural sequences, leading to the oviposition of unfertilized eggs which hatch into males only. This would depress the population, a phenomenon normally observed immediately after the rainy season.

1.21 FIELD POPULATIONS, DISTRIBUTION AND MORTALITY OF CASSAVA GREEN SPIDER MITE

M. O. Odindo

Field studies to determine the prevalence of fungi and other pathogenic mortality factors started at the Ungoye field site in July 1988. These studies will form the basis of the field application of several fungal isolates, especially *Hirsutella thomposii,* for the management of the cassava green spider mite.

Table 1.16 Observed mortality among cassava green spider mites *M. tanajoa* at the Ungoye field site in 1988

Sampling date	Sample size	Infestation (mean/plant)	Mortality (%)
6 Jul.	294	85.3	0.0
3 Aug.	4929	308.1	2.8
6 Sep.	2062	128.9	4.6
4 Oct.	2649	190.6	13.0
15 Nov.	2317	83.8	10.5
27 Dec.	4071	254.5	2.9

Natural mortality was low over most of the sampling period, although mite populations were high (Table 1.16). The first and second leaves had a high mite population throughout the sampling period. When mite cadavers were placed on wet filter paper and incubated at 35°C, most of the dead mites did not show any fungus mycelial growth, although there were some as yet undetermined fungi. So far no *Hirsutella* isolations have been made from the sampling site and it is apparent that *H. thomposii* does not occur widely in the field.

1.22 INTEGRATED PEST MANAGEMENT: PILOT TRIALS

K. N. Saxena, K. V. Seshu Reddy, E. O. Omolo, A. Pala-Okeyo and L. Ngode

As explained in the introduction, the components for integrated pest management (IPM) of the target insect pests are being developed in 3 stages, the third stage involving pilot trials in farmers' fields under their own management. The work has now advanced to a stage where such pilot trials could be undertaken on two major components for the management of crop-borers of sorghum and maize: (a) intercropping and other cultural practices like adjustment of planting time and crop residue disposal and (b) plant resistance to insect pests.

Table 1.17 Mean (± s.e.) grain yield (g/25m²), showing the advantage of using ICIPE IPM components, in trials in farmers' fields in 2 divisions in Western Kenya. Two strains of sorghum were monocropped, and also intercropped with cowpea (ICV 2); long rainy season 1988

Crops grown	Oyugis(6)*	Kendu Bay(16)*
Farmers' own sorghum monocrop	4045 ± 877	3176 ± 271
LRB 5 monocrop	5103 ± 677	4857 ± 472
ICV 2 monocrop	325 ± 65	742 ± 160
LRB 5 intercrop	5992 ± 741	5140 ± 485
ICV 2 intercrop	277 ± 57	437 ± 85
LRB 8 monocrop	4589 ± 566	4579 ± 464
LRB 8 intercrop	4924 ± 604	5119 ± 515
ICV 2 intercrop	192 ± 37	321 ± 56

* Number of participating farmers.

During 1988, pilot trials of these components were continued in collaboration with the ICIPE's Social Science Interface Research Unit and extension staff of the Ministry of Agriculture in Kenya. On the basis of previous surveys, 50 resource-poor small-scale farmers were selected for these trials in western Kenya, 25 each in Kendu Bay and Oyugis Divisions. Each farmer adopted the farming practices and cropping patterns recommended (see Table 1.17). During the 1988 long rainy season, these cropping patterns showed the advantages of intercropping itself, as well as the use of selected sorghum cultivars, in keeping stem-borer attack low and increasing the grain yield. For example, the population density of the larvae plus pupae of *Busseola fusca* and *Chilo partellus* on LRB 5 and LRB 8 monocrops was almost as low as on the farmers' own sorghum both at Kendu Bay and Oyugis. This density was significantly reduced on intercropping each cultivar with the cowpea ICV 2 in Oyugis. But in Kendu Bay such an intercropping effect was not marked, and the percentage of sorghum plants damaged by the two stem-borer species was not affected.

The effects of the two IPM components on the sorghum grain yield were also quite evident in the detailed results obtained from 22 of the cooperating farmers (Table 1.17). The percentage increases in the yield are given in Table 1.18. The advantages of the sorghum cultivars as monocrops over the farmers' own sorghum monocrop varied from 13% in Oyugis for LRB 8 to 53% in Kendu Bay for LRB 5. Intercropping these sorghum cultivars with the cowpea ICV 2 resulted in a further increase in the grain yield over that of the farmers' own sorghum monocrop from 22% in Oyugis for LRB 8, to 62% in Kendu Bay for LRB 5. These intercrops also gave an additional yield of 5–17% relative to monocrops of the same sorghum cultivar.

Table 1.18 Percentage increase in sorghum grain yield, showing the advantage of using ICIPE IPM components, in trials in farmers' fields in 2 divisions in Western Kenya. Two strains of sorghum were monocropped, and also intercropped with cowpea (ICV 2); long rainy season 1988

Crops grown	Oyugis (6)*	Kendu Bay (16)*
LRB 5 monocrop v. farmers' monocrop	26.2	53.0
LRB 5 intercrop v. farmers' monocrop	48.1	62.0
LRB 5 intercrop v. monocrop	17.4	5.4
LRB 8 monocrop v. farmers' monocrop	13.4	44.2
LRB 8 intercrop v. farmers' monocrop	21.7	61.2
LRB 8 intercrop v. monocrop	7.3	11.8
Intercropping advantage (LER)**		
LRB 5	1.9	1.6
LRB 8	1.7	1.6

* Number of participating farmers.
** Land equivalent ratio.

These trials thus demonstrate the effectiveness of the two IPM components in combination for reducing attack and yield loss due to stem-borers on sorghum. These trials also provide a basis for intensifying our work in the same area and extending it to other areas in near future.

INSECT MASS-REARING TECHNOLOGY UNIT

The Insect Mass-Rearing Technology Unit (IMRT) and Animal Breeding Section are basically research support services, which handle the breeding of insects and laboratory animals. Research is undertaken to develop practical techniques in insect rearing. In 1988, this section worked on the predatory mite Neoseulus teke *and the sorghum stem-borer* (Chilo partellus), *achieving significant developments.*

1.23 THE PREDATORY MITE *N. TEKE*

R.S. Ochieng' and F. Onyango

An artificial diet (ICD 286) developed for *Neoseulus teke* in 1986 was successful and made a significant breakthrough in rearing predacious mites artificially, providing an important contribution to their use as predators in biological control of the cassava green spider mite (CGSM).

It was, however, noticed that the fifth and succeeding generations reared on the diet could not recognise their prey (CGSM and the red spider mite). It was therefore necessary to develop a diet without this drawback. After a series of experiments ICD 387 was formulated. This was basically ICD 286, modified by adding prey mite material.

Mites of the prey species were washed from the leaves on which they were reared in the screenhouse, weighed and then blended with ICD 286 which consists of egg yolk, milk powder, honey and salts in distilled water.

Mite cultures that had lost the ability to recognize their prey were fed on diet ICD 387 for one complete generation, and were then able to recognize the prey again in the next generation. Observations on several generations of *N. teke* reared on this diet have not shown any biological abnormality that was observed on diet ICD 286. Samples of eggs were collected from generations 40, 45, 50, 55 and 60 and raised on ICD 286. Replicates of diet ICD 387 were inoculated with the eggs. The eggs hatched and their development was recorded.

The results confirmed the finding given in the *ICIPE 1987 Annual Report* and showed that these predators not only recognised and went back to feeding on the host, but their development was very similar to that in wild populations. Fecundity per female was 32.9 eggs on the diet compared to 36.0 on a natural mite diet.

1.24 OTHER INSECTS AND LABORATORY ANIMALS REARED

R.S. Ochieng' and F. Onyango

1.24.1 Phytophagous insects

Phytophagous insects were reared at MPFS and the production and use of *Chilo partellus* during the year is shown in Table 1.19.

Table 1.19 Quantities (in thousands) of *C. partellus* supplied in 1988 to CPRP sections, African Regional Postgraduate Programme in Insect Science (ARPPIS) and ICIPE Nairobi (Nbi)

Stage	BAE	PRIP	BC	ARPPIS	Nbi	Total
Egg masses	0	30	243	65	1	339
Larvae (1st instar)	1	50	163	1	1	216
Larvae (2nd instar)	0	0	119	0	0	119
Larvae (3rd instar)	0	4	58	5	1	68
Larvae (4th instar)	1	2	125	8	1	137
Larvae (5th instar)	3	7	115	1	0	126
Pupae	18	1	74	1	0	94
Adult pairs	5	2	1	0	0	8
Total	28	96	898	81	4	1107

Use of fruit jelly to rear C. partellus. During 1988 agar, used almost exclusively as the gelling medium in artificial insect diets, became very difficult and expensive to obtain. It was essential to find a suitable locally available substitute. Several substances were tried before a brand of vanilla-flavoured fruit jelly was found which was the best in terms of both gelling and keeping quality. It has

been incorporated in the new diet shown in Table 1.20. The keeping quality is, in fact, better than that of the standard agar-based diet routinely used in our insectary to rear *C. partellus*. The fruit jelly diet has shown few instances of mould development — which is the major problem with the standard agar-based diet D3B.

Table 1.20 Fruit jelly diet for *C. partellus*

Ingredient	Amount
Fraction A	
1. Bean powder *(Phaseolus vulgaris)*	100g
2. Sorghum leaf powder (Serena)	100g
3. Brewer's yeast (Yestermin)	36g
4. Vitamin mix (Vanderzant)	3g
5. Wesson's salt mix	2.5g
6. Sorbic acid	1.2g
7. Methyl-*p*-hydroxybenzoate	2.2g
8. Formalin 40%	2ml
9. Distilled water	500ml
Fraction B	
10. Fruit jelly	60g
11. Distilled water	500ml

In addition to being locally available, the fruit jelly is cheaper than agar. It is envisaged that by using it, the cost of rearing *C. partellus* could be reduced in our laboratory by approximately KShs 50,000/- per year. Larvae reared on this diet had a mean adult emergence of 56%; the females laid a mean of 294 ova with 95% hatchability. However, research to determine the effect of this diet on successive generations of *Chilo partellus* continues.

1.24.3 Material supplied

In our Nairobi branch, work is basically centred on research support services to supply larger insects and laboratory animals to various Research Programmes and Units. The colonies of laboratory animals were maintained for feeding haematophagous insects, besides supplying animals for use in research work. During 1988, the Nairobi branch reared and supplied the following:

Haematophagous insects	*Glossina morsitans*
	Glossina pallidipes
	Aedes aegypti
Phytophagous insects	*Chilo partellus*
	Eldana saccharina
	Spodoptera exempta
Small laboratory animals	rabbits
	rats
	mice
	hamsters

1.24.2 Rearing G. pallidipes

In the last quarter of 1988, the rearing of *Glossina pallidipes* was started in the new facilities in Nairobi, with a stock of pupae from the colony at Mbita Point Field Station.

The indications so far are that *G. pallidipes* is adapting to controlled conditions in Nairobi. The adults that emerge feed very well, there is no difficulty in mating and 5 generations have already been obtained.

It is envisaged that at the present rate of development, the unit will be able to produce and supply all the *G. pallidipes* requirements from the Nairobi colony by the end of 1989.

ICIPE-IRRI RESEARCH PROJECT

1.25 RESEARCH ON LEAFFOLDERS AND OTHER RICE PESTS

Z. R. Khan

1.25.1 Feeding of first-instar larvae of rice leaffolders on selected wild rices

Wild rices are valuable sources for resistance to several insect pests of rice. We measured the leaf area consumed and the weight gained by two rice leaffolder species — *Cnaphalocrocis medinalis* and *Marasmia patnalis* — feeding on wild rice leaves in the laboratory. The wild rices were *Oryza officinalis, O. perennis, O. punctata, O. nivara* and *O. australiensis*. The susceptible and resistant check varieties were IR36 and TKM6, respectively.

Larvae of *C. medinalis* consumed a significantly bigger leaf area of susceptible IR36 and *O. officinalis* than of TKM6, *O. punctata, O. nivara* and *O. australiensis* (Table 1.21). Larvae of *M. patnalis* consumed significantly less leaf area of all the wild rices and TKM6 than of IR36. Larvae of *C. medinalis* consumed significantly more than *M. patnalis* of all the wild rices except *O. nivara*.

Larvae of both species gained weight on all host plants tested. Weight gained by *C. medinalis* larvae on wild rices was comparable to that gained by those feeding on susceptible IR36. *Marasmia patnalis* gained less weight on *O. officinalis, O. australiensis* and TKM6 than on IR36. Although *C. medinalis* larvae consumed more than did *M. patnalis* larvae, percent weight gain increase in *M. patnalis* was higher than in *C. medinalis* on resistant TKM6 and *O. perennis* wild rice.

1.25.2 Isoenzyme differentiation of two species of rice leaffolders

The rice leaffolders *C. medinalis* and *M. patnalis* are usually found in association on rice plants. Studies on the comparative biology and population dynamics of these species when they are sympatric require precise identification of their larvae and pupae, which are similar in morphology and are difficult to differentiate. Spinning and feeding behaviour of the larvae of the two species on rice plants are also very similar.

We used horizontal starch gel electrophoresis to differentiate the larvae and pupae of the two species. Second to fifth-instar larvae and pupae of the two species were accurately identified with the enzymes esterase and isocitric dehydrogenase. Esterases of these leaffolders were investigated for possible genetic differentiation. A maximum of 6 zones of activity were observed after starch gel electrophoresis using ∝-naphthyl and ß-naphthyl esters as substrates. Differences in the activity and nature of expression of isoenzymes were observed in the different stages of development of the two species (Figure 1.1).

Table 1.21 Mean (± s.d.) leaf area consumed and mean (± s.d.) percent weight gain by 1-day-old, first-instar leaffolder larvae after 48 h on selected wild rices (*Oryza* spp.)

Food plant	Leaffolder species		
	C. medinalis	*M. patnalis*	Difference#
Leaf area consumed (cm^2)			
O. officinalis	$0.92 \pm 0.21^{ab\#\#}$	$0.52 \pm 0.04^{b\#\#}$	0.40**
O. perennis	0.90 ± 0.35^{abc}	0.41 ± 0.14^{bc}	0.51**
O. punctata	0.70 ± 0.20^{c}	0.52 ± 0.05^{b}	0.18*
O. nivara	0.31 ± 0.11^{d}	0.34 ± 0.05^{cd}	-0.03^{ns}
O. australiensis	0.73 ± 0.21^{bc}	0.23 ± 0.11^{d}	0.50**
IR36 (susceptible check)	1.10 ± 0.20^{a}	0.98 ± 0.16^{a}	0.12^{ns}
TKM6 (resistant check)	0.68 ± 0.15^{c}	0.38 ± 0.14^{c}	0.30**
Percent weight gain			
O. officinalis	178 ± 50^{a}	151 ± 37^{c}	27^{ns}
O. perennis	155 ± 38^{ab}	198 ± 37^{ab}	−43**
O. punctata	134 ± 41^{bc}	177 ± 67^{abc}	-43^{ns}
O. nivara	160 ± 52^{ab}	148 ± 56^{a}	12^{ns}
O. australiensis	163 ± 50^{ab}	168 ± 34^{bc}	-5^{ns}
IR36 (susceptible check)	161 ± 61^{ab}	203 ± 49^{a}	-42^{ns}
TKM6 (resistant check)	102 ± 46^{c}	166 ± 42^{bc}	−64**

\# *C. medinalis* compared with *M. patnalis*.
\#\# Within-column means followed by the same letter do not differ significantly ($P > 0.05$) by Duncan's Multiple Range Test.
** $P < 0.01$, * $P < 0.05$, ns = not significant, by *t*-test.

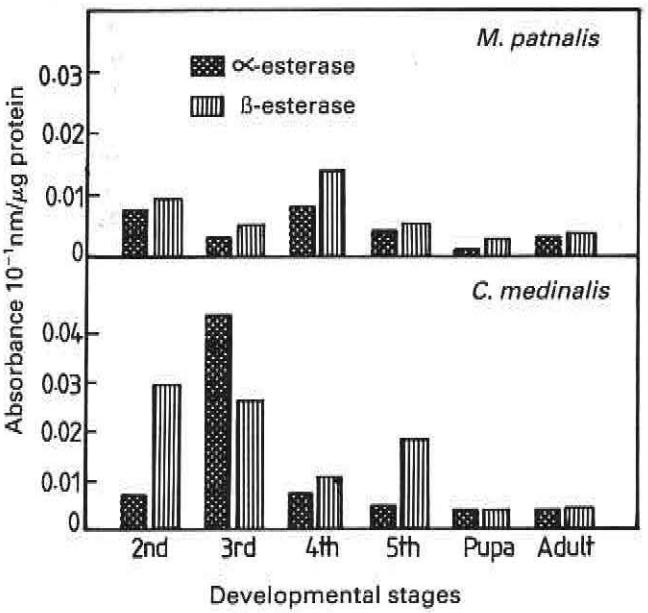


Figure 1.1 Esterase activity in different larval, pupal and adult stages of two species of rice leaffolders.

These results revealed the existence of genetic differentiation in the esterase loci during the separate evolution of the two leaffolder species.

1.25.3 Antifeedants from resistant varieties of rice

Extracts were prepared of rice plants in petroleum ether, dichloromethane, methanol and water from the leaffolder-susceptible varieties IR36 and Rexoro, the resistant variety TKM6 and a resistant wild rice *Oryza perennis*. The extracts were bioassayed in a no-choice test for their antifeedant activity against *C. medinalis* larvae. Methanol extractables from TKM6 and *Oryza perennis* effectively reduced larval feeding as compared to the extractables from IR36 and Rexoro plants. Petroleum ether, dichloromethane and water extractables were not active. Preliminary separation of the methanol extract by high-performance liquid chromatography (HPLC) under reverse phase conditions showed consistent qualitative differences among susceptible and resistant varieties (Figure 1.2). Isolation of the fractions is in progress.

1.25.4 Profiles of volatiles from susceptible and resistant varieties of rice

Profiles of rice plant volatiles were obtained by head space analysis, in collaboration with the Cereal Chemistry Department of IRRI, of the leaffolder-susceptible varieties Rexoro and IR36 and resistant TKM6 and *O. perennis*. The results indicated significant qualitative and quantitative differences among these varieties. Preliminary chemical characterization by gas chromatograph mass spectrometry (GC-MS) indicated the presence of 4 major groups of compounds: low molecular weight oxygenated (C_5–C_8) hydrocarbons, monoterpenoids, sesquiterpenoids and low molecular weight waxes (C_8–C_{16}). Characterization of these substances revealed the presence of several green odour compounds such as 2-pentanone, 2-pentanol, heptane, (Z)-2-hexenal, (Z)-3-hexen-1-ol, ethyl-benzene, 2-heptanone and 2-heptanol. The monoterpenes present were ∝-phelandrene, ∝-pinene, ∝-thujene, ß-myrcene, 3-carène, 4-cymene, limonene, cineole and menthol. The sesquiterpenes were copaene, elemene, caryophyllene and -caryophyllene. Bioassays of these chemicals against leaffolders and stem borers is currently underway.

1.25.5 Bioassay of volatile components from rice plants

In close collaboration with the Cereal Chemistry Department of IRRI, chemical profiles were examined to elucidate the basis of resistance to the rice leaffolder *C. medinalis*. Candidate chemicals based on their qualitative and quantitative differences in resistant and susceptible rice varieties were screened in a variety of bioassays, such

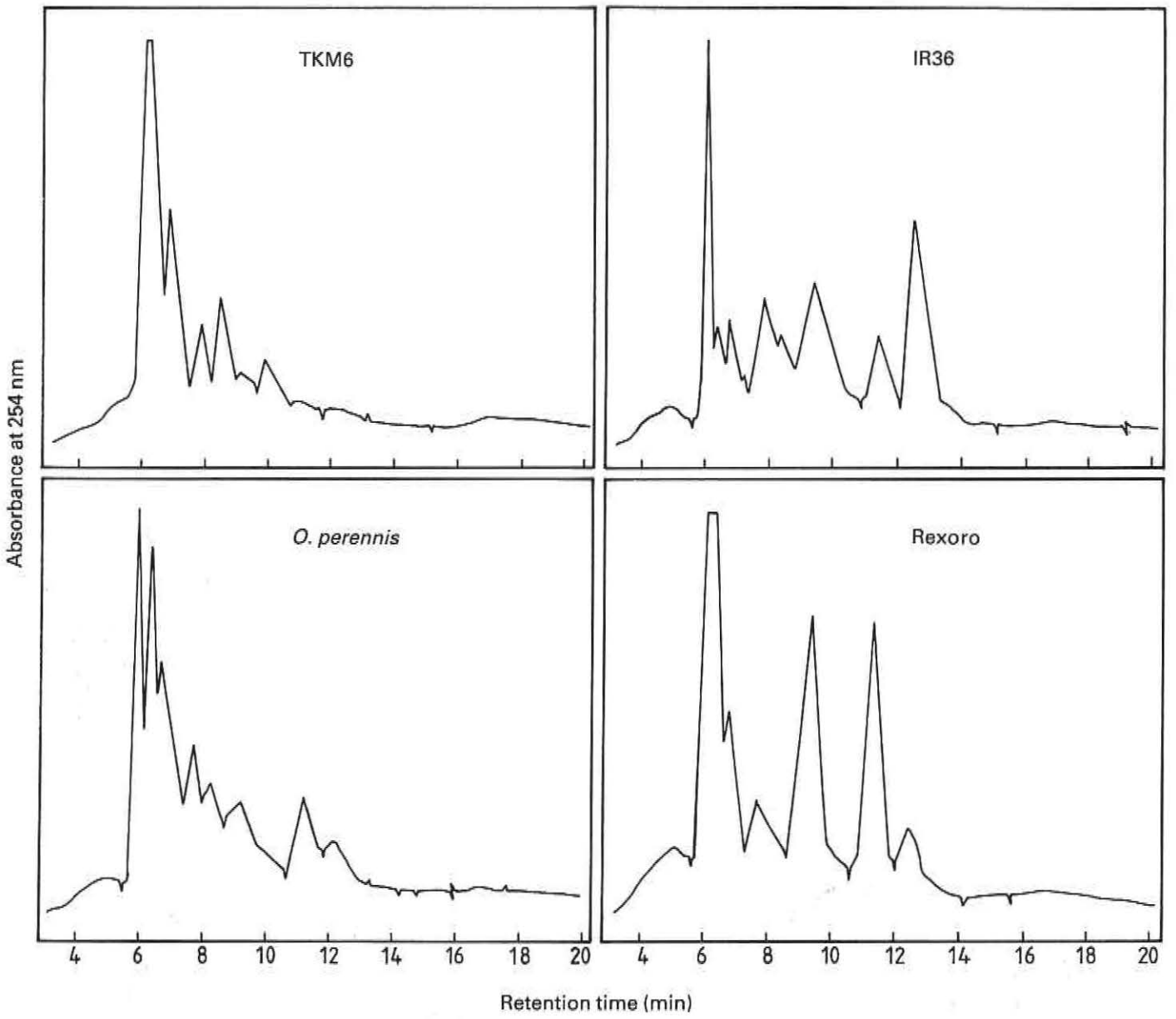


Figure 1.2 Ultraviolet absorbance patterns by HPLC of methanol extracts of four rice varieties.

as first-instar larval orientation, settling response and weight gain of the first and third-instar larvae. The oxidized form of *trans*-caryophyllene adversely affected the settling behaviour of the first and third-instar *C. medinalis* larvae in laboratory bioassays (Figure 1.3). The presence of this compound may contribute to the resistance of TKM6 and some wild rices to this pest. First-instar larvae of *C. medinalis* were attracted to one of the more volatile chemicals from rice plants — hexanal — in a bioassay designed to evaluate chemicals involved in the orientation of lepidopterous larvae.

1.25.6 Electroantennogram (EAG) response of rice leaffolder to volatiles from rice plants

Identification of the volatile compounds from rice plants responsible for attraction or repulsion of insect pests will open new avenues for manipulation of insect behaviour for use in pest management programmes, besides serving as useful indicators for breeding resistant rice varieties. The EAG, which measures the drop in electrical potential across an insect's antenna in response to a stimulus, was used to study the reaction of rice leaffolder moths *(C. medinalis)* to total volatile extracts from resistant and susceptible rice plants, see Figure 1.4(a). The volatiles were extracted cryogenically under high vacuum from leaffolder-susceptible IR36 and Rexoro, and resistant TKM6 and *O. perennis* rice plants. Significantly higher EAG responses, compared to equivalent quantities of water, were recorded with all the plant extracts tested. The extracts of susceptible plants generally evoked a higher response than resistant ones. Pure compounds such as hexanal, 1-hexanol, *cis*-3-hexen-1-ol and ß-myrcene, which have been identified as volatilizing from rice plants, also elicited very high EAG responses at one-tenth concentration shown in Figure 1.4(b). We are currently screening the EAG response to other identified volatile compounds from rice plants in some major lepidopterous pests of rice.

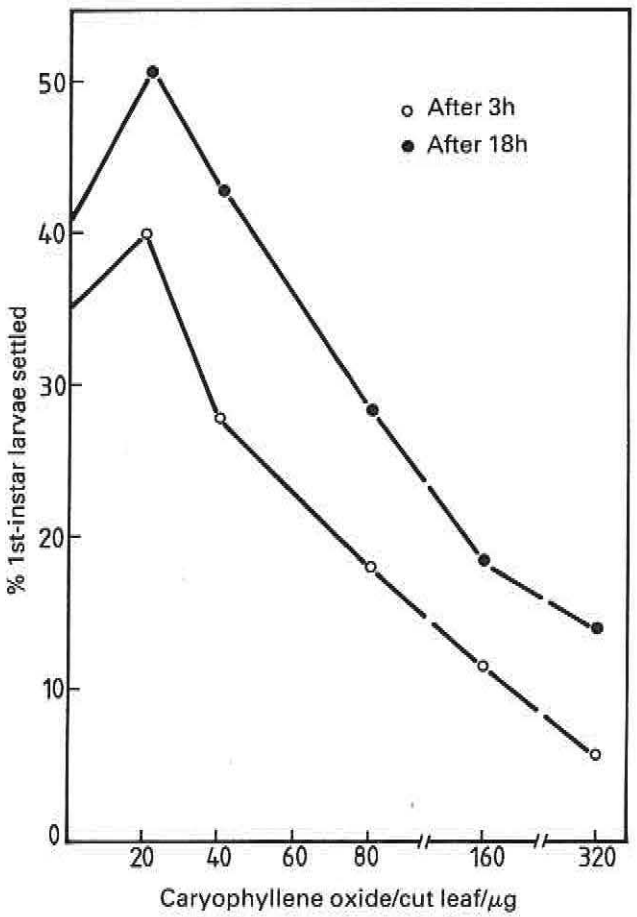


Figure 1.3 Effect of caryophyllene oxide on the settling behaviour of first-instar *C. medinalis* larvae.

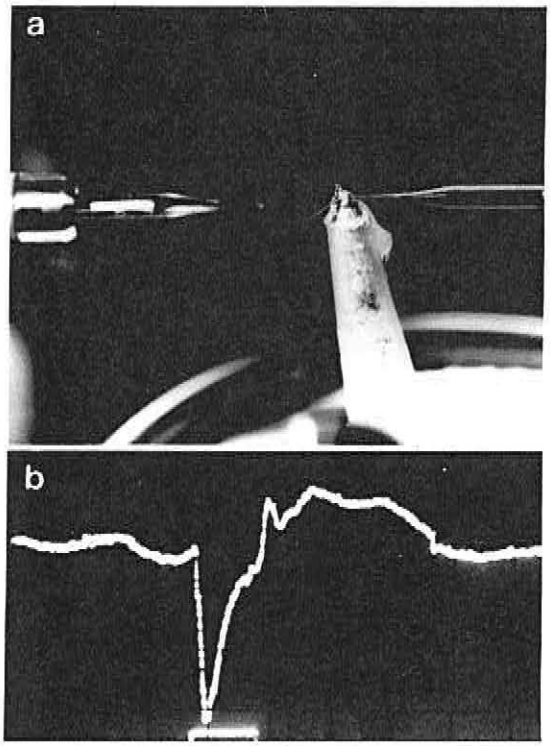


Figure 1.4 (a) Antennal preparation of a female *C. medinalis* moth for electroantennogram response measurement. (b) Negative potential of 2.5 mV recorded for *C. medinalis* in response to hexanal.

1.25.7 Use of tissue culture as a technique in studies of rice resistance to leaffolders

The development of *C. medinalis* on callus from the susceptible variety Rexoro was normal and similar to its development on plants in natural conditions. After 17 days of infestation, *C. medinalis* developed to the fifth instar on Rexoro and susceptible Ptb 10, to fourth instar on moderately resistant IR5865-26-1 callus, and to only the second instar on highly resistant wild rice *O. ridleyi* callus (Figure 1.5). Analysis of protein, total amino acids, carbohydrates and starch in the calluses of the rice varieties clearly indicated that nutrition is not a factor involved in the resistance to *C. medinalis*.

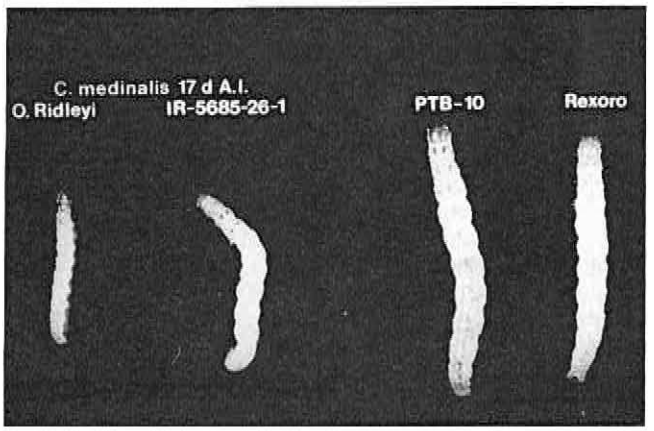


Figure 1.5 *Cnaphalocrocis medinalis* larvae after 17 days growth on callus of susceptible and resistant rice varieties.

1.25.8 Protease activity in leaffolder larvae reared on susceptible and resistant rice varieties

Digestive protease activity was highest in *C. medinalis* larvae reared on susceptible IR36, significantly lower in larvae reared on resistant TKM6, and lowest in larvae reared on resistant wild rice *O. perennis* (Table 1.22). This raises the possibility that protease inhibitors are present in resistant rice plants and suppress growth and development of *C. medinalis* larvae reared on them.

1.25.9 Protein patterns of leaffolder larvae reared on susceptible and resistant rice varieties

Significant differences were recorded in protein bands among third-instar larvae of *C. medinalis* reared on the susceptible rice variety IR36, resistant TKM6 and *O. perennis* plants. This result provides a possible biochemical explanation for the significantly different biological responses of insects feeding on resistant and susceptible host plants. Out of 24 protein bands detected, a total of 18 bands (both anodal and cathodal proteins) were present in larvae reared on IR36 while only 14 bands (mostly cathodal proteins) were present in larvae reared on TKM 6 and *O. perennis* plants. Variations in width and colour intensity of protein bands were also observed.

1.25.10 Attraction of insect pests and their natural enemies to different coloured lights in rice fields

In a field experiment leafhoppers and planthoppers, and their natural enemies such as *Microvelia* sp., *Cyrtorhinus*

Table 1.22 Analysis of protease activity in *C. medinalis* third-instar larvae reared on susceptible IR36 and resistant TKM6 and *O. perennis* plants, using pooled samples of 100 larvae

Food plant	Protease/10ml sample (μg)	Protein/ insect (μg)	Trypsin/ insect (μg)	Trypsin units/μg protease/insect
IR36	12.93	69.51	0.136	0.272
TKM6	11.03	56.44	0.094	0.188
O. perennis	11.35	38.94	0.069	0.138

Table 1.23 Insect pests of rice and their natural enemies attracted to different colours of light in a rice field

	Insects attracted/20 days								
Insect	White	Red	Orange	Yellow	Green	Blue	Violet	U.V.	Total
Pests									
Nilaparvata lugens	2461	267	1081	793	1277	534	422	481	7316
Sogatella furcifera	188	68	15	53	77	27	18	48	494
Nephotettix virescens	3843	228	330	427	415	172	99	124	5638
Cnaphalocrocis medinalis	4	5	0	11	1	2	15	3	41
Marasmia patnalis	0	1	0	1	1	1	0	2	6
Chilo suppressalis	284	221	286	310	160	108	153	148	1670
Scirpophaga incertulas	42	33	9	51	18	0	37	61	251
Predators									
Microvelia sp.	334	46	73	187	81	90	24	100	935
Cyrtorhinus sp.	240	0	24	52	44	19	26	18	423
Formicidae	532	118	101	378	379	279	163	173	2123
Opius sp.	358	32	29	78	58	88	36	92	771

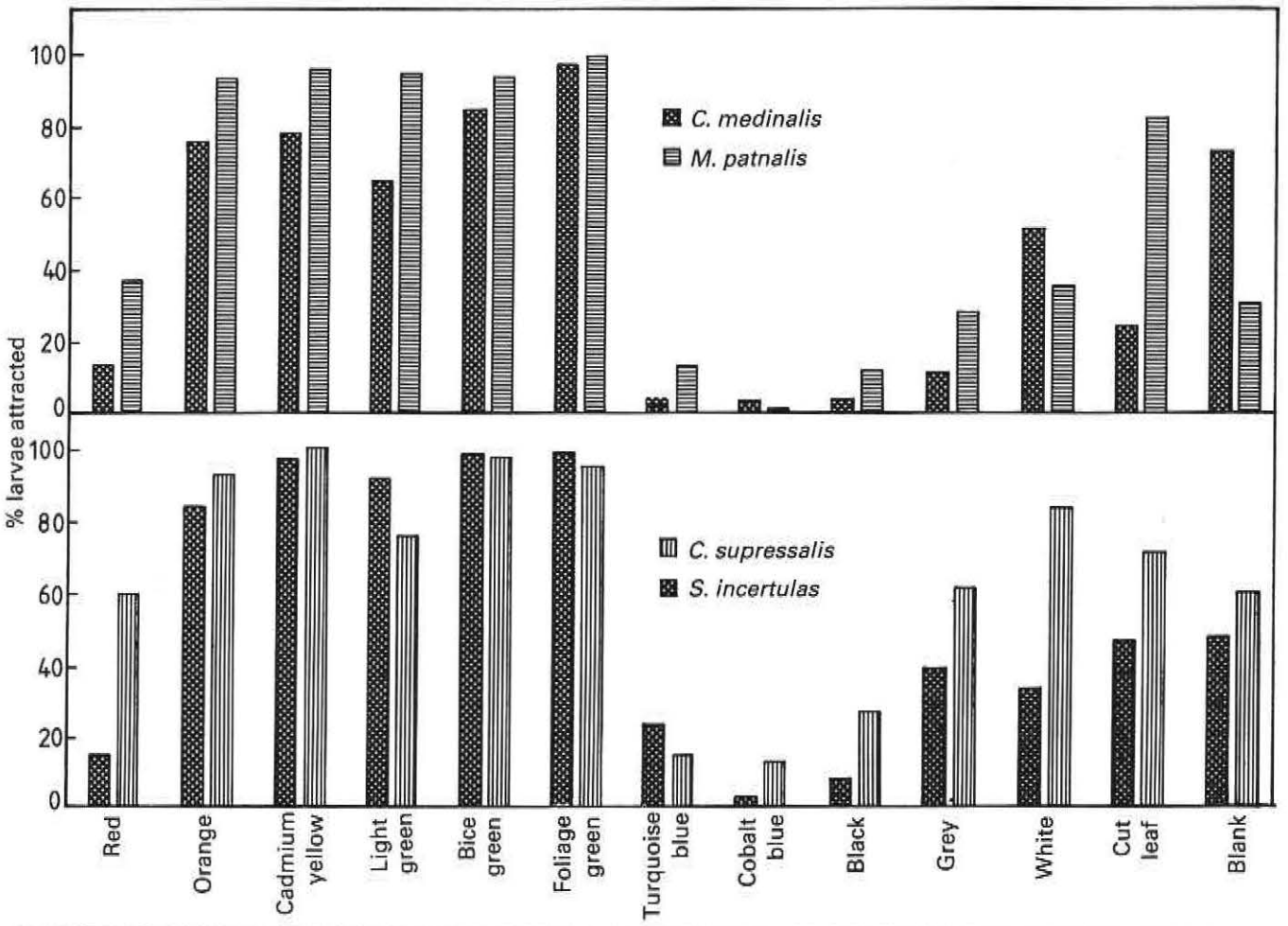


Figure 1.6 Percentages of first-instar larvae of leaffolders *(C. medinalis* and *M. patnalis)* and stem-borers *(C. suppressalis* and *S.incertulas)* attracted by 11 colours of known reflectance spectra tested against an IR36 cut leaf and a blank control.

sp., *Opius* sp., and members of the Formicidae were most attracted to white light (Table 1.23). Of the two stem-borers, *Chilo suppressalis* was most attracted to yellow light and *Scirpophaga incertulas* to ultra violet light. Leaffolders were not strongly attracted to any of the light colours tested. The experiment is being repeated.

1.25.11 Attraction of first-instar larvae of selected lepidopterous rice pests by coloured surfaces

The response of first-instar larvae of the rice leaffolders *C. medinalis* and *M. patnalis,* and the rice stem-borers *S. incertulas* and *C. suppressalis,* were tested on several coloured surfaces with known reflectance spectra (Figure 1.6). Results are given from a preliminary screening of 11 colours against a blank, and a cut leaf of a preferred rice variety (IR36).

In general, orange, cadmium yellow, light green, bice green and foliage green were highly attractive; white, blank, cut leaf, and in some cases red and grey, were approximately neutral; in other cases red and grey were repellent, as were turquoise blue, cobalt blue and black.

The results of this study are, at present, being further tested in choice tests between the cut leaf and the most attractive colours for each species, to determine which colours are suitable for trapping first instar larvae out of infested plants.

1.25.12 The spinning behaviour of two species of rice leaffolders

The spinning behaviour of the rice leaffolders *C.medinalis* and *M. patnalis* was compared. Thirty larvae of each species were observed one at a time as they stitched a 15-cm cut length of IR36 leaf into a roll.

Cnaphalocrocis medinalis larvae made 5–11 stitch sets in 0.3–8.1 min while *M. patnalis* larvae made 5–14 sets in 0.5–11.0 min along the piece of leaf. Analysis of variance on the average time taken to finish each stitch set showed a significant difference between the species.

Feeding behaviour in relation to spinning was also observed. Feeding occurred before the start of spinning, between two stitch sets, after spinning, not at all, or in any combination of these, and chi-squared testing of this data did not reveal any significant differences between the two species.

It was observed that the stitch sets could be spun one after another and always outside the sets previously spun. This pattern was designated as orderly. On the other hand, stitch sets could be spun randomly, such as on top of old sets and between two previously spun sets, in addition to orderly sets.

The chi-squared test of independence of the spinning order showed that the species differ in the proportion of larvae spinning orderly, as opposed to random, stitch sets. Further testing of the fixed ratio hypothesis showed that almost equal numbers of *C. medinalis* larvae spun orderly compared to random stitch sets, while most *M. patnalis* larvae spun randomly in a given population.

Further observations, perfection of methodology, and more appropriate statistical analysis may lead to a new way of identifying the two species of rice leaffolders. The present methods of differentiating the larvae, which are morphologically very similar, involve tedious procedures or require electrophoretic studies (see above).

1.25.13 Mating behaviour of M. patnalis

The mating behaviour of *Marasmia patnalis* was observed in the laboratory under a 12 h light: 12h dark cycle. Of 36 same-aged pairs 28% mated, while 20% mated in 10 pairs with a 1-day age gap and in 5 pairs with a 2-day gap. Mating usually occurred 2 and 3 days after emergence, and 6 h into scotophase (darkness). Mating activity commences with the calling behaviour of the female, followed by the male approaching the female from the rear, curving its abdomen and turning 180° for copulation which lasts from 30 min to about 2 h.

Preliminary field trials have shown that, on average, traps baited with one female moth attract 2 males, while those baited with 2 and 4 females attract 4 and 5 male moths, respectively.

1.25.14 Nitrogen levels and leaffolder infestation

In a second experimental trial, at Lian, Batangas (see *ICIPE 1987 Annual Report),* 5 levels of nitrogenous fertilizer (0, 30, 60, 90 and 120 kg/ha) were applied to IR36 (susceptible), IR5865-16-1 (moderately resistant) and TKM6 (resistant) rice varieties to reconfirm the results on incidence of damage by rice leaffolders. At 65–75 days after transplanting, damage by leaffolders on IR36 and on IR5865-16-1 plants increased significantly with increasing amounts of fertilizer applied, but only on TKM6 at 120 kg/ha nitrogen 75 days after transplanting.

ICIPE-IITA RESEARCH PROJECT

Work continued in Nigeria during the second main cropping season since the project began. The research sites at Alabata in Oyo State, Bida in Niger State and Minjibirr in Kano State were maintained. The possibility of carrying out one or two on-farm tests in Borno and Imo States in collaboration with their agricultural development projects was also explored. These new collaborative arrangements have experienced some logistic problems. They will be reviewed with the objective of strengthening them with greater participation on the part of ICIPE. We continued to have inputs into the three IITA commodity-based working groups. Inputs included joint experiments on cassava/cowpea intercropping and insect pest management at Ohosu, Bendel State, and on-farm testing of cowpea insecticide technology for maize-based cropping systems at Alabata and Ijaiye, Oyo State.

The major experimental studies undertaken by the project continued along the following lines:

- *Insect pest profiles and resultant crop losses*
- *Advantages of intercropping in cowpea insect pest management*
- *On-farm verification of preliminary IPM component technology. Some results are reported here; others will be reported on completion of data analyses.*

1.26 ICIPE/IITA COLLABORATIVE RESEARCH PROGRAMME ON COWPEA IMPROVEMENT

A. M. Alghali

1.26.1 Studies on grain yield losses due to cowpea insect pests

Studies on insect pest profiles and yield losses caused by these pests continued for the second year. The experiment was designed to assess the grain yield losses due to various cowpea insects at the sites and to determine the cost-effectiveness of 1–4 insecticide sprays at different plant growth stages. The experiment was conducted at Alabata and Minjibirr. Individual plots measuring 3.75 × 4 m were sprayed with Cymbush Super ED 1–4 times depending on the prescribed treatment. Three cowpea cultivars were used: TVx 3236 for its moderate level of thrips resistance, IT84E-108 for its high level of aphid resistance and a local bold white-seeded cultivar "Dan ilan".

The key insect pests were a species of *Ootheca* (a foliage beetle) in the vegetative stage, flower thrips in the bud initiation and flowering stages, and a complex of pod-sucking bugs. In general, pest pressures were lower in Minjibirr than in Alabata, except for the pod-borer *Maruca testulalis*. However, the *M. testulalis* populations were low. Results are presented for the studies at Minjibirr (Table 1.24).

Table 1.24 Percent change in grain yield in 3 cowpea cultivars under insect pest attack at Minjibirr, Kano State

Insect pests	TVx 3236	IT84E-108	"Dan ilan"
Foliage pests	−34.5	–	−25.6
Thrips at bud initiation	−21.7	23.9	−28.7
Thrips at flowering	+11.5	+39.2	−1.2
Thrips at bud initiation + flowering	−42.1	−3.0	−56.4
PSB[1]	−34.6	+30.2	−31.5
Foliage pests + thrips at bud initiation	−30.0	−15.7	−31.5
Foliage pests + thrips at flowering	−30.3	+19.0	−26.3
Foliage pests + PSB	+6.7	−7.8	−7.9
Thrips at bud initiation + PSB	−36.0	+51.9	−28.7
Thrips at flowering + PSB	−40.8	−0.4	−36.4
Foliage pests + thrips at bud initiation + PSB	−29.1	+16.4	−27.3
Foliage pests + thrips at flowering + PSB	−35.8	−22.0	−20.1
All pests combined	−73.3	−57.5	−59.1

[1] Pod-sucking bugs.

The percentage grain yield losses due to insects attacking the crop at the various growth stages were assessed. At both locations, thrips damage was the most serious, followed by pod-sucking bugs and varietal differences were observed for these pests. (In Minjibirr flower thrips damage was more acute at bud initiation; while in Alabata it was more acute at flowering.) Combinations of two or more pests decreased the grain yield by 50–80%. Results are presented for Minjibirr (Table 1.25).

Table 1.25 Marginal returns (in naira) for every naira spent on each additional insecticide spraying of 3 cowpea cultivars at Minjibirr, Kano State (3 scenarios)

Spray occasions	TVx 3236	IT84E-108	"Dan ilan"
Scenario 1			
Bud initiation	3.75	1.63	3.27
Bud initiation + flowering	8.84	1.36	2.14
Bud initiation + flowering + podding	−8.77	0.75	−3.10
Foliage + bud initiation +flowering + podding	7.38	–	4.49
Scenario 2			
Flowering	4.42	3.39	2.67
Flowering + podding	1.21	−3.07	−0.75
Bud initiation + flowering + podding	−1.14	1.50	1.03
Foliage + bud initiation + flowering + podding	7.38	–	4.49
Scenario 3			
Bud initiation	3.75	1.63	3.27
Bud initiation + podding	1.14	3.92	−1.07
Bud initiation + flowering + podding	−1.07	−1.82	0.11
Foliage + bud initiation + flowering + podding	7.38	–	4.49

The marginal returns for every naira invested in one additional insecticidal spray in plots sprayed 1–4 times were also worked out for the 2 test sites. Three different scenarios of 1–4 schedules were mapped out; one spray at either bud initiation or flowering appeared to be the most profitable for most cultivars. This is in contrast to last year's observation that 3 sprays (at bud initiation, flowering and podding) were recorded as the optimum spraying level. Possible explanations are the increase in spray costs accompanying the price increases after the devaluation of the naira and the lower pest incidence in 1987. Other profitable spray regimes were obtained with some cultivars at both sites. Four sprays against foliage pests, thrips at bud initiation and flowering, and pod-sucking bugs showed profitable returns for all cultivars and in all locations. But the differences in grain yield between this regime and one spray at either bud initiation or flowering were not very high. The marginal yield increase due to 4 sprays compared with 3 was sufficiently high to result in positive returns.

1.26.2 Pest and damage levels of cowpea under monocropping and intercropping systems in Minjibirr

An experiment to determine the pest status and level of damage for cowpeas intercropped with sorghum was undertaken in Minjibirr. Five elite cowpea cultivars were planted in monocropped and sorghum intercropped plots measuring 4 × 5 m. Half of each plot was protected by spraying Sherpa Plus at bud initiation, flowering and podding growth stages. Insect numbers and

grain yields were recorded from each of the protected and unprotected sub-plots (Table 1.26).

Table 1.26 Damage levels and grain yields for five cowpea cultivars planted in monocrop and sorghum intercropping systems on sprayed and unsprayed plots, Minjibirr, Kano State

	No. of *M. testulalis* per 20 flowers	No. of thrips per 20 flowers	Seed damage by PSB[1] (%)	Grain yield (kg/ha)
Cropping system				
Monocrop	5.3	34.7	24.7	515.6
Intercrop	5.7	27.7	29.3	255.3
LSD[2]	1.9	7.9	4.7	98.7
Spray level				
Sprayed	3.6	9.7	25.0	531.7
Unsprayed	7.4	52.6	29.0	239.2
LSD	1.0	5.3	5.2	129.1
Cultivars				
IT84E-124	5.5	33.4	30.4	383.0
IT84S-2246-4	9.3	23.6	25.6	513.6
TVx 3236	5.6	37.8	25.2	441.6
IT84E-108	3.8	20.8	25.8	362.2
"Dan ilan"	3.3	40.2	28.0	226.8
LSD	2.3	14.5	10.0	172.2

[1] Pod-sucking bugs.
[2] Least significant difference ($P = 0.05$)

The insects observed on this experiment were *M. testulalis*, flower thrips and pod-sucking bugs. The number of *M. testulalis* larvae and seed damage due to pod-sucking bugs were not influenced by cropping systems. Intercropping cowpeas gave lower numbers of flower thrips. Spraying the cowpeas with Sherpa Plus was effective in reducing insect numbers. There were varietal differences for the numbers of insects recorded on different cultivars. "Dan ilan" and IT84E-108 had the lowest numbers of *M. testulalis* larvae while IT84E-108 and IT84S-2246-4 had the least flower thrips.

Damage by the insect pest complex reduced grain yields by 55%. Similarly, intercropped plots yielded 51% less grain compared with monocropped plots. Yield differences were observed in different cultivars, with IT84S-2246-4 and TVx 3236 being the top yielders.

1.26.3 The efficacy of two insecticidal sprays for cowpeas grown in monocrop, or in intercrop with maize

Preliminary studies have indicated that insect numbers are reduced when cowpea is grown as an intercrop with cereals. Similarly, 3 timely sprays have been observed as the optimum for growing cowpeas in this region. Therefore, in order to reduce the spray requirements for the resource-poor farmers who grow cowpeas and derive a sizeable portion of their protein from it, studies were designed to investigate the usefulness of intercropping cowpea with maize for reducing pest attack. This was done by reducing the optimum number of sprays to two.

The same 5 elite cultivars were planted alone or intercropped with maize in plots measuring 4 × 5 m at Alabata. Half of each plot was protected with 2 sprays of Cymbush Super ED applied either at the foliage and flowering stages, or at the bud initiation and podding stages.

Intercropping maize and cowpea reduced the number of flower thrips and pod-sucking bugs on the cowpea, while the number of *M. testulalis* larvae was similar for both the sole and intercropped cowpea. Spraying the cowpea at the foliage and flowering growth stages was more effective in reducing *M. testulalis* and flower thrips numbers than spraying at bud initiation and podding. The later spray schedule was more effective against pod-sucking bugs. The various cultivars did not differ significantly for the different insect numbers and in grain yields except for IT84S-2246-4 which out-performed the others. Grain yields were similar for the 2 spray schedules, but differed significantly for the cropping systems. Monocropped cowpeas produced 60% more than those intercropped.

1.26.4 Efficacy of three sprays for cowpea grown on-farm in both monocropping and intercropping systems

Biotic pressures such as insect pests are known to depress cowpea grain yields considerably. In an effort to minimise this, insecticidal applications have been very useful with most spray recommendations based on regular calendar intervals. Calendar schedules are sometimes ineffective with cultivars of varying maturity periods, and especially with indeterminate local cultivars whose growth cycles cannot be predicted in order to fit into a calendar. Thus, spray schedules based on plant growth stages which coincide with attack by the key insect pests would be most effective.

Preliminary studies to determine an optimum spray level for cowpea indicated that 3 sprays at bud formation, flowering and podding were most suitable. On-farm trials to verify the appropriateness of three insecticidal sprays for sole and intercropped cowpeas were therefore carried out at Alabata and Minjibirr. Intercropping cowpea with cereals has been shown to reduce the numbers of some insect pests, but not enough to offset grain yield reduction. Farmers fields ranging in size from 1000–2500 m^2 were planted in equal proportions to sole and intercropped cowpeas using a 3 cultivars. Thirteen fields were planted at Minjibirr with TVx3236, IT82D-699 and "Dan ilan" in the sole and intercropped plots with sorghum. Similarly, in Alabata, 10 fields were planted with IT81D-994, IT84E-124 and a local cultivar intercropped with maize. An equal number of fields were sprayed with Cymbush Super ED using the Electrodyn sprayer and Sherpa Plus using the knapsack sprayer. In both locations, plots of each cultivar were divided equally into sprayed and unsprayed portions. The insecticides were applied to the sprayed subplots at the 3 major plant growth stages of bud initiation, 50% flowering and 50% podding.

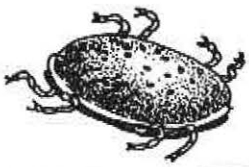
The insecticides were effective in reducing insect numbers, but they gave varying responses in the different insects, cultivars, locations and cropping systems. Cymbush Super ED was more effective against aphids,

Ootheca foliage beetle and flower thrips, while Sherpa Plus was more effective against pod-sucking bugs in Alabata. In Kano, Sherpa Plus was more effective against the two predominant pests, flower thrips and pod-sucking bugs. In both locations, the intercropped cowpeas had lower numbers of aphids, *Ootheca* beetle, flower thrips and pod-sucking bugs than the monocropped cowpeas. There was no significant difference for the number of *M. testulalis* recorded from the intercropped and monocropped cowpeas in Alabata, probably because of their low numbers.

Grain yields were increased substantially with 3 sprays of both Cymbush Super ED and Sherpa Plus, in both locations, and in the two cropping systems for most of the cultivars used. In general, control of insect pests by insecticidal applications was profitable for both locations and both cropping systems. Application of insecticides to monocropped cowpeas was more profitable for Sherpa Plus than Cymbush Super ED in terms of absolute profits, but their cost:benefit ratios were similar. The performance of the local cultivar was similar to, or slightly better than, that of the elite cultivars, both in terms of additional yields and cost:benefit ratios.

LIVESTOCK TICKS RESEARCH PROGRAMME

2



Livestock Ticks Research Programme

The primary goal of the Livestock Ticks Research Programme (LTRP) is to control ticks through the application of a library of integrated tick management packages. These will consist of models to be applied eventually to any of the various farming systems in Africa. They must be technically feasible, economically affordable, and also sustainable by resource-poor subsistence farmers. It is anticipated that the components of these integrated tick management packages will include an anti-tick vaccine, limited or zero acaricide application, improved management and, probably, genetic methods. This multi-disciplinary approach is being advanced through research into host immunology, host and vector genetics, tick biology and tick ecology.

Laboratory studies have concentrated on the isolation, characterization and purification of antigens derived from tick salivary glands, tick midgut and the whole tick which have shown varying degrees of protection against tick feeding, especially on rabbits. Monoclonal antibodies were also raised against tick polypeptide antigens. An enzyme-linked immunosorbent assay (ELISA) technique to measure antibody levels in animals following immunization with purified tick antigens was developed during the year. Research has also progressed towards the use of tick gustatory receptor proteins, tick haemolymph and ecdysone (moulting hormone) as protective antigens with which to generate antibodies in the host that would interfere with tick feeding and metabolism.

Tick ecological studies were intensified in 1988. Survival and development studies on Rhipicephalus appendiculatus *and* Amblyomma variegatum *continued in three biotypes with concurrent use of data loggers. Studies on Rusinga Island continued with increased emphasis on tick population studies and the further participation of the ICIPE Social Science Interface Research Unit. It is hoped that data from these studies will be used to establish a reliable model for predicting tick populations in the field. Work continued on the natural enemies and pathogens of ticks and possible methods of biological control. Several predators have been identified and their effects quantified. A parasitoid,* Hunterellus *sp., was regularly found in about 33% of* A. variegatum *nymphs at one study site.*

The ICIPE/Kenya Government tick project in Mariakani and Mtwapa took off by the end of 1988. The aim is to establish cost-effective control of ticks on cattle populations protected from tick-borne diseases. The main research activities are located in the Livestock Productivity Centre at Mariakani and the Coast Agricultural Research Station at Mtwapa. Investigations have started on the prevalence of ticks, estimation of their populations on pasture, and their survival and development off the host.

The move to new laboratories at Duduville has at last given the LTRP good facilities for housing experimental animals and the important tick colony. We can now plan experiments with adequate numbers of cattle or rabbits that have been reared free of ticks.

In February the Programme underwent one of the regular in-depth triennial reviews, with very favourable results. A brainstorming session was held in November and programme priorities were critically assessed and a timetable drawn up for their achievement.

2.1 IMMUNIZATION OF RABBITS WITH SOLUBILIZED TICK-DERIVED ANTIGENS

A. O. Mongi, E. I. P. Kamanga-Sollo, C. A. Aganyo and G. K. Otieno

Two possible approaches to tick control are the utilization of acquired host resistance to tick infestation, and immunization with tick-derived immunogens. Both are being followed. In each case the induced resistance is expressed by the immunized host's ability to interfere with the reproductive potential of the ticks feeding upon it.

We have recently shown that immunizing rabbits with solubilized whole-tick extract from *Rhipicephalus appendiculatus,* or with the derived antigen-antibody complexes, confers protection against subsequent tick challenge. In the present study we have employed both gel permeation and ion-exchange chromatography in the purification of some of the tick antigenic components.

Gel permeation chromatography was performed by fractionating an extract in 1% deoxycholic acid of fully-fed, solubilized, female *R. appendiculatus*. The chromatogram revealed the presence of 5 peaks (Figure 2.1). The individual peaks from several replicates were pooled and concentrated. Fraction V contained the highest concentration of protein and was therefore selected for further study. This peak was also selected because of an earlier (unpublished) observation using solubilized tick midgut extract on Sephacryl S-300 in which the protein profiles obtained were similar to that obtained by fractionating whole tick extract. In addition, ticks feeding on rabbits immunized with the midgut fraction showed a slight reduction in engorgement weight and in the weight of eggs produced.

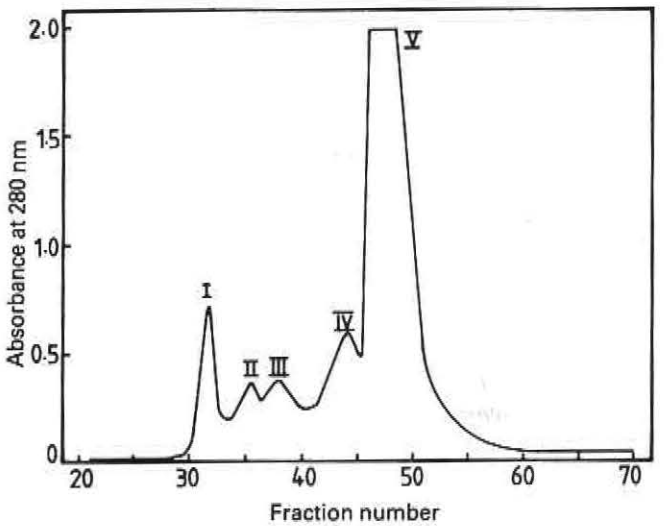


Figure 2.1 Elution profile of solubilized whole tick extract of *R. appendiculatus* in 1% deoxycholic acid fractionated on Sephacryl S-200. The eluted peak fractions are numbered I–V.

The ion-exchange chromatography employed was in diethylaminoethyl (DEAE)-Sephacel medium. When the Sephacryl S-200 peak fraction V was completely adsorbed on the DEAE-Sephacel medium, the column was eluted with a gradient of 0.01–0.5M NaCl. At least 7 new fractional peaks (Figure 2.2) were obtained

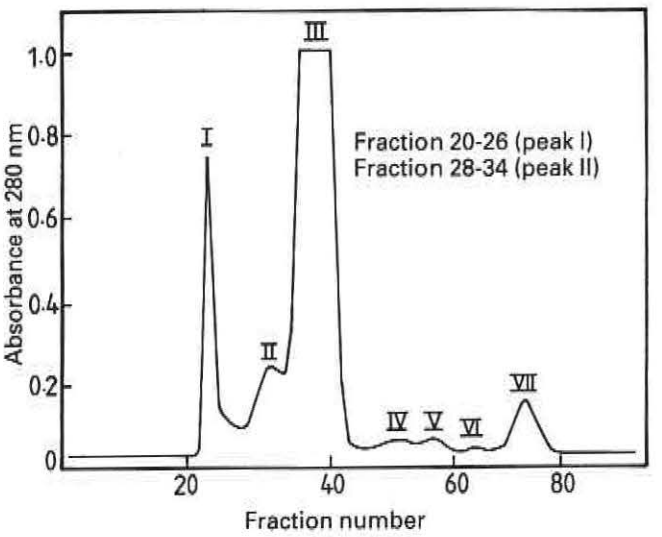


Figure 2.2 Elution profile of peak fraction V (see Figure 2.1) on a DEAE-Sephacel ion-exchange column. The eluted peak fractions are numbered I–VII.

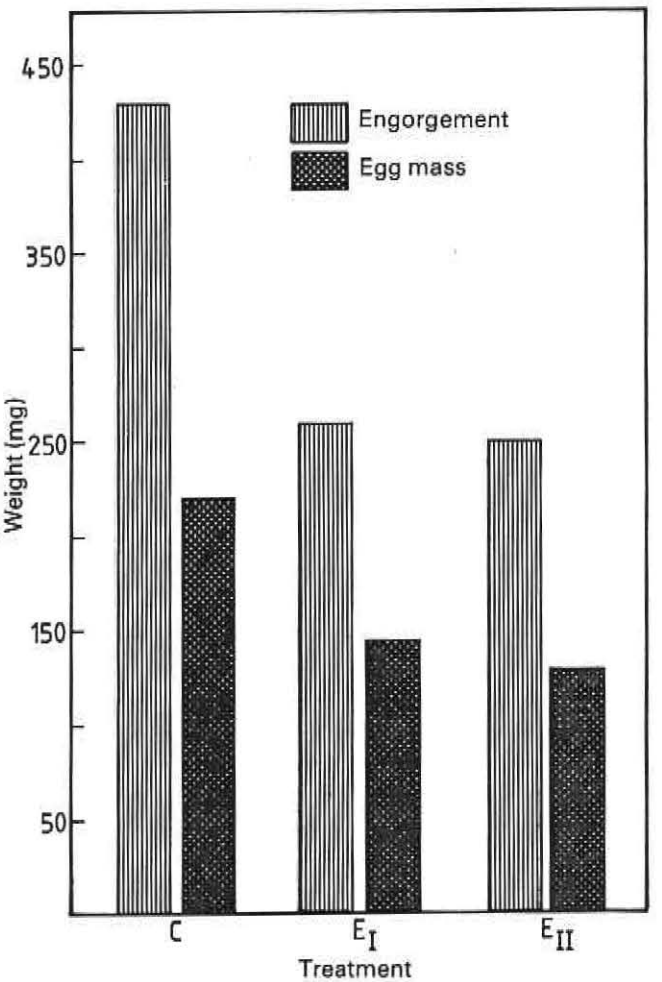


Figure 2.3 Mean performance of *R. appendiculatus* fed on rabbits immunized with DEAE-Sephacel peak fractions I and II (see Figure 2.2); C = untreated control; EI, EII = immunised rabbits.

from the protein profile. Peak fractions I and II were used separately for immunization. Each antigenic fraction was mixed with an equal volume of Freund's complete adjuvant (FCA) and 5 rabbits were immunized 3 times with these immunogen preparations. Test feeds with adult *R. appendiculatus* were done and the effects of the immunizations are shown in Figure 2.3. There were statistically significant reductions in both engorgement weight and egg weight compared with the control rabbits injected with FCA and PBS alone.

The antibody titres in all the rabbits were assayed using both immunodiffusion and enzyme-linked immunosorbent assays (ELISA). In ELISA, antibody titres of 1:200 and 1:300 for DEAE-Sephacel fractions I and II were detected one week post immunization, rising to peak titres of 1:2000 and 1:2500 after the second booster injections. However, there was no significant change in the antibody titres after the third booster.

The polypeptide antigens involved in these protective immune responses against feeding ticks are also being investigated by immunoblotting studies. It is intended to isolate these polypeptides for further immunizations studies.

2.2 ESTABLISHMENT OF AN ASSAY SYSTEM FOR EVALUATING THE EFFICACY OF IMMUNIZATION WITH TICK ANTIGENS

E. I. P. Kamanga-Sollo, M. Nyindo, A. O. Mongi, S. K. Mbogo, H. A. Kutima and O. O. Dipeolu*

Antibody levels in cattle immunized with tick midgut antigens correlate with the degree of protection given. They are therefore a good indicator of the degree of immunity conferred on animals immunized with purified antigens. Preliminary results using sera from rabbits immunized with antigens isolated from the whole-tick extract (see section 2.1) also show that antibody titres are good indicators of the degree of immune response. The technique used in this study was the enzyme-linked immunosorbent assay (ELISA).

The specificity and sensitivity of ELISA depend on the nature of the antigen (pure or semi-purified) and the avidity of the antibodies produced against the immunizing antigens. The first step in establishing an assay system for evaluating the efficacy of immunization of rabbits and cattle with selected antigens was, therefore, the purification of the antigens. Proteins derived from the midgut of *Rhipicephalus appendiculatus* were therefore subjected to ammonium sulphate precipitation and gel chromatography. The results of these studies are currently being evaluated.

The antigens that are isolated will be used for coating the plates in the ELISA system. Earlier observations using antigens from the whole tick extract indicated that 0.15M phosphate-buffered saline containing 2% polyethylene glycol 6000 would be the buffer of choice in adsorbing tick antigens to the polyvinyl plates. Efforts are also underway to make conjugates which will be used in ELISA.

* *Postgraduate student from the Department of Zoology, Kenyatta University, Nairobi.*

2.3 PROTECTION OF ANIMALS FROM TICKS BY INTERFERENCE WITH THE TICK FEEDING PROCESS

E. I. P. Kamanga-Sollo, S. M. Waladde, D. Ben-Yakir, R. Galun*, P. B. Capstick and O. O. Dipeolu*

Tick gustatory receptors are located in the paired cheliceral digits. They are chemoreceptors stimulated by phagostimulant components in the host blood. It has been established that "cheliceral sensing" is an essential component in the tick feeding process. Two phagostimulants are involved in the stimulation of the cheliceral receptors; these are glutathione (GSH) and adenosinetriphosphate (ATP) in combination with glucose.

The work so far has concentrated on GSH which is a tripeptide (glutamyl cysteinyl glycine). Initial attempts to polymerize GSH in order to make an immunoaffinity column for the isolation of cheliceral proteins were not very successful. Attempts are now being made to develop a standard technique to conjugate GSH with a larger protein molecule (e.g. bovine serum albumin), which can then be coupled to an insoluble matrix to form an immunoaffinity column. This column will then be used to isolate the cheliceral proteins binding to the GSH as these are probably the receptor proteins. The work has concentrated on GSH because earlier observations by both Waladde and Ben-Yakir showed that GSH induces faster feeding and larger blood meals in ticks.

Work on the isolation of tick cheliceral proteins continued in order to assess the changes, if any, in protein profile during the course of feeding. Ticks *(Rhipicephalus appendiculatus)* were fed on tick-naive rabbits for 1–8 days. The cheliceral digits were cut off and the protein extracted by solubilizing with different detergents. The estimated protein concentrations are presented in Table 2.1, from which it can be seen that there was no significant difference in the amount of pro-

Table 2.1 Estimates of total protein content of cheliceral digits of *R. appendiculatus* on each day during the course of feeding. Protein content in μg/ml by u.v. absorbance at 260 and 280 nm; 1 ml of extract per 100 pairs of chelicerae

	Days of feeding								
Ticks	0	1	2	3	4	5	6	7	8
Males	130	250	300	70	30	110	110	40	20
Females	120	140	130	140	80	50	90	110	160
Mated Females	100	70	60	130	120	180	120	100	300

tein obtained from the cheliceral digits in relation to the days of feeding. It was therefore decided to collect the chelicerae on day 5 since the same ticks could then be used for harvesting tick midgut.

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2.4 INDUCTION OF RESISTANCE TO TICKS BY IMMUNIZING WITH ECDYSONE AND OTHER TICK ANTIGENS

S. K. Mbogo, E. O. Osir, and A. O. Mongi

Experiments were conducted to evaluate ß-ecdysone tick haemolymph proteins and *Rhipicephalus appendiculatus* gut antigens as potential tick immunogens.

2.4.1 Beta-ecdysone

Beta-ecdysone and other ecdysteroids have been detected at all stages in the life cycle of some ixodid ticks and are thought to control moulting, oogenesis, sex pheromone activity, salivary gland degeneration and the termination of diapause. These processes are physiologically vital for ticks, hence blocking them would produce fatal effects.

Attempts were made at blocking the above processes immunologically by feeding ticks on rabbits immunized with ß-ecdysone. Since ß-ecdysone has a low molecular weight it was conjugated with bovine serum albumin as a carrier to render it immunogenic. It was hypothesized that when the antibodies to ß-ecdysone entered the tick haemolymph they would neutralize the physiological functions of the ß-ecdysone.

2.4.2 Solubilized tick gut membrane proteins (STGMP)

Previous work has shown that ticks feeding on hosts immunized with tick gut antigens are adversely affected. The tick gut wall is thought to be damaged by anti-tick antibodies in the blood meal which increase the passage of other antibodies and harmful substances across the gut barrier into the haemolymph. It is likely that not more than a few polypeptides cause these effects and their isolation and use would allow otherwise protected targets to be attacked, e.g. internal organs and haemolymph components.

Tick guts were harvested from 5-day fed female *R. appendiculatus* and solubilized using Triton X-100 and deoxycholic acid. The STGMP were characterized using sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and their protein profiles were determined.

To facilitate the entry of ß-ecdysone antibodies into the haemolymph, rabbits were immunized with conjugated ß-ecdysone in combination with STGMP. For control purposes, other rabbits were immunized with STGMP alone and conjugated ß-ecdysone alone. All immunizations were made using Freund's incomplete adjuvant.

Two weeks after the final booster, the rabbits were challenged by feeding larvae, nymphs and adults of *R. appendiculatus* on them. The results are still being accumulated.

2.4.3 Haemolymph proteins

Haemolymph bathes all tick tissues and contains physiologically vital proteins including enzymes. Neutralization of these proteins would therefore be likely to cause severe anti-tick effects.

Experiments were conducted to evaluate the anti-tick potential of using tick haemolymph as an immunogen. Initial studies concentrated on determining the presence or absence of age-specific and sex-specific proteins in order to determine from which ticks the haemolymph should be used for immunization.

Haemolymph was harvested from both male and female *R. appendiculatus* at different stages of development, i.e. from unfed to 6 days of feeding. The haemolymph was characterized using SDS-PAGE and its protein profiles observed.

It was demonstrated that ticks have proteins specific for both sex and age. The protein concentration (and probably diversity) is maximal in 5–6 day fed ticks and males and females show different protein profiles (Figure 2.4).

Haemolymph was harvested from 5-day fed male and female ticks and will be used to immunize rabbits. Any anti-tick effects will be assessed by challenging the immunized rabbits with all stages of *R. appendiculatus*.

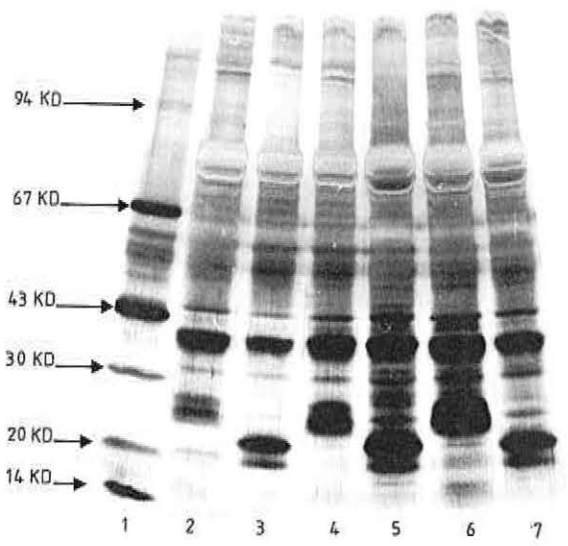

Figure 2.4 Silver stained protein profiles obtained by SDS-PAGE electrophoresis of haemolymph from *R. appendiculatus* males and females fed for 4–6 days.

Lane	*Sample applied*
1	Mol. wt markers
2	Male, 4-day fed
3	Female, 4-day fed
4	Male, 5-day fed
5	Female, 5-day fed
6	Male, 6-day fed
7	Female, 6-day fed

2.5 IMMUNIZATION USING SOLUBILIZED TICK GUT ANTIGENS: CROSS-PROTECTION BETWEEN SPECIES

H. A. Kutima and E. I. P. Kamanga-Sollo*

Livestock are subject to challenge by more than one species of tick under natural conditions. There are, however, few reports of cross-protection between different tick species in the immunity that develops. Gut antigens have been used by several workers to immunize animal hosts, with attractive results. The objective of this study, therefore, was to immunize rabbits with solubilized gut antigens derived from *Rhipicephalus appendiculatus, Rhipicephalus evertsi* and *Amblyomma variegatum* and to assess the immunity engendered both within and between species.

Female *R. appendiculatus* and *A. variegatum* were fed on rabbits' ears and removed on day 5 post attachment. Tick guts for antigen preparation were obtained by dissection in phosphate-buffered saline (at 4°C) containing protease inhibitors and stored at −20°C until use. Twenty-four batches of *R. appendiculatus* and 13 of *A. variegatum* gut material were harvested. We are now in the process of preparing and characterizing the gut antigens.

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2.6 COMPARISON OF *RHIPICEPHALUS APPENDICULATUS* STRAINS

J. W. Chiera

It has been shown previously that the laboratory strain of *R. appendiculatus* that we use is more severely affected by host resistance to tick infestation than field strains *(ICIPE 1986 Annual Report)*. The present investigation is to compare 3 field strains of *R. appendiculatus,* collected in ecologically different parts of Kenya, with respect to their ability to survive under various controlled conditions of temperature and humidity.

The strains under study are from: Rusinga Island (RS) near Mbita Point Field Station; Kikuyu (KS) in Kiambu District; Intona (IS) in the Trans-Mara Division, Narok District, and the Muguga laboratory strain (LS). Results to date have shown some differences between strains. Eggs of LS had the poorest hatchability at all humidities and temperatures tested, especially the lowest humidities (Table 2.2). The hatchability of IS eggs was similar to that of LS at 22°C but differed at 28°C. Hatchability of the eggs of all the strains was positively correlated to both temperature and humidity, unlike the survival of unfed larvae, nymphs or adults which is known to be negatively correlated to temperature, but positively correlated to humidity.

The preliminary results of a comparison of the survival of unfed nymphs of KS, IS and LS have also shown differences between strains under stress conditions. At 18°C and 44% r.h. KS nymphs survived better than both

Table 2.2 Mean percentage hatchability (± s.e.) of eggs of 4 strains of *R. appendiculatus* under controlled conditions of temperature and humidity (r.h.)

R.h. (%)	Strains			
	Rusinga	Kikuyu	Intona	Lab.
Temperature 22°C				
44	0	0	0	0
55	0	0	0	0
75	$33 \pm 3^{c*}$	22 ± 4^{bc}	13 ± 4^{bc}	6 ± 2^{a}
93	82 ± 2^{b}	83 ± 3^{b}	67 ± 6^{ab}	55 ± 5^{a}
Temperature 28°C				
44	2 ± 2	0	0	0
55	2 ± 1^{a}	3 ± 1^{a}	0	0
75	73 ± 3^{c}	62 ± 3^{bc}	54 ± 4^{b}	30 ± 5^{a}
93	96 ± 2^{b}	90 ± 4^{b}	95 ± 4^{b}	74 ± 5^{a}

*Percentages in each row having the same letter do not differ significantly ($P > 0.05$).

LS and IS nymphs, which were similar. At 28°C and 44% r.h. survival of LS nymphs was poorer than that of KS and IS nymphs. For the combinations at 22°C and 44% r.h., 22°C and 55% r.h. and 28°C and 55% r.h., however, there were no differences between the strains.

This work is still in progress and it is hoped that the results will lead to a better understanding of the ecological characteristics of the field strains.

2.7 INVESTIGATIONS ON RUSINGA ISLAND

D. K. Punyua and A. A. Latif

A number of experiments which were started in 1986 and 1987 were continued in 1988. These included observations on cattle productivity and a tick-borne disease survey on calves *(ICIPE 1987 Annual Report)*.

2.7.1 Effect of age on tick burden

This experiment was started in 1986 using 10 cattle in Farm 22, when 5 of the animals were adult and the other 5 were calves. The observations will stop as soon as these calves reach reproductive maturity. The animals were all de-ticked each month and the ticks were identified and counted. During the 1985 Rusinga Island tick survey *(ICIPE, 1986 Annual Report)* it was found that calves carried significantly less ticks than adult cattle. It is, therefore, the purpose of the present experiment to compare the infestation levels of adults with calves over a long period. The results for *Rhipicephalus appendiculatus* are shown in Figure 2.5. Although infestations on calves were generally lower than on adult cattle the difference was not significant.

2.7.2 Feeding performance of four strains of R. appendiculatus *on Rusinga Island cattle*

An index of tick resistance has recently been developed and tested. It is based on the engorgement weight of the local strain of *R. appendiculatus* (RS) females and nymphs feeding on resistant cattle from Rusinga Island and some susceptible Friesian steers reared in an area

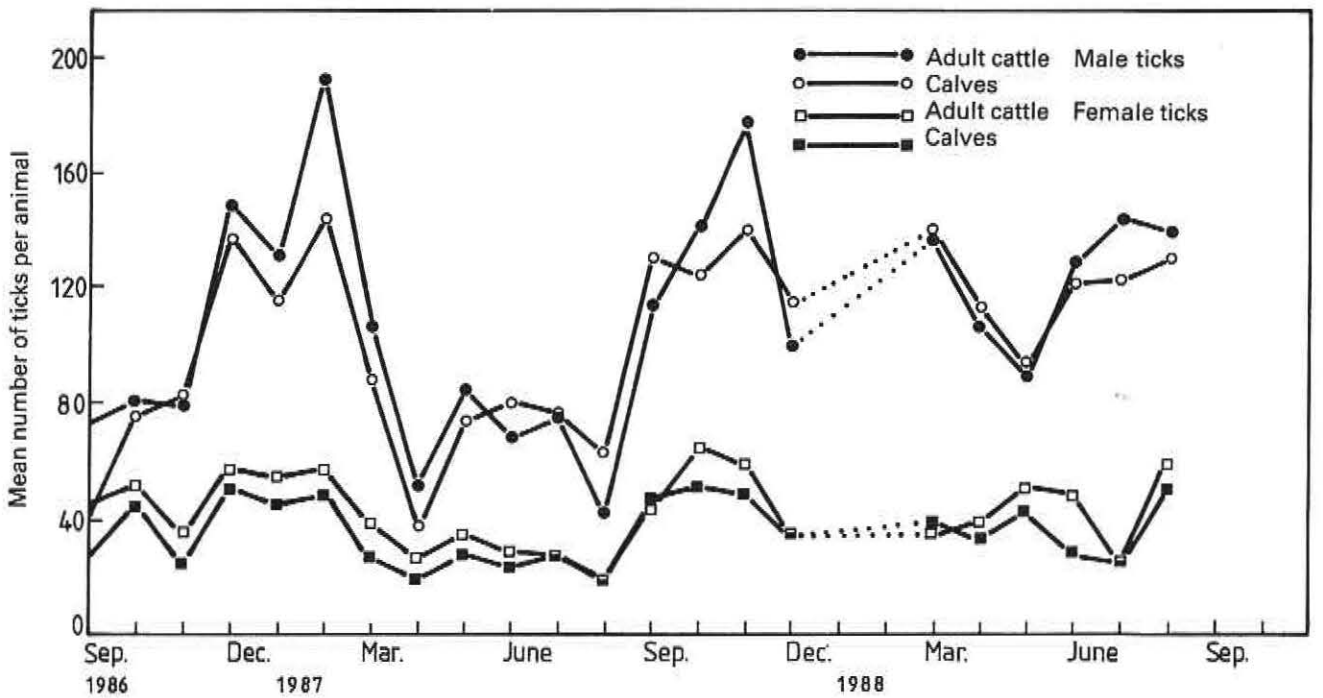

Figure 2.5 Monthly infestation levels of *R. appendiculatus* males and females on mature cattle and calves on Farm 22 Rusinga Island.

with intensive tick control. While the index could be said to be applicable to the Rusinga Island populations of *R. appendiculatus* and cattle, it was necessary to test its applicability to other situations.

Using the same resistant Rusinga Island cattle, strains of *R. appendiculatus* originating from Intona (IS) (Trans-Mara Division, Narok District), Ukunda (US), (Kwale District, on the Coast) and the Muguga Laboratory strain (LS) were compared with RS. Twenty-five (RS) females, 25 males and 50 nymphs were applied together on the left ear of each of 18 animals. For comparison, the same number of ticks was applied to the right ear of each animal, with 6 receiving IS, US and LS, respectively.

The animals were examined twice daily and the engorged ticks were collected and counted. The females were weighed individually, and the nymphs from each animal were weighed in a group. The results are given in Table 2.3.

Table 2.3 Feeding performance of 3 strains of *R. appendiculatus* on resistant cattle from Rusinga Island compared with the Island strain; mean weight (± s.e.) and percentage fed (*n* = sample size)

	Comparison					
Ticks	Rusinga	Intona	Rusinga	Ukunda	Rusinga	Lab.
Females						
Weight (mg)	257.4	250.6	303.2	314.8	295.3	275.0
± s.e.	13.1	11.9	10.4	13.6	11.0	8.6
% fed	34.7	47.3	52.7	43.3	47.3	56.7
n	52	71	79	65	71	85
Nymphs						
Weight (mg)	6.0	6.2	6.2	6.1	6.9	6.2
± s.e.	0.5	0.2	0.3	0.3	0.4	0.4
% fed	44.7	42.3	69.3	56.0	47.7	51.3
n	134	127	208	168	143	154

No significant differences were observed between the Rusinga Island strain of *R: appendiculatus* and any of the other 3 strains when fed on the resistant Rusinga Island cattle. The index of resistance may therefore be applicable to any strain of *R. appendiculatus* fed on resistant cattle. This, however, also needs to be confirmed using susceptible cattle.

2.7.3 The effect of cattle management on the tick population

Tick counts on Rusinga Island cattle increase steadily from September, reaching a peak in January–February, followed by a marked and sudden decline in March–April which continues until July–August when the lowest infestations occur. These population changes could not be related to such recorded climatic factors as rainfall, temperature or relative humidity *(ICIPE 1987 Annual Report)*. Instead, these changes appear to be a result of the traditional pattern of husbandry and management. As soon as the fields are ploughed and the crops are planted in March and April, cattle grazing is controlled and the animals are either tethered along road sides and in small uncultivated areas, or are kept at the homestead until midday. At this time the children return from school and take the cattle out to graze under supervision on restricted areas. This practice continues until the crops are harvested in August–September. During the cropping season, therefore, the cattle are restricted in both their grazing space and the daily duration of exposure to ticks. After harvest, grazing becomes unrestricted and time unlimited, increasing both the areas grazed and the exposure time (see also Chapter 10 of this report).

To test this hypothesis, in September–October 1988 (at the time of increasing tick numbers) 6 animals from one herd (group I) were kept in the homestead each day until midday when they were allowed to graze until 1800

hours. Another 6 cattle from the same herd (group II) were released at 0800 hours each morning and grazed until 1800 hours when they are driven home together with the first group. Both groups were de-ticked twice each month and their infestation levels compared (Table 2.4). More ticks were found on group II than group I. The experiment will be repeated in February–March 1989 (peak season) and July–August 1989 (low season).

Table 2.4 Mean numbers (± s.d.) of 4 species of ticks collected from cattle on Rusinga Island during September–October 1988, under 2 daily grazing regimes: group I (6 hours grazing), group II (10 hours grazing)

Stage	*Rhipicephalus appendiculatus*	*R. evertsi*	*Amblyomma variegatum*	*Boophilus decoloratus*
Group I				
Adults	104.2 ± 55.8	4.2 ± 2.4	7.2 ± 4.0	19.0 ± 26.5
Nymphs	7.3 ± 4.6	0	26.2 ± 11.8	3.0 ± 3.5
Larvae	2.7 + 3.6	2.0 ± 4.0	36.2 ± 36.0	0.5 ± 0.8
Group II				
Adults	128.8 ± 24.3	6.2 ± 3.4	19.3 ± 12.3	50.3 ± 46.0
Nymphs	26.0 ± 7.2	1.5 ± 3.2	50.2 ± 15.9	9.2 ± 7.4
Larvae	58.7 ± 57.9	8.8 ± 21.2	105.7 ± 78.2	3.0 ± 3.5

2.8 HISTOLOGY OF ATTACHMENT SITES OF *RHIPICEPHALUS APPENDICULATUS* AND *AMBLYOMMA VARIEGATUM* ON CATTLE IN RELATION TO RESISTANCE TO TICKS

A. A. Latif, D. K. Punyua, S. K. Nokoe, P. B. Capstick, A. R. Walker and J. D. Fletcher**

Ten East African Zebu cattle from Farm 6 on Rusinga Island were ranked for total tick counts for 12 months in succession. The 4 cattle with consistently lowest tick counts were judged to be of high resistance (HR) to natural tick infestation, and the 4 with consistently highest tick counts were designated as being of low resistance (LR). Figure 2.6 shows the mean tick counts per head for these 2 groups for *R. appendiculatus* and *A. variegatum*. The cattle were brought to the Mbita Point Field Station animal accommodation unit and de-ticked, special care being taken to remove all ticks on both sides of the neck. Each animal received simultaneously 20 nymphs each of *R. appendiculatus* and *A. variegatum* on opposite sides of the neck, confined in cloth bags glued to the skin. On day 3 the bags were removed and feeding ticks were identified for sampling.

This bias in sampling was necessary in order to reduce variation in the period for which the ticks had fed, since ticks attach and detach several times on resistant animals. The cattle were sedated and 4 attachment sites were biopsied for each tick species. Samples were fixed in Karnovsky's fixative, embedded in plastic, sectioned and the sections were stained with acidic Giemsa.

At attachment sites of *R. appendiculatus* (See Figure 2.7a) there were significantly fewer neutrophils, eosinophils and basophils in the LR group compared with the HR group. The numbers of mononuclear cells were not significantly different and the numbers of mast

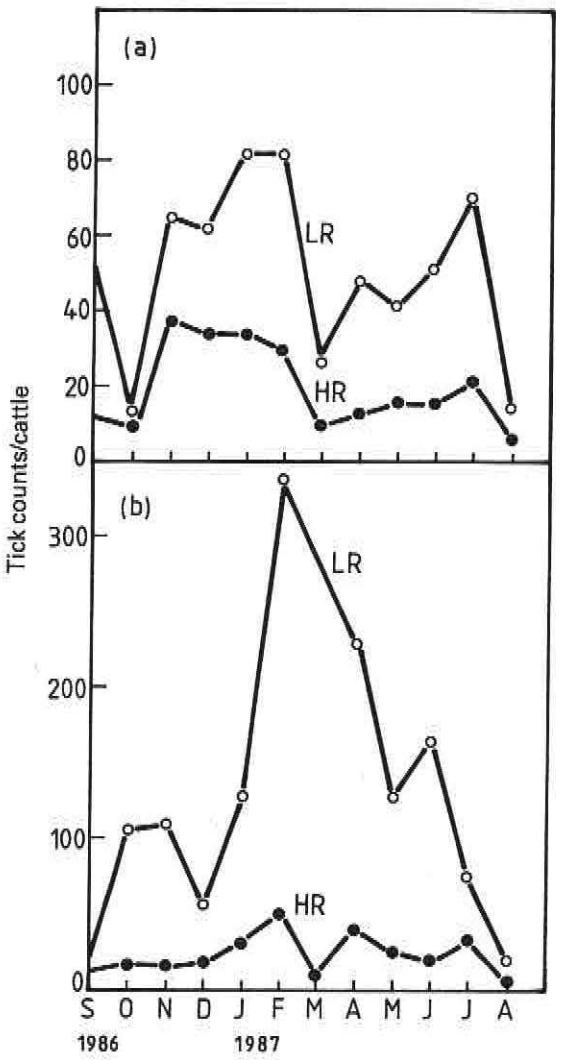

Figure 2.6 Monthly mean tick counts on cattle of high resistance (HR) and of low resistance (LR) on Farm 6 Rusinga Island. (a) *R. appendiculatus* females and nymphs combined; (b) *A. variegatum* larvae and nymphs combined.

cells and the amount of collagen reflect less disruption of the dermis at the attachment sites on LR cattle. With *A. variegatum* (Figure 2.7b) the numbers of neutrophils were not significantly different between the groups but there were significantly more eosinophils at the attachment sites on the HR group. For basophils there was no significant difference between the HR and LR. The reduction in numbers of basophils at the sites of tick attachment on HR cattle is likely to reflect the greater degree of degranulation (and therefore disappearance) of these cells, rather than fewer active cells. In contrast there did appear to be fewer mononuclear cells in the lesions on the HR cattle, leading to the speculation that they are not directly involved in the reactions accompanying tick resistance. The results indicate the obvious differences in cells contributing to resistance between the 2 groups of cattle.

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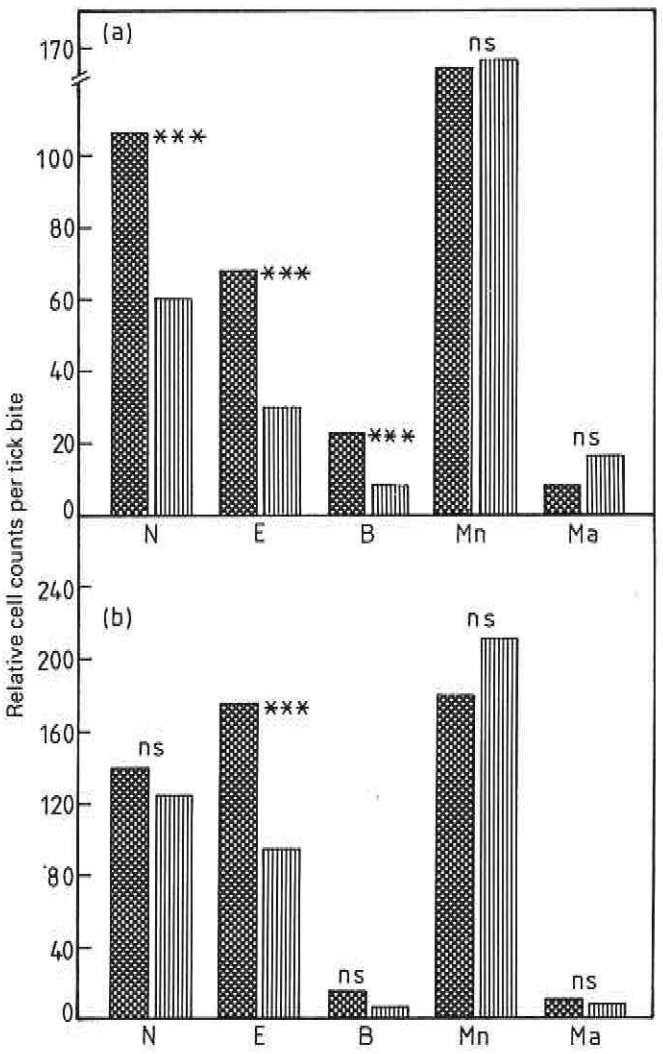

Figure 2.7 Histograms showing mean counts of 5 types of cell in histological examination of tick bite sites, comparing highly resistant cattle (HR, closed columns) with low resistant cattle (LR, open columns). (a) *R. appendiculatus* (b) *A. variegatum;* N = neutrophils; E =eosinophils; B = basophils; Mn = mononuclear leucocytes; Ma = mast cells; ***difference significant ($P < 0.001$); ns, non-significant.

2.9 MODELLING THE IMPACT OF TICK INFESTATION ON CATTLE PRODUCTIVITY UNDER NATURAL FIELD CONDITIONS ON RUSINGA ISLAND

H. M. Oranga and S. K. Nokoe

Many third world countries are experiencing an acute shortage of protein of animal origin, despite the fact that they have the highest densities of livestock and wildlife. The persistent decline in per capita protein intake is a matter of concern to many governments, particularly in Africa. In addition to meat and milk, ruminants in tropical Africa also provide hides and hair, traction, transportation, fertilizer and fuel. They also serve as a vehicle for investment, savings and capital formation. In most societies, livestock also feature prominently in social relationships and such rituals as payment of bride price.

Livestock in Africa are characterized by numerous diseases, poor nutritional regimes and heavy endoparasite burdens as well as exposure to a harsh social environment. All these factors simultaneously take their toll, especially in terms of productivity, reproductive potential and efficiency.

This study was started on Rusinga Island in late 1987. The long-term objective is to identify the relevant factors that are associated with productivity losses in cattle within the environment of the resource-poor farming community on the Island. Once these factors have been identified, they will be used to develop stochastic models which could be used for predicting cattle productivity losses, particularly those attributed to ticks and tick-borne diseases. The productivity factors considered here are milk yield and liveweight gain.

Although the study area is Rusinga Island, it is our belief that these models would also be applicable in other areas in similar agro-climatic zones where comparable cattle and the same tick species are found. The objectives of this study are: (a) To develop stochastic predictive models for cattle productivity factors as functions of tick population dynamics. (b) To develop a general stochastic model for predicting productivity losses based on tick population dynamics and climatic data. (c) To illustrate the use of such predictive models in the management decision-making process.

The study is based on 127 East African Zebu cattle *(Bos indicus)* from all over the island including calves born in 1986 and 1987. Data were collected at monthly intervals on tick numbers (by size, sex and species), plus faecal sample, rectal temperature and liveweight. Twenty-seven dams out of the total sample calved in 1987. From these dams additional data on milk yield were obtained. No faecal samples were taken from the dams. The weigh-suckle-weigh technique was used for measuring milk yield. In addition, pasture and soil samples were collected on the same sampling days from the respective grazing pastures.

Because of the complex nature of the study, many variables are involved. By the end of the present reporting period some of the data, particularly faecal samples for endoparasites and pasture samples for nutritive quality analysis, had not been fully analysed. The models will be developed within the next year.

2.10 NATURAL ENEMIES OF TICKS

E. Mwangi, R. M. Newson and G. P. Kaaya

Very little is known about the predators, parasites and pathogens of ticks. Since all stages of ticks, with the probable exception of one-host ticks, spend the major part of their life on the soil or in the vegetation rather than on a host, it can be expected that their interaction with the environment will result in mortality from natural enemies. A knowledge of these enemies will be valuable within the context of biological control which can be expected to feature in any integrated tick control packages that will be developed.

Several experiments were undertaken to identify predators, pathogens and parasitoids of ticks. In experiments to study predation, engorged nymphs and females of *Rhipicephalus appendiculatus* were exposed every month in an experimental pasture plot. Predation of the females averaged 43% before oviposition began, while that of engorged nymphs was 55% over the same 8-day period. The predators were ants, spiders, rodents, shrews, lizards, birds and domestic chickens. When some of these predators were subsequently offered ticks in the laboratory, some of the rodents and the spiders were observed to readily attack and eat them.

In a study of tick parasitoids, engorged nymphs of *Amblyomma variegatum* and *R. appendiculatus* were collected from cattle in Trans-Mara Division, Narok District, and from Rusinga Island. They were kept until they moulted to see if any parasitoids emerged. A parasitoid, *Hunterellus* sp., was found in one-third of *A. variegatum* from Trans-Mara only, but none has been found from large samples of *R. appendiculatus* from either area. Attempts were made to infest *A. variegatum* nymphs in the laboratory as a start to mass rearing of the parasitoid. About 40% of unfed nymphs confined in the same container with the parasitoid became infested at a ratio of one parasitoid to 3 ticks. Work is in progress on ways to get more of the ticks infested.

During studies on pathogens of ticks, engorged *R. appendiculatus* females were exposed in the field for 8 days. Of these, 11% were eventually found to harbour bacteria while only 1% contained fungi. The bacteria isolated were *Enterobacter cloacae, Staphylococcus aureus* and *Escherichia coli,* and the fungi were *Aspergillus* sp, *Mucor* sp. and *Fusarium* spp. Subsequently, laboratory infections of *R. appendiculatus* with the entomophagous fungi *Beauveria bassiana* and *Metarhizium anisopliae* were carried out. The results showed that 70% of unfed adults were killed by *B. bassiana* but only 30% were killed by *M. anisopliae.*

2.11 ECOLOGICAL STUDIES ON *AMBLYOMMA VARIEGATUM*

F. Gigon

Ecological studies on *A. variegatum* were started in 1986, in order to provide a better understanding of the species ecology, and more specifically to acquire the vast amount of data necessary to feed mathematical models simulating tick population dynamics and their fluctuations under different possible control regimes and strategies. There are 3 general objectives of the studies, plus a study of host-seeking behaviour to better understand the crucial transition between the off-host and on-host phases.

2.11.1 Survival

A study on the survival of the various stages of *A. variegatum* is being made under field conditions in 3 contrasting biotopes selected from its potential range of distribution. (a) Nairobi area (eco-zone III-5): temperate with two relatively well defined rainy seasons. (b) Trans-Mara Division (eco-zone II-4): also temperate but lacks real dry seasons, having a rather homogeneous rainfall pattern. (c) Lake Victoria shore (Rusinga Island, eco-zone IV-3): represents a hot climate with two rainy seasons under constant influence of the lake in terms of humidity and wind.

During the last 18 months, 4 releases (at 3-monthly intervals) have been made. At the Nairobi and Trans-Mara sites, nymphs reached 50% survival after 8–9 months, whereas adults took over one year. At Mbita, survival of both nymphs and adults appeared to be shorter by approximately 3–4 months. For larvae, a comparable technique was previously shown to be unsuitable, giving a reduced larval survival of less than 3 months; however, new preliminary experiments indicate that larvae may live up to about 5 months. The design of these new experiments (enclosing newly laid, undisturbed egg clutches) indicates that larval clustering is indeed a major factor affecting survival.

2.11.2 Meteorological conditions

A survey of the meteorological and micro-meteorological field conditions in conjunction with the survival experiments is required. Data loggers are now recording temperature and humidity at different heights above ground level, as well as rainfall.

2.11.3 Life cycle

Data are needed in order to test suitable models. Monthly tick collections in the Trans-Mara, from a typical Masai cattle sample, have been going on for one and a half years. The previously suspected lack of seasonality in the tick population appears to be confirmed by the 1988 data. Tick populations are known to fluctuate according to rain patterns in other tropical areas; it is not surprising that the lack of fluctuation observed here correlates with the lack of marked rainy seasons at this site. However, there is still a slight increase in adult numbers around July–August, which happens to be a little drier. Such a strategy could ensure that subsequent larval populations would encounter more favourable environmental conditions.

It is hoped that further work will be carried out on the larval stage, because this is a critical part of the life cycle which may affect the potential increase of any tick population. Statistical procedures are being designed to correlate survival and population dynamics with meteorology.

MEDICAL VECTORS RESEARCH PROGRAMME

3

Medical Vectors Research Programme

The Medical Vectors Research Programme (MVRP) continued work on various aspects of leishmaniases epidemiology in both the field and the laboratory. The field activities were mainly in West Pokot, Baringo and Kitui Districts. In West Pokot, epidemiological studies on visceral leishmaniasis were continued. These included vector behaviour and reservoir studies, particularly the screening of domestic ungulates. Furthermore, screening for possible vectors of leishmaniasis was initiated in Trans-Mara, Narok District. In the Baringo focus of leishmaniasis, investigations were concentrated on the disease in domestic ungulates. In the Kitui focus, both vector and animal reservoir studies were continued.

The investigations of leishmaniasis in the laboratory comprised:

- *Characterization of leishmanial parasites*
- *Biochemical characterization of vectors*
- *Parasite-host interactions.*

3.1 LEISHMANIASES IN DOMESTIC ANIMALS: FIRST DESCRIPTION OF CLINICAL LEISHMANIASIS IN A GOAT IN KENYA

M. J. Mutinga, S. M. Kihara, A. Lohding*, C. M. Mutero, A. Ngatia**, F. Karanu*, F. Amimo, D. Omogo, F. M. Kyai, P. Munguti and P. Mutua*

Extensive research has been carried out in Eastern Africa on potential animal reservoirs of both visceral and cutaneous leishmaniasis. The dog is the only domestic animal so far implicated as a possible reservoir for visceral leishmaniasis. The hyrax and the giant rat are the proven reservoirs of cutaneous leishmaniasis (caused by *Leishmania aethiopica),* while several other species of rodents have been demonstrated to harbour *Leishmania major.*

Recent studies conducted on domestic animals in West Pokot led to the first isolation of visceral leishmanial parasites from a goat, always a close associate of man in the vast endemic leishmaniases foci.

We have now encountered a clinical case in a female goat aged about 6 months, originating from a Maasai owner in the neighbourhood of the Trans-Mara Veterinary Research Sub-Centre, Lolgorien, where it was taken for treatment. The animal was diagnosed and treated for parasitic *otitis externa* of the left ear, but 2 weeks later was brought back suffering from lameness.

The animal was examined and the following findings were made: (a) The packed cell volume of the blood was 20%, but blood smears contained no visible parasites. (b) Faecal samples showed *Strongyloides* eggs, many *Strongylus* eggs and *Coccidia* oocysts. (c) Smears were made from cutaneous lesions on the left ear, chin, brisket, the affected hoof, vulva and superficial lymph nodes. All except the brisket revealed leishmanial amastigotes (Leishman-Donovan bodies) in high concentrations, and all yielded promastigotes after 5 days' culture in diphasic NNN medium. (d) Aspirates from the ear, vulva and lymph nodes were suspended in sterile physiological saline and inoculated subcutaneously into 2 goats in the tip of the nose, and the ear and vulva. One developed lesions of the upper lip, nose and vulva that were clinically similar to those of the sick goat.

The original naturally infected animal died and additional information was obtained post-mortem. Histological studies showed massive concentrations of parasites in the epidermis immediately beneath the cutaneous lesions and in the prescapular lymph nodes. The reticuloendothelial cells of the spleen, liver and lungs contained amastigotes, but their numbers were extremely low.

The Lolgorien area is higher in altitude, much colder, and experiences higher rainfall than the West Pokot and Baringo leishmaniases foci where both *Leishmania donovani* and *L. major* are endemic. *Leishmania aethiopica,* however, occurs at altitudes of about 2000m. Although the parasites have not yet been typed, because they occurred within the climatic zone for high altitude cutaneous leishmaniasis, they are most likely to be *L. aethiopica.*

This appears to be the first report of a clinical case of leishmaniasis in the goat, not only in this region, but probably in the world.

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3.2 EPIDEMIOLOGICAL INVESTIGATIONS OF KALA-AZAR IN WEST POKOT DISTRICT

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

The field studies on sandfly population dynamics which were initiated in 1987 were continued . Sampling was carried out in 3 different sites around Kacheliba. These were Sangakai, Kongelai, and Pole. At each site, sandflies were collected weekly from a variety of habitats including houses, termite hills, rodent burrows, tree holes, and cattle and goat enclosures. Sampling was done using a standard $1m^2$ sticky polythene sheet trap which was set up at 1800 hours and removed at 0600 hours the following day. Two such traps were placed in each habitat.

Flies removed from the traps were washed in normal saline solution with 1% detergent added. A predetermined, representative sample of the trapped flies was dissected and the gut area examined for leishmanial parasites. The contents of guts positive for parasites were inoculated into NNN culture medium and incubated for parasite growth and subsequent characterization.

The most common sandfly species were *Sergentomyia antennatus, S. bedfordi* and *S. schwetzi. Phlebotomus martini,* the vector of *Leishmania donovani* in Kenya, occurred in comparatively low numbers. The termite hill habitat yielded the highest number of sandflies in Kongelai, while animal burrows were more productive in Sangakai and Pole.

The highest number of sandflies was collected around houses at Sangakai, while the lowest was at Pole. The relative density per month of all species showed a major peak in numbers in January, then declined steadily until June, when the lowest density was recorded. This population trend was most evident in termite hills and houses.

Investigations were extended to live animals manifesting cutaneous sores and led to the isolation of leishmanial parasites from 2 sheep and one goat. Several hundred sheep and goats were screened for cutaneous leishmaniasis in Baringo District and cultures made from those animals with sores. One isolate was made from a sheep and one from a goat and the identity of these parasites is under investigation.

3.3 DEVELOPMENT OF LEISHMANIAL PARASITES IN MOSQUITOES

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

Laboratory studies on the experimental infection of *Aedes aegypti* with *Leishmania* spp. were continued. Results on dissection of *A. aegypti* previously fed on RPMI medium infected with *L. donovani* showed an absence of promastigotes during the first 3 days post infection. During the fourth and fifth day, actively swimming promastigotes were detected in the pylorus area of the hind gut. These initial infections were light and averaged 3 promastigotes per microscope field under ×40 objective. Massive infections consisting of hundreds of actively swimming promastigotes were observed in the pylorus and rectum, and to a lesser extent in the ileum, between the sixth and seventeenth days following the infective feed (Figure 3.1).

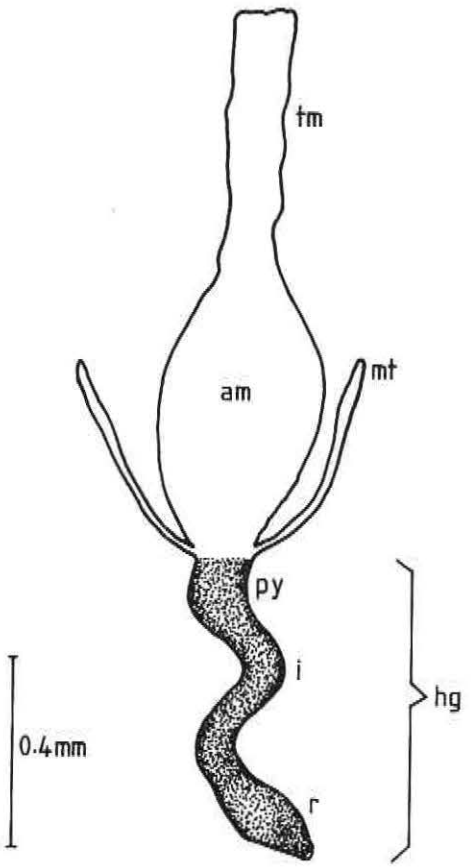


Figure 3.1 Distribution of *L. donovani* promastigotes (shaded area) in the gut of the mosquito *A. aegypti;* thoracic midgut *(tm),* abdominal midgut *(am),* malpighian tubule *(mt),* pylorus *(p),* ileum *(i),* rectum *(r),* hindgut *(h).*

In order to confirm the absence of other parasitic infections (e.g. *Crithidia* spp.) in the gut of *A. aegypti,* a second experiment was set up using mosquitoes from the same colony. Two batches were fed on parasite-free RPMI in addition to clean mice. Subsamples from each group were dissected on days 0, 3, 6, 9 and 12. Eighty mosquitoes from each batch were all free of flagellate parasites on dissection, thus confirming the absence of other parasites that could be confused with *Leishmania* promastigotes.

3.4 A WIDER SEARCH FOR BREEDING HABITATS OF SANDFLIES IN THREE KALA-AZAR ENDEMIC FOCI

M. J. Mutinga, C. C. Kamau, F. M. Kyai and D. M. Omogo

Phlebotomine sandflies are the known vectors of the 3 forms of leishmaniasis reported in Kenya: namely a visceral form caused by the parasite *Leishmania donovani,* and two cutaneous forms caused by either *Leishmania aethiopica* or *L. major.* After more than 30 years, studies to determine sandfly breeding sites have not been very successful, apart from our work on the breeding sites of *Phlebotomus pedifer* on Mount Elgon, and the recent investigations in Baringo District which shed light, for the first time, on the major breeding habitats of phlebotomine sandflies in East Africa. A search for breeding sites in a wider range of habitats than previously investigated was carried out in three leishmaniases foci in Baringo, Kitui and Machakos Districts (Figure 3.2).

Figure 3.2 Map of Kenya showing the locations of three foci of kala-azar (Marigat, Kalawa and Tseikuru) where studies on breeding sites of phlebotomine sandflies were carried out, and the districts in which they lie.

Three methods of investigation were used: saturated sugar solution flotation, direct soil examination and soil incubation. The first two were unsatisfactory so work was concentrated on the third method.

Soil samples were excavated from termite hills and animal burrows and collected from human dwellings, animal enclosures, chicken coops, tree holes, rock crevices, under tree canopy and open ground. The samples were collected all the year round in order to investigate both the perennial and the seasonal breeding habitats. In field laboratories, the samples were placed in rectangular containers for incubation, covered with sandfly netting. Black polythene sheeting was placed over the netting to provide a dark environment. The containers were opened twice daily at 0600 hours and 1800 hours to check for emerged sandflies. Water was then sprayed on the soil (using a hand-sprayer) to maintain moist and humid conditions, before the containers were recovered. The sandflies which emerged were washed in 1% detergent saline solution and mounted on glass slides for species identification.

A total of 473 soil samples weighing over 4 tonnes was collected and examined. Of these samples, 267 weighing 3002 kg were positive and produced 6419 sandflies comprising 17 species.

This study resulted in the identification of both dry season and wet season breeding sites of most of the phlebotomine sandfly species found in these three endemic leishmaniases foci. It was also found that these sandflies breed almost exclusively in termite hills and animal burrows during the dry season, perhaps because suitable environments are restricted to such sites. In the rainy season, however, the sandflies utilise a wide variety of alternative breeding sites like those listed above.

3.5 ABILITY OF THE SANDFLY *S. INGRAMI* TO FEED ON PLANT JUICES

J. B. Kaddu, M. J. Mutinga, S. Nokoe and R. Musyoki

The diet of sandflies is likely to affect the development of *Leishmania,* the causative agent of leishmaniasis which they carry, and thus influence the transmission and distribution of the disease. Plant sugars are part of the diet of sandflies, and it is suspected that many species obtain sugars directly from plants. We therefore investigated the ability of *S. ingrami* to feed on various species of plants. A total of 4838 female and 3509 male, teneral, laboratory-reared sandflies 1–2 days old were caged in a number of replicates and offered shoots of a total of 46 plant species, both indigenous and exotic, belonging to 21 families of Kenyan plants. The tests lasted for 16 hours at 25 ± 1°C and 90% r.h. Control sandflies were kept under the same conditions, but were not offered plants. The sandflies were then dissected and tested for the presence of sugars using the Anthrone test. The feeding performance of the sandflies was measured by the percentage feeding rate (PFR):

$$PFR = 100\ (b + c + d)/a + b + c + d$$

where a = number of sandflies which failed to feed, b = number of fed sandflies with faint blue staining intensity, c = number of fed sandflies with intermediate blue staining intensity and d = number of fed sandflies with deep blue staining intensity in the Anthrone test. A statistical analysis was carried out and significance was determined at the 5% level.

There was no overall significant difference between the sexes when comparisons were made on the combined plant families. Among the families most preferred

(with mean PFR ± s.e.) were: Musaceae (21.3 ± 2.8), Araceae (5.3 ± 0.4), Compositae (5.1 ± 0.1), Umbelliferae (4.5 ± 0.5), Myrtaceae (4.6 ± 0.1), while the least preferred were Camelineae (0.7 ± 0.1) and Alliaceae (0.1 ± 0.04). The control sandflies were all negative in the Anthrone test.

The data were also examined for species preferences within families. For example in the Compositae, *Bidens pilosa* (86.8 ± 4.3) and *Galinsoga parviflora* (78.0 ± 2.1) were the most preferred, compared to *Chrysanthemum coccineum* (2.0 ± 0.2) which was the least preferred. Similarity in feeding preference by male and female sandflies was confirmed, for instance, for the following species (with mean PFR values for female and male, respectively, in parenthesis): *Asystasia schimperi* (31.2 and 30.2), *Bidens pilosa* (67.1 and 70.3), *Conyza floribunda* (20.0 and 22.1), *Vernonia lasiopus* (36.2 and 38.2), *Ficus natalensis* (19.2 and 19.9), *Neonotonia wightii* (33.1 and 33.6), *Lycopersicon esculentum* (5.0 and 5.0) and *Solanum incanum* (2.0 and 2.0).

Detailed chemical analysis of the host plants will be needed to elucidate the feeding performance of *S. ingrami* and other sandflies. This is essential if we are to understand the influence of sandfly diet on the transmission of *Leishmania*.

3.6 THE INTERACTION OF *LEISHMANIA* AND FUNGI

J. B. Kaddu and M. P. Nyamori

The observed lack of *Leishmania* in the guts of sandflies which have fungal infections raises the suspicion that mycosis is an important factor inhibiting the development of *Leishmania* in sandflies. We therefore investigated the interaction of a fungus (MVRP-2, *Metarhizium* sp.) previously isolated from Kenyan sandflies in this laboratory (and identified by the C.A.B. International Mycological Institute, Kew, England) with biochemically confirmed *Leishmania donovani, L. major* and *L. adleri,* as well as an uncharacterized reptilian *Leishmania.*

Tissue culture flasks, in groups of five, containing nutrient broth (Oxoid CM1) were each inoculated with a loopful of fungal growth. One to five days later the flasks were each inoculated with 100–250 promastigotes suspended in nutrient broth. Control flasks were inoculated with *Leishmania* alone. The cultures were incubated at 22° C and their growth monitored by means of daily cell counts using a haemocytometer for 13–19 days.

The flasks containing *L. donovani, L. adleri* and the reptilian *Leishmania* maintained high parasitaemias throughout, but in the flasks containing *L. major* the cultures died out on day 3 post inoculation. The results indicate interactions of *Leishmania* and fungus depending on the species of *Leishmania* involved. It remains to be investigated whether the inhibition of *L. major* by the fungus MVRP-2 is through competition for nutrients in the culture medium, or production of substances toxic to *Leishmania.* Similarly, the apparent lack of growth inhibition in the other 3 species may be either through lack of competition for nutrients, or by production of substances that are of nutritive value to them. In both cases the final results could be of value, either in designing control strategies or for the improvement of *Leishmania* culture techniques.

3.7 IDENTIFICATION AND CHARACTERIZATION OF LEISHMANIAL PARASITES

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

The development of improved methods for identifying parasites has mainly concentrated on the search for species-specific DNA hybridization probes. Studies were continued in 1988 to identify and clone DNA sequences which could be used as probes to distinguish *Leishmania* species and subspecies. DNA sequences can be divided into two broad classes, single-copy sequences and multiple-copy sequences, which are repeated within the genomes. Species differences can occur in either class, but it is among the repetitive DNA that the most useful diagnostic sequences are detected easily in the DNA extracted from microorganisms such as leishmanial parasites.

Restriction endonuclease digests of DNA from cloned *Leishmania* isolates, IC-235 and IC-236 (WHO *Leishmania* marker strains), were fractionated on 0.8% agarose gel electrophoresis. After staining the gels with ethidium bromide, visualization under ultraviolet light revealed prominent bands ranging from 0.3–1.8 kilobases (kb), indicating the presence of repetitive sequences. In order to determine whether any sequence homologies exist among the repetitive sequences observed in clone IC-235 and those of clone IC-236, the differential hybridization method with radio-labelled total DNA from the two cloned *Leishmania* isolates was applied. Under specific hybridization and post-hybridization conditions, the repetitive sequences are detectable only when homologous total DNA is used as a probe. Investigations are in progress on the possible existence of genetic differences between isolates IC-235 and IC-236 and their potential use as probes for the distinction and characterization of leishmanial parasites.

The patterns of chromosomal DNA of a number of closely related *Leishmania* species are sufficiently distinct that it is possible to diagnose the organism infecting a patient by a single pulsed field gradient electrophoresis analysis. This new approach has been introduced into our investigations and should provide useful additional information for studies on taxonomy and epidemiology of leishmaniasis.

Isoenzyme analysis using cellulose acetate electrophoresis has been used for the identification and characterization of *Leishmania* parasites. Six enzymatic systems which could differentiate between WHO *Leishmania* marker strains were selected and used to establish the typical reference enzymatic patterns (zymograms) for comparison with unknown wild *Leishmania* isolates. These are: ALAT, G6PD, LDH, MDH, MPI and SOD (described in Table 3.1).

Table 3.1 Enzymes examined by cellulose acetate electrophoresis (see sections 3.7 and 3.9) and their Enzyme Commission (EC) numbers and abbreviations

Enzyme	EC number	Title
Alanine amino transferase	2.6.1.2	ALAT
Aspartate amino transferase	2.6.1.1	ASAT
Glyceraldehyde phosphate dehydrogenase	1.2.1.12	GAPDH
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PD
Glucose phosphate isomerase	5.3.1.9	GPI
Hexokinase	2.7.1.1	HK
Isocitrate dehydrogenase	1.1.1.42	ICD
Lactate dehydrogenase	1.1.1.12	LDH
Malate dehydrogenase	1.1.1.37	MDH
Mannose phosphate isomerase	5.3.1.8	MPI
6-phosphogluconate dehydrogenase	1.1.1.44	6PGD
Phosphoglucomutase	2.7.5.1	PGM
Superoxide dismutase	1.15.1.1	SOD

Eleven wild *Leishmania* isolates were unequivocally identified and characterized applying the enzyme systems G6PD, MDH and MPI in comparison with the WHO *Leishmania* marker strains. Work is also in progress using other enzymatic systems.

3.8 MOLECULAR KARYOTYPE ANALYSIS OF *LEISHMANIA* SPECIES

V. C. S. Nyambati

Morphological techniques alone are inadequate for the identification of *Leishmania* species, yet it is critical that the identity of *Leishmania* isolates is established in order to incriminate vectors and reservoirs, among other aspects of the epidemiology of leishmaniasis. The objective of this study is to distinguish *Leishmania* species by examination of their molecular karyotype (the patterns of chromosomal DNA bands shown by ethidium bromide fluorescence) and molecular hybridization. These methods make it possible to localize and identify particular genes useful for species differentiation.

Chromosomes from different species are being analyzed in this study. Pulsed field gel (PFG) electrophoresis techniques are applied to allow the separation of entire chromosomes or large DNA molecules. The conditions (ie. temperature, pulse frequency, number of parasites) affecting the running of PFG electrophoresis have been determined. The chromosome standard profiles of WHO reference strains for species differentiation and characterization of *Leishmania* parasites have also been established. Trials using cloned wild *Leishmania* isolates have shown similarities to some of the WHO reference strains.

3.9 ISOENZYMES AND CUTICULAR COMPONENTS IN SANDFLY TAXONOMY

H. Mahamat

These techniques have shown promise as taxonomic tools for a number of insect species. The objectives of this study are to examine the patterns of isoenzymes and cuticular components of phlebotomine sandfly species found in Kenya with a view to identifying useful taxonomic features.

3.9.1 Isoenzyme analysis

Seven species were analyzed, namely *Sergentomyia africanus, S. bedfordi, S. garnhami, S. ingrami* and *Phlebotomus duboscqi, P. elgonensis* and *P. pedifer.* They were collected from the field and reared in the MVRP sandfly colony. Eighteen isoenzymes were screened for their biochemical characterizations by thin layer starch gel electrophoresis. The following (described in Table 3.1) were found to be promising: ASAT, GAPDH, GPI, G6PD, HK, ICD, MDH, 6PGD and PGM. Some of these, GPI, ICD, MDH and PGM gave consistent banding patterns and were able to differentiate between males and females of the same species. Furthermore GPI, MDH and PGM showed polymorphism in their patterns and GPI and PGM gave banding patterns which were specific for all the species of sandflies investigated. It is envisaged that this study will help in developing a zymogram based on GPI, MDH and PGM for isoenzyme characterization.

3.9.2 Cuticular component analysis

Pattern analysis of cuticular components has been used for insect-specific identification in the past. This technique is now being adapted for identification of the sandflies of East Africa. Methods have been developed for extracting cuticular hydrocarbon components in hexane, for analysis on a gas chromatograph, from sandflies reared in the laboratory and also obtained from field collections. The cuticular components obtained in preliminary screening showed qualitative and quantitative differences between species, and quantitative differences between males and females of the same species.

TSETSE RESEARCH PROGRAMME

4

Tsetse Research Programme

African trypanosomiasis is one of the major constraints facing the development of the livestock industry in sub-Saharan Africa. This is a disease complex, of which the main vector is the tsetse fly, that attacks both man and his domestic livestock. Trypanosomiasis of cattle, for example, severely limits their production in Africa. It should be remembered that cattle play diverse economic and social roles in the economy of many African countries. They produce milk and meat and supply draught power and manure, apart from direct income obtained from the sale of animals. The combined detrimental effects of trypanosomiasis on food production in the affected areas are therefore considerable.

The Tsetse Research Programme (TRP) contributes to national and international efforts in vector management by investigating tsetse control strategies which are easily managed, adaptable to various situations, and within the means of the local community. Towards this end, the TRP has emphasized studies on tsetse trapping, population dynamics and, especially, population suppression; it also works on tsetse reproductive biology, including pathogens of tsetse, and disease-vector/animal-host relationships. Concentrated efforts in these areas are likely to pay dividends in the foreseeable future for the whole of Africa, by reducing the transmission of trypanosomiasis.

4.1 POPULATION SUPPRESSION OF *G. PALLIDIPES* AND *G. LONGIPENNIS* AT NGURUMAN

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

The experimental suppression of tsetse populations on the Olkeramatian Group Ranch using odour-baited NGU traps has continued throughout 1988. The traps, deployed in February 1987, have maintained a reduction in the population of *Glossina pallidipes* ranging from 90% in the rainy season to 99.5% in the dry season (Figure 4.1). Partial re-invasion, mainly by females, from the top of the escarpment and the northern area, was the reason for the lower level of control during the rains. This ingress has been reduced, but not entirely eliminated, by barrier traps at 100-metre intervals. For most of the year, the effect of the traps extends several kilometres outside the suppression zone. The impact of the traps has been less dramatic on the *G. longipennis* population, although the reduction has exceeded 90% in the dry season. Scientists from the Kenya Trypanosomiasis Research Institute (KETRI) have been collaborating with the ICIPE in the Nguruman area by monitoring trypanosome infection rates in sentinel herds of cattle, both inside and outside the suppression zone. So far, the results look very promising, with no infections recorded in those cattle grazing where tsetse numbers have been reduced.

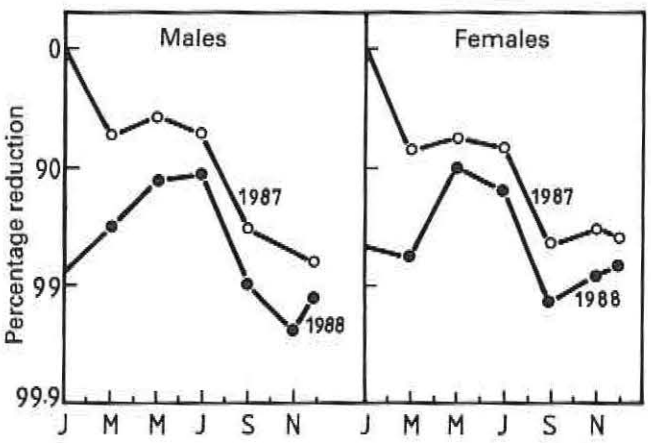


Figure 4.1 Percentage reduction of *G. pallidipes* in the suppression zone at Nguruman in 1987 and 1988 relative to apparent densities in 1986.

This manipulation of the study population by trapping has also provided considerable insight into its dynamics. Since the start of the experiment the density, age distribution and infection rate of tsetse inside and outside the suppression zone have been closely monitored.

From this information, mortality rates have been estimated and incorporated in the simulated population model. Mark-release-recapture each month has enabled us to quantify rates of interchange between subpopulations. Results from sampling populations on the escarpment suggest that seasonal changes are out of phase with those in the lowland areas (Figure 4.2). Whereas catches on top of the escarpment are lowest in the rains, rising to a peak in the cool dry season, those in lowland areas peak in the rains and decline in the dry season. These different trends seem to result in part from climatically mediated movement between the two zones.

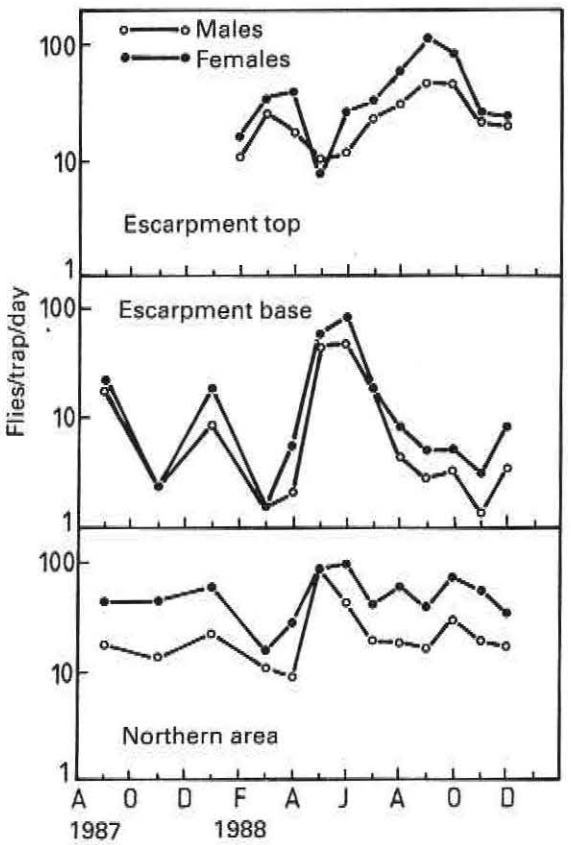


Figure 4.2 Catches of *G. pallidipes* in baited biconical traps outside the suppression zone in three different areas, 1987–1988.

4.2 IMPROVEMENT OF LOW-COST TRAPPING TECHNOLOGY

R. Brightwell and R.D. Dransfield

Further development of the trap/odour bait system has pursued two distinct, but complementary, objectives. Firstly to increase catch per unit cost, and secondly to improve trap reliability, by reducing failure rate and servicing requirements. Underlying both approaches is the need of resource-poor farmers for cheap, simple tsetse control.

Our most exciting development is the "winged" NGU series of traps, of which the NG2G (the "lop-sided" NGU) is the most effective. Compared to the standard NG2B, this design increased catches of male and female *Glossina pallidipes* by 34% and 57% respectively. In addition *G. longipennis* catches were increased by 69% for males and 66% for females. Whilst requiring an additional 25% cloth compared to the standard NG2B, other costs remain the same. When maintenance costs are included, this new trap design offers a considerable saving.

Further experiments have also been carried out on odour attractants which can be used with, or in place of, the presently used acetone and cow urine. Combinations of 4-cresol and 3-n-propyl phenol, dispensed from tubes or polythene sachets, have proved as good as, but no better than, cow urine. Given the expense of these chemicals, they have not been incorporated in the system. Results for 1-octen-3-ol using these dispensers were much more promising, with a dramatic (3–4×) increase in *G. longipennis* catch compared to the known slight increase (1.3–1.5×) for *G. pallidipes*. Sachets of octenol are now therefore being used with acetone and cow urine.

Changes to improve reliability have been outwardly less spectacular, but are transforming what was an interesting possibility into a control technology suitable for widespread application.

4.3 SUPPRESSION OF *G. PALLIDIPES* IN THE LAMBWE VALLEY

L.H. Otieno, P. Onyango and E. Mpanga

Monitoring the *Glossina pallidipes* population in the thickets of the Ruma National Park in the Lambwe Valley was continued during the year. Since spraying operations by the Ministry of Livestock Development of the Kenya Government had virtually stopped in 1987 for technical reasons, the fly population has returned to its pre-spray level. A comparison of the age structure of flies sampled during 1988 and in 1985–6, when the spraying operations were carried out with few problems, showed that the invading flies were mostly from older age groups. This observation has confirmed our suspicion that there was a constant invasion of the National Park by flies from outside. A survey around the National Park has confirmed that *G. pallidipes* is widespread in South Nyanza District.

4.4 RESPONSES OF *G. PALLIDIPES* IN THE LAMBWE VALLEY TO ODOUR BAITS

L.H. Otieno, P. Onyango and E. Mpanga

Biconical traps odour-baited with cow urine and acetone were used to determine the presence of *Glossina pallidipes* along the Lambwe River. The traps were deployed along the river starting from the edge of the Ruma National Park and extending for a distance of 4 km. Baited traps caught significantly more flies than non-baited traps. When the flies were examined for the presence of trypanosomes, it was found that the non-baited traps caught a higher number of flies infected with trypanosomes compared to the baited traps (Figure 4.3). These preliminary observations show that baited and

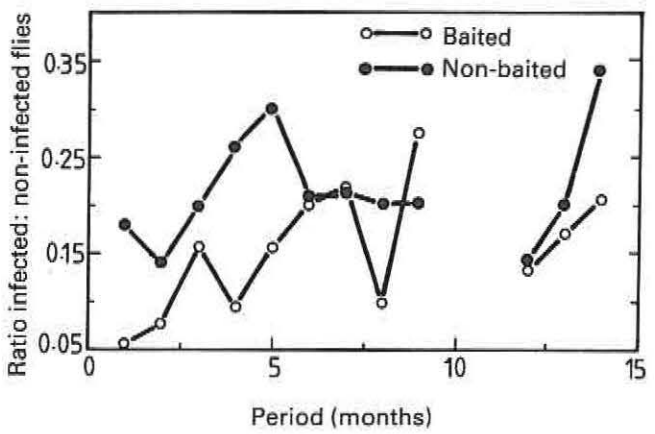


Figure 4.3 Response of infected *G. pallidipes* to odour baited traps in the Lambwe Valley.

non-baited traps catch flies with different physiological or genetic traits, indicating substructuring of the population.

4.5 ANALYSIS OF *T. BRUCEI* STOCKS FROM THE LAMBWE VALLEY

L.H. Otieno and N. Darji

The Lambwe Valley, situated close to the shores of Lake Victoria in Western Kenya, remains an active focus of human trypanosomiasis in spite of repeated insecticide spraying campaigns to control the tsetse vectors. Since this is an isolated place it was interesting to see the range of *Trypanosoma brucei* zymodemes circulating in the area. It was also important to see whether a particular zymodeme could be associated with human infectivity.

Forty-three *T. brucei* stocks were isolated: 18 from cattle, 17 from *Glossina pallidipes* and 8 from humans. These parasites were processed for isoenzyme analysis using 11 enzymes, as detailed in the *ICIPE 1987 Annual Report*. Eight zymodemes were observed from the 18 cattle stocks; 8 stocks belonged to one zymodeme, all from a single focus (Ruri Hills). In addition, 5 single stocks were each unique zymodemes, 3 more stocks comprised a single zymodeme, and 2 stocks belonged to a different zymodeme and both came from God Jope. Eleven zymodemes were identified from the 17 fly isolates. Most isolates represented different zymodemes, except 4 which belonged to one zymodeme and 3 to another. Seven of the 8 human stocks had been described previously; only one stock belonged to a zymodeme new to this area.

It would appear from these studies that more and more new zymodemes are appearing in the Lambwe Valley — from cattle, fly and man. We speculate that the repeated suppression of the fly population and the constant re-invasion (pointed out earlier) have introduced these new trypanosomes to the area. The role that they may play in disease outbreaks is yet to be determined.

4.6 EPIDEMIOLOGY OF SLEEPING SICKNESS IN THE LAMBWE VALLEY

S. Mihok, L.H. Otieno and N. Darji

Since 1959 the Lambwe Valley in Western Kenya has been a focus of human sleeping sickness caused by the parasite *Trypanosoma brucei*. Ruma National Park occupies part of the valley, and hence provides a potential wildlife reservoir for the disease, as well as large populations of the vector, *Glossina pallidipes*. To understand better the forces maintaining this endemic focus, we analyzed the relationships among parasite stocks circulating in wildlife, cattle, tsetse flies and humans. Published and unpublished data on enzyme polymorphisms were used to characterize 321 isolates using numerical techniques.

Parasites isolated from humans were the least genetically diverse, and overlapped considerably with parasites isolated from all other sources. Stocks from non-human sources were much more diverse, and contained a large number of types or "zymodemes" not found in humans. The results suggest that perhaps only 20% of the parasites circulating in tsetse flies, wildlife, and cattle are infective to man. Human stocks could be differentiated from all others, with almost complete confidence, based on a few key enzymes.

The relationships among the various parasite stocks indicate that human-infective parasites have multiple origins. Transmission cycles are also quite complex and are not stable through time. After the World Health Organization's spraying effort in the early 1970's the Lambwe Valley transmission cycle was probably one of chronic human-wildlife-cattle contact. The parasites present in the 1970's were mostly characteristic of East African wildlife isolates. As the geographical extent of these parasites is large, this chronic cycle is probably a continual threat, wherever tsetse flies and wildlife are present in association with humans. During the epidemic starting in 1980, new parasite stocks emerged in the Lambwe Valley, coincident with civil unrest in neighbouring Uganda. Introduction of new genes into the parasite population appears to have occurred at this time through cattle. Wildlife was probably not involved in this epidemic, but tsetse flies may have been responsible for the generation of novel genetic types. Following the aerial spraying of the valley in 1981, stocks characteristic of the epidemic mostly disappeared. Recent isolates appear to be part of yet another transmission cycle involving cattle and man. It is possible that the continual use of insecticides in the Lambwe Valley has resulted in the selection of new parasite stocks. The eventual fate of these stocks in terms of human infectivity remains to be determined.

Our results suggest that the epidemiology of the Lambwe Valley sleeping sickness focus is continuing to evolve. The current situation is, unfortunately, difficult to interpret as few recent isolates from humans have been analyzed electrophoretically. At present, the wide variety of parasite stocks in tsetse flies and cattle could represent either of two alternatives: (1) a dead-end

transmission cycle not involving man, or (2) a potentially dangerous system with high parasite diversity, only awaiting an upsurge in tsetse numbers and increased human-wildlife-cattle contact to spark off another outbreak of human sleeping sickness.

4.7 ECOLOGY AND VECTORIAL CAPACITY OF *G.F. FUSCIPES* AROUND LAKE VICTORIA

M.I. Mwangelwa, R.D. Dransfield and L.H. Otieno

Glossina fuscipes fuscipes is an efficient vector of human trypanosomiasis. This subspecies was mainly responsible for the transmission of sleeping sickness which resulted in epidemics of great proportions on offshore islands and along the shores of Lake Victoria early this century. Although the disease was cleared from its foci in Kenya, recent observations have indicated continued occurrence of *G. f. fuscipes*. In some localities within the environs of Lake Victoria, *G. f. fuscipes* is presently involved in the transmission of sleeping sickness. This subspecies, therefore, may act as a potential vector should the disease be re-introduced into previously cleared areas. The role of *G. f. fuscipes* in the transmission of animal trypanosomiasis, in particular, caused by *Trypanosoma congolense* requires further investigation.

Tsetse surveys conducted from November 1987 to January 1988 indicated that *G. f. fuscipes* occurs all round Rusinga Island and also in some areas on the mainland in dense thickets fringing the lake. Studies on population dynamics indicate that heavy rainfall and periodical clearing of vegetation for cultivation are prime factors that influence the populations, as reflected by monthly apparent densities of the flies, estimated using standard biconical traps. Mark-release-recapture experiments have shown that *G. f. fuscipes* has restricted mobility, hardly covering 1.5 km in a month. This implies that traps might be used in control programmes against this subspecies. Various odour baits were tested for *G. f. fuscipes*, including cow and human urine, acetone, 1-octen-3-ol and phenols known to be attractive to *G. pallidipes*. Results indicated that incorporation of these odour baits in the traps did not significantly raise catches of *G. f. fuscipes*. Studies are continuing on population dynamics, determination of likely attractive odours and on improved trap designs. A colony of *G. f. fuscipes* has been established at the ICIPE Mbita Point Field Station to provide the specimens which will enable transmission studies to commence.

4.8 SIMULTANEOUS INFECTION OF TSETSE FLIES WITH DIFFERENT TRYPANOSOME SPECIES IN THE LAMBWE VALLEY

P. A. O. Majiwa and L. H. Otieno*

The utility of recombinant DNA probes in the detection of natural trypanosome infections in tsetse flies was assessed in the Lambwe Valley. The flies were sampled at two separate seasons in 1988. Three different probes were used; each contained highly repetitive DNA sequences specific for a species or subspecies of trypanosomes of the *Nannomonas* subgenus. A fourth probe was used which contained repetitive sequences common to trypanosome species of the *Trypanozoon* subgenus. In all cases where present, mixed mature or immature infections were detected in various combinations in individual tsetse. Such infections were detected in both the gut and the mouthparts of some tsetse. Other tsetse flies were shown to be simultaneously infected with both savannah-type *Trypanosoma congolense* and Kilifi-type *T. congolense*, or *T. congolense* and *T. brucei*.

The probes have thus been used to demonstrate the presence in western Kenya of a type of *T. congolense* first discovered among trypanosome isolates obtained from sentinel cattle exposed to natural infection on a ranch at Kilifi on the Kenyan coast. We conclude that this type of *T. congolense* is not confined to coastal Kenya and may contribute significantly to livestock morbidity in other areas of eastern Africa. In the coastal situation, *T. congolense* is transmitted primarily by *Glossina austeni;* in the lake region it is transmitted by *G. pallidipes*.

**International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya.*

4.9 ROLE OF NUTRITIONAL AND HORMONAL FACTORS ON SEXUAL MATURATION AND BEHAVIOUR IN *G. PALLIDIPES*

M.F.B. Chaudhury

Male and female *G. pallidipes* are both unique among tsetse species so far studied, in terms of sexual maturation and sexual activity culminating in copulation and insemination. The male *G. pallidipes* becomes sexually mature and capable of inseminating the female about 11–12 days after emergence, as opposed to 4–6 days for other *Glossina* species. Most females of *G. pallidipes* become sexually receptive about 8 days after emergence under laboratory conditions (25 ± 1°C, 75 ± 5% r.h.), whereas the females of other species of *Glossina* become receptive on the second or third day after emergence. Moreover, it appears to be extremely difficult to achieve 100% insemination, even when the insects are sexually mature. In order to determine the role of nutritional and endocrine factors on sexual maturation and sexual behaviour, studies were undertaken to observe the effects of change in blood meal frequency, reduction of total blood intake, removal of corpus allatum, application of juvenile hormone analogue (JHA) and injection of "brain extract" as a source of neurohormone.

The results of some of these studies were described previously *(ICIPE 1987 Annual Report)*. Additional observations on the nutritional and endocrine control of sexual development and copulatory behaviour of male *G. pallidipes* are now described.

4.9.1 Development of male accessory reproductive gland (ARG) and mating

The mean ARG diameter is about 0.05 mm at the broadest region at emergence and increases to about 0.12 mm on day 6, after offering 4–5 blood meals. In the next 6-day period the males take 3–6 blood meals and the ARG increases to a mean diameter of about 0.24 mm. Only 15% of the 6-day-old males are able to mate, and they fail to inseminate the female; however, their inseminating capability increases as they become older. About 80% of 12–13-day-old males can successfully inseminate females. The mean spermathecal value (MSV) of the females mated with 7-day-old males was 0.10, whereas MSVs of the females mated with 12–13-day-old males ranged from 1.80–2.00. (MSV 2.00 indicates the presence of full spermathecae.)

4.9.2 Feeding frequency

A change in feeding frequency results in reduction of the total blood meal, which in turn adversely affects ARG development. This condition obviously reduces the inseminating capability of the males. These 12-day-old males appear to be sexually appetitive and capable of copulating for the normal duration, but are unable to inseminate the females fully, as indicated by the low MSV obtained.

A comparison of the development of the male ARG in *G. pallidipes* and *G. m. morsitans* indicates that the rate of ARG development is faster in *G. m. morsitans* males. They appear to synthesize a full complement of ARG secretion earlier than *G. pallidipes* males.

4.9.3 Effect of allatectomy

Surgical removal of the corpus allatum 4 h after emergence of the males does not have any effect on the duration of sexual maturation, mating behaviour or inseminating capability of the operated males.

4.9.4 Effect of applying JHA

Administration of 2–5 μg of a JHA on alternate days from day 1 to day 4 increases the diameter of the ARG. The mean ARG diameter of 9-day-old treated flies was shown to be 0.22 mm, which is about the same as that of sexually mature, 12-day-old, untreated males. However, the mating and inseminating performance of the JHA-treated males was comparable to that of untreated controls of the same age group.

4.9.5 Effect of JHA and brain extract

It was observed previously that injection of a brain extract from sexually mature males into younger males induced increased copulatory behaviour. Experiments were conducted in which repeated applications of 2–5 μg of JHA were administered from days 1–4 (see previous section), plus an injection of brain extract (half brain equivalent). The treated males were then allowed to mate on either day 6 or day 8. Six-day-old males which had received a total of 11 μg of JHA plus brain extract, were able to inseminate 25% of females, whereas males receiving 14μg JHA and brain extract inseminated 35%. Eight-day-old males treated similarly were able to inseminate 40–45% of females.

These results clearly indicate that the male sexual maturation, mating behaviour and inseminating capability are all regulated by total blood intake, and by complex endocrine mechanisms involving both juvenile hormone (JH) and brain neurohormone. The failure of allatectomy to influence ARG development is probably because the JH from the corpus allatum (CA) is no longer required once the ARG tissue has been primed, most probably before the time of allatectomy. The ARG then continues the normal developmental process without any endocrine component from the CA. Addition of JHA probably enhances the synthetic activity of the ARG secretion. The injection of brain extract clearly increases copulatory behaviour, indicating a prostaglandin effect that is exhibited by some insects treated with the hormone.

4.10 THE CONTROL OF SEXUAL RECEPTIVITY IN *G. M. MORSITANS*

J.O. Davies-Cole

Some factors that are known to induce refractoriness in the mating of dipterans were investigated in *Glossina morsitans morsitans*. The results so far indicate that the degree of impregnation of the spermathecae does not significantly affect receptivity if females are mated again with mature males aged 7 days and over. However, when males younger than this mate with 3-day-old females, 53.9% are still receptive after 72 hours, compared with 20.0% for females that mate with mature males. When mated with younger males, 46.2% of females had partly filled spermathecae, compared to 19.3% for females mating with mature males.

The situation was examined in two further experiments. In the first, females were mated with mature, but aspermic, males. In the second, females copulated with normal mature males until the start of the jerking phase, when they were immediately separated. With aspermic males, only 14.3% of females remained receptive 72 hours later. This means that even if copulation did not result in insemination, a high percentage still became refractory. In the second experiment, only 18.8% of females were still receptive after 72 hours. It is therefore suggested that the onset of the jerking phase is the crucial element in the mechanical stimulation of the female which turns off receptivity. It should be noted that none of the females was inseminated if the male had reached the jerking phase but they were then separated. The duration of copulation also showed no effect on receptivity. When females were given the opportunity to mate for 50, 60 or 80 minutes, but had not reached the jerking phase, it was observed that 86–100% were still receptive and mated again after 72 hours.

4.11 TSETSE SURVEY IN MUHAKA AREA, SOUTH COAST OF KENYA

M.L.A. Owaga

A survey was conducted in July, August and November 1988 in an area of about 100 km^2 lying south-east of the Shimba Hills National Reserve and including Muhaka Forest. The main objective was to determine the distribution of tsetse in the settled area around Muhaka Forest and its immediate surroundings. The main species is *Glossina pallidipes* but the project also aimed to establish whether *G. brevipalpis* and *G. austeni,* which occur at very low densities inside the forest, pose a measurable disease challenge to domestic livestock.

Five existing tracks, of which 4 traverse the area, were used as transects, while the fifth forms the southern boundary. A sixth transect was cut inside the forest. Biconical traps were laid out at intervals of 1 km on either side of each transect. Some were baited with cow urine (5 years old) and acetone, but in some sections only unbaited traps were used. Ten traps per transect were used at a time and they were emptied every 2 days.

Tsetse were captured on all transects, even in a very densely settled area. Non-baited traps caught tsetse only near the boundary of the Shimba Hills National Park and in the Muhaka Forest area. The highest catches were made on the transect bordering the Shimba Hills and along the forest edge, where the mean catch was 5 flies per trap per day (t/d); on other transects it was 0.5–1.0/t/d. Inside the forest the catch rate for all three species, *G. pallidipes, G. brevipalpis* and *G. austeni,* was 2.0–2.5/t/d. The two latter species were only encountered on the transect in Muhaka Forest. In the northern section, bordering the Shimba Hills, a single *G. austeni* was captured in August and a single *G. brevipalpis* in November.

This preliminary survey indicated that tsetse are widely present on the south coast, albeit at very low densities. Traps set next to homes captured tsetse, although the villagers were not even aware of their presence. However, most villagers, especially those who came originally from upcountry, admitted that they have not been able to keep cattle in the area due to frequent attacks of fatal disease.

4.12 PATHOGENICITY AND BIOCONTROL POTENTIAL OF ENTOMOPATHOGENIC FUNGI FOR TSETSE

G.P. Kaaya

The control of tsetse has relied heavily on the use of chemical insecticides and very little effort has been made to evaluate the potential of natural enemies in tsetse control strategies.

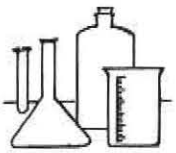
We have therefore started to study the pathogenicity and biocontrol potential of several strains of 4 genera of entomopathogenic fungi, i.e. *Beauveria, Metarhizium, Paecilomyces* and *Hirsutella,* using mostly wet spore suspensions in distilled water of 2×10^7 spores/ml. Wide differences were observed when these fungi were screened for pathogenicity to adult tsetse, even between strains of the same species. Strains of *B. bassiana* usually gave the best results, followed very closely by *M. anisopliae,* causing mortalities of 90–100% by 14 days post-exposure. *Paecilomyces farinosus, P. fumosoroseus* and *Hirsutella* species caused low mortalities of 29–36%. A dose-mortality relationship was demonstrated for both *B. bassiana* and *M. anisopliae* but no difference in mortality was observed between adult tsetse maintained at 65% and 90% r.h., suggesting that r.h. has little effect on the pathogenicity of these two fungi.

No increase in abortions occurred in pregnant females infected with *B. bassiana* or *M. anisopliae.* However, pupae from infected females suffered significantly higher mortality than those from untreated controls, although the longevity of the adults that emerged was comparable in both groups. On the other hand, when freshly deposited larvae were directly infected with dry spores of *B. bassiana* or *M. anisopliae* there was no increase in mortality in the resulting fungus-infected pupae. However, the longevity of adults emerging from *B. bassiana*-infected pupae was significantly reduced compared to that of adults emerging from control pupae and from pupae infected with *M. anisopliae.* By 10 days post-emergence, over 90% of adults emerging from the *B. bassiana*-infected pupae had died, compared to only 2–5% in both the *M. anisopliae*-infected and the control groups.

When males infected with *B. bassiana* or *M. anisopliae* were maintained in cages with non-infected females they transmitted infection to the females which resulted in high mortality. Similarly, infected females could infect males, resulting in mortalities of 60–75% in the latter over a period of 32 days. Likewise, when infected and non-infected members of the same sex were mixed, the infection was readily transmitted. Indeed, transmission of infection through physical contact, by releasing fungus-infected flies, may provide a way to initiate epizootics of fungal disease in wild tsetse populations.

CHEMISTRY AND BIOCHEMISTRY RESEARCH UNIT

5



Chemistry and Biochemistry Research Unit

The primary role of the CBRU is to undertake collaborative research with Core Programmes in areas of chemical ecology and biochemistry pertinent to their goals. The current research activities fall under the following three themes:

- *Chemical ecology of ICIPE's target pests and disease vectors, currently focussed on tsetse flies (in collaboration with TRP and SPRU), the stem-borer* Chilo partellus *and the pod-borer* Maruca testulalis *(in collaboration with SPRU and CPRP).*
- *Protein biochemistry focussed on collaborative work with LTRP on the development of anti-tick vaccine and with CPRP on the diapause phenomenon in* Busseola fusca.
- *Exploratory studies on selected anti-arthropod natural products from African plants, and on biochemical phenomena of special significance and interest.*
- *In addition, a taxonomic project based on pattern analysis of cuticular components of different species of sandflies has been initiated by an ARPPIS student and is being carried out in collaboration with MVRP.*

5.1 SYNOPSIS OF THE UNIT'S MAJOR ACCOMPLISHMENTS

A. Hassanali

5.1.1 Semiochemicals, pheromones and phagostimulants of C. partellus

Several earlier projects on the chemical ecology of *Chilo partellus,* were continued during the year. The purging-trapping technique used previously to identify volatiles from intact host plants was further optimised and is now considered to be sufficiently effective for all studies involving the identification of volatile semiochemicals. Details of the technique are described in this report. In the moth pheromone project, synthetic samples of all the 6 components of the pheromone of *C. partellus* identified to date have been made available for evaluation in screenhouses at MPFS. Lastly, several phagostimulatory cinnamic acids present in sorghum cultivars were identified and the two major groups of phagostimulants (sugars and phenols) were shown to act synergistically. A series of sorghum varieties now needs to be chromatographically analyzed, and the phagostimulants quantitated to determine if such a method could be routinely used in screening for resistance.

5.1.2 Tsetse kairomone studies

Work on tsetse kairomones included the synthesis of phenolic analogues derived from buffalo urine (buffinol), designed on the basis of an emerging structure-activity relationship; the isolation, purification and confirmation of the structures of pro-attractants; and the screening of pure cultures of microbial isolates for their ability to release phenolic attractants from buffalo urine. Some of the key phenolic homologues that are currently being evaluated by electroantennogram (EAG) and antennal movement assay include 3-n-butylphenol , 4-n-pentylphenol, 5-methyl-3-n-propylphenol, 4-indanol and 5-indanol. A detailed study of the nuclear magnetic resonance of purified precursor fractions showed that these are made up of more than 80% glucuronate and smaller amounts of another conjugate, probably sulphate. Two of the microbial isolates screened have shown definite ability to release phenols from buffalo urine samples and are now being identified. The precursor-microbe combination that is envisaged is expected to provide a useful model for controlled release of these tsetse attractants in the field.

5.1.3 Tick antigen studies

The major focus of collaborative research with LTRP during the year has been on the fractionation of membrane-bound proteins from whole ticks and tick midgut, and on the quantification and characterization of the immune effects on the midgut of ticks fed on rabbits that have been immunised with detergent-solubilised midgut proteins. Fractionation of whole tick membrane was undertaken using different concentrations of detergents of varying liphophilicities. Quantification of immune effects of anti-midgut proteins was

based on the analysis of the proportion of antibodies that crossed into the haemolymph using the enzyme-linked immunosorbent assay (ELISA) and radio-immunoassay (RIA) techniques. These experiments are at an advanced stage and the results are expected to lay down the groundwork for future strategies in the search for effective antigens.

5.1.4 Diapause in B. fusca

A protein associated specifically with *Busseola fusca* larvae was isolated and characterised. The protein is synthesised and secreted into the haemolymph prior to the onset of diapause and is detectable in the larvae of *B. fusca* in the prediapausing instar. The occurrence of diapause in this insect appears to be associated with feeding on older host plants, and the protein is expected to provide a useful marker in the search for allelochemical cues which may be involved in inducing this phenomenon. In addition, the possible role of the protein in maintaining high juvenile hormone (JH) titres in the diapausing larvae needs to be investigated.

5.1.5 Natural stored product protectants against weevils

In an exploratory study two plant materials (leaves of the shrub *Ocimum suave* and the flower buds of *Eugenia caryophyllata),* used traditionally in protecting stored grains against weevils, were shown to contain constituents that repelled the maize pest *Sitophilus zeamais.* Since *O. suave* (and other *Ocimum* species) thrives under various growing conditions, it may have a role as a small-scale protectant of stored products in rural homes.

5.2 A NEW TECHNIQUE FOR THE ANALYSIS OF AIRBORNE VOLATILE COMPOUNDS OF PLANTS

W. Lwande

Volatile compounds originating from plants may play a role in the orientation of their insect pests towards the plant, and in ultimate recognition of the plant for feeding and oviposition. Knowledge of the volatile compounds associated with plants of economically important crops may therefore be useful in studies of insect-pest/host-plant relationships.

For insect-plant interaction studies, collection of airborne plant volatiles is preferred to the use of traditional isolation methods such as steam distillation and distillation under reduced pressure. These methods lead to destruction of the plant tissue which may result in enzyme-catalyzed oxidation products, not normally present in the intact plant, which may mask the original plant volatile compounds. Some of the plant volatiles may also be degraded by enzyme action.

A technique has been developed for the analysis of airborne volatiles of plants. The apparatus is shown in Figure 5.1. Charcoal-filtered air is drawn over the plants in a glass chamber and then through a trap containing Tenax TA absorbent to retain the volatiles. The trapped volatiles are subsequently released directly into a capillary gas chromatograph-mass spectrometer (GC-MS) by heating the trap. The GC is modified to include a second injection port (B in Figure 5.1) and employs a 6-port Valco valve.

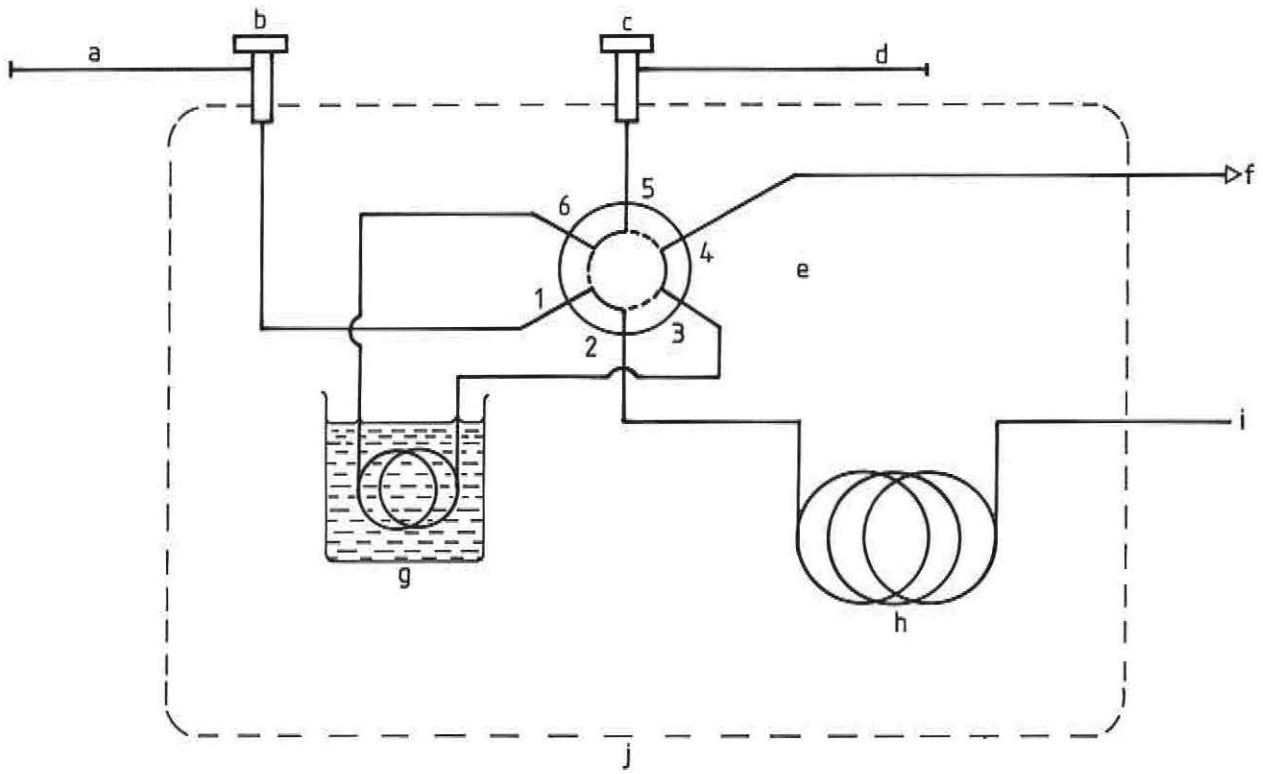


Figure 5.1. Diagram of the system for desorption of plant volatiles from the Tenax trap and subsequent injection into the gas chromatograph-mass spectrometer (GS-MS); (a) helium carrier, (b) injection port A, (c) injection port B, (d) helium carrier, (e) Valco valve, (f) to vent, (g) dry ice/acetone mixture, (h) capillary column, (i) to MS, (j) GC oven.

During desorption, the trap is placed at 35°C in injection port B, which is then heated to 200°C to release the volatiles. These are trapped again in the cooled sample loop ([g] in Figure 5.1). Helium is used as the carrier gas and flows through port B and the sample loop to vent, and also through the normal injection port A and the capillary column ([h] in Figure 5.1).

In the course of sample injection the dry ice/acetone mixture surrounding the sample loop is first removed and the loop is then heated to 250°C in programmed steps. Helium flows through port A, the sample loop and the capillary column to the MS, and also vents via port B. Identification of the volatiles by MS is confirmed by comparing their mass spectra and retention times with those of authentic samples.

A major problem with thermal desorption of analytes from porous polymer traps onto a capillary column is that flow rates of more than 20 ml/min are required for efficient desorption, and capillary column flow rates are typically much lower. The use of a 6-port valve and a second injection port in the present method enables desorption of the volatiles from the Tenax trap at a flow rate of 30 ml/min.

5.3 SORGHUM FEEDING ALLELOCHEMICS FOR *C. PARTELLUS*

B. Torto, A. Hassanali and K.N. Saxena

Studies on the feeding response of third-instar larvae of *Chilo partellus* to major phenolic components (*p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) in the ethyl acetate extract of the whorls of sorghum cultivar IS 18363, and to the analogues of these compounds, were reported in the *ICIPE 1987 Annual Report.*

During the year under review, a further examination was carried out on the ethyl acetate extract to identify other minor phenolic components present in the extract. Acetylation of the extract and gas chromatograph-mass spectrometer (GC-MS) analysis of the product revealed the presence of caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid (4-hydroxy-3 methoxycinnamic acid) and *p*-coumaric acid (4-hydroxycinnamic acid) among other components which are yet to be identified. The identities of these cinnamic acids were confirmed by co-injection with authentic samples by high performance liquid chromatography (HPLC) on a reverse phase column (Zorbax ODS).

Feeding assays were carried out on these cinnamic acids and on other phenolic constituents of sorghum reported previously, including vanillic acid (4-hydroxy-3-methoxybenzoic acid), protocatechuic acid (3,4-dihydroxybenzoic acid) and gentisic acids. Chlorogenic acid and quinic acid were also tested.

The results show that all but *p*-coumaric and gentisic acids stimulated larval feeding. The responses of larvae to increasing doses of the feeding stimulants followed broadly the same pattern, reaching a maximum and then dropping (Figure 5.2). Feeding rates were similar for ferulic and caffeic acids. Comparison of the hydroxybenzoic acid analogues with the cinnamic acids showed that

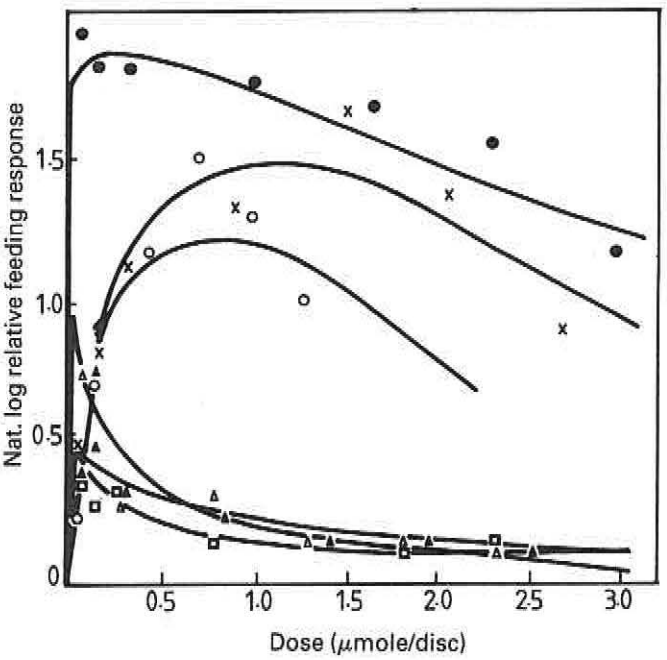


Figure 5.2 Dose response curves for some hydroxybenzoic and cinnamic acid compounds; • protocatechuic acid, X vanillic acid, ○ chlorogenic acid, △ quinic acid, ▲ caffeic acid, □ ferulic acid.

the former were generally more stimulatory than the latter. Thus vanillic acid was more stimulatory than ferulic acid; protocatechuic acid was also more stimulatory than caffeic acid; and whereas 4-hydroxybenzoic acid was a strong stimulant, *p*-coumaric acid was not

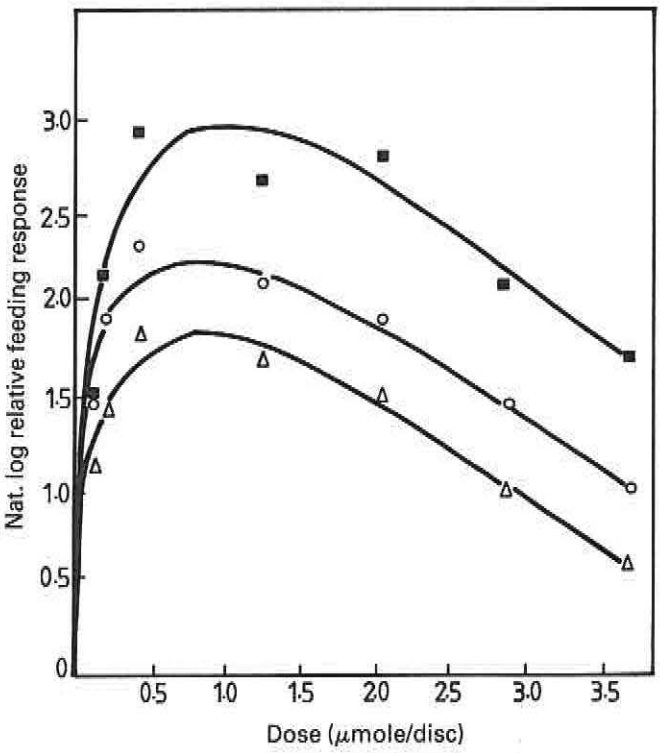


Figure 5.3 Dose response curves for *p*-hydroxybenzaldehyde alone and a blend of sucrose and *p*- hydroxybenzaldehyde; sucrose + *p*-hydroxybenzaldehyde, ○ sucrose + *p*-hydroxybenzaldehyde (theoretical plot), △ *p*-hydroxybenzaldehyde, (←) activity of 0.03 mole sucrose.

stimulatory. Chlorogenic acid and quinic acid were both stimulatory, but the former was a better stimulant than the latter.

Tests were performed for evidence of synergism between *p*-hydroxybenzaldehyde and sucrose, the two major phagostimulants reported last year. The results show that the addition of 0.03 μ mole sucrose to different doses of *p*-hydroxybenzaldehyde increased larval feeding response by increments which were significantly greater than a simple summation of the activities of the two compounds would suggest (Figure 5.3). The results show that the sugar and the phenol act synergistically to stimulate feeding in *C. partellus* larvae.

5.4 PHENOLIC TSETSE ATTRACTANTS

M. Okech, W. Ouma, H. Amiani and A. Hassanali

During the year, investigations continued on the identification of pro-attractants, and on the isolation and screening of microbes from buffalo urine involved in their formation. In addition, structure-activity studies on the phenolic components and their analogues were continued in collaboration with SPRU.

5.4.1 Pro-attractant compounds

The procedure described previously *(ICIPE 1987 Annual Report)* was used to obtain relatively pure samples of the major fraction from a high performance liquid chromatograph (HPLC) reverse phase column. Examination of nuclear magnetic resonance (NMR) spectra of this fraction confirmed the presence of the glucuronate of 4-cresol as the major product. However, integration of the aromatic protons suggested that a second derivative of 4-cresol was also present in smaller amounts (about 20%); lack of corresponding high field proton signals suggests that this derivative is either a sulphate or a phosphate. The synthesis of these compounds is now in hand.

5.4.2 Isolation and screening of microbes

Isolation of microbes was carried out as described previously *(ICIPE 1987 Annual Report)*. Eight microbial isolates have been screened for their ability to release phenols from buffalo urine. The release was monitored by extracting 10-ml samples of the fermenting urine every day and analysing for 4-cresol by gas chromatography. Of the isolates screened, three organisms (A, L and M) have demonstrated varying ability to produce 4-cresol. The phenol is detectable within 24 hours of incubation with A or L, reaching peak production on day 2 for A and day 6 for L. Thereafter, in both cases the amount of 4-cresol declines and then stabilises. Organism A continued to grow after 20 days but L was dead by day 8. Urine samples inoculated with M gave much smaller amounts of 4-cresol compared with those inoculated with A or L. Identification of organisms A and L is now underway.

5.4.3 Structure-activity studies

The results of a dose-response study based on electro-antennogram (EAG) and antennal response assay in *Glossina pallidipes* and *G. morsitans* are reported under SPRU (section 7.3 of this report). The study shows that an attempt at optimising 4-cresol production is unlikely to be very fruitful. On the other hand, the increase in activity in the series 3-cresol, 3-ethylphenol and 3-n-propylphenol suggests the need for evaluating the higher 3-alkyl homologues. The flexibility of the propyl group means that one of several topological variants of 3-n-propylphenol may be involved in interaction with the active site. In addition, the possibility that there may be a generalised receptor for both 4-alkyl and 3-alkyl compounds suggests that compounds alkylated at both positions might show some interesting activities. Accordingly, the analogues shown in Figure 5.4 were among those prepared during the year and are currently being evaluated. The results obtained are expected to provide further insight into the structural requirements for optimal interaction at the active site and a basis for further optimisation work.

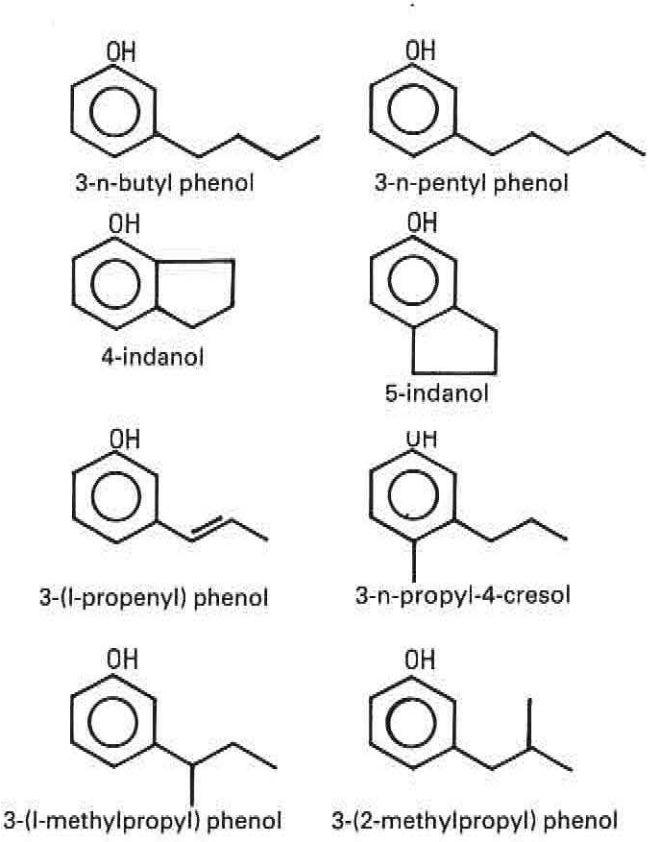


Figure 5.4 Eight analogues of phenol currently being evaluated for attractancy to *G. pallidipes* and *G. morsitans.*

5.5 THE DIAPAUSE PHENOMENON IN THE STEM-BORER *B. FUSCA*

E.O. Osir, G.C. Unnithan and L.V. Labongo

We have initiated a new research project on the diapause phenomenon in *Busseola fusca,* an economic-

ally important pest of maize and sorghum. The majority of the population of this pest enters diapause as mature larvae and survives the dry season in maize and sorghum stubble and dead stalks. Diapause is apparently induced by larvae feeding on mature host-plants and it can be prevented by rearing the insects on young plants. The overall objectives of the study are (i) to identify a suitable biochemical marker for diapause and (ii) to use the marker in the identification of host-plant chemical stimuli responsible for the induction of diapause.

5.5.1 Identification and purification of the diapause-associated protein

On non-denaturing polyacrylamide gels, haemolymph from diapausing larvae shows a distinct protein band which is clearly lacking in the haemolymph obtained from non-diapausing insects (Figure 5.5). The name "*Busseola* diapause protein" (BDP) was coined for this protein. The isolation of BDP was achieved by a three-step procedure. Haemolymph from diapausing insects was subjected to density gradient ultracentrifugation in a KBr gradient, and BDP was found in the subphase. The subphase was applied onto a concanavalin A-Sepharose column and BDP was recovered in the flow-through fractions. These fractions were applied onto a gel permeation column and a homogeneous sample of BDP resulted.

Figure 5.5 Polyacrylamide gel electrophoresis of *B. fusca* haemolymph; (1) from diapausing larvae (20μg protein), (2) from non-diapausing larvae (15μg protein). Arrow shows the position of the BDP band.

5.5.2 Physical-chemical and immunological properties of isolated BDP

The native molecular weight of BDP was determined to be approximately 500,000 by electrophoresis as well as by gel permeation chromatography. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), BDP yielded two closely migrating subunits, I (M_r ~ 88,000 ± 4000) and II (M_r ~ 79,000 ± 1000) (Figure 5.6). These two subunits are not joined by disulphide bridges.

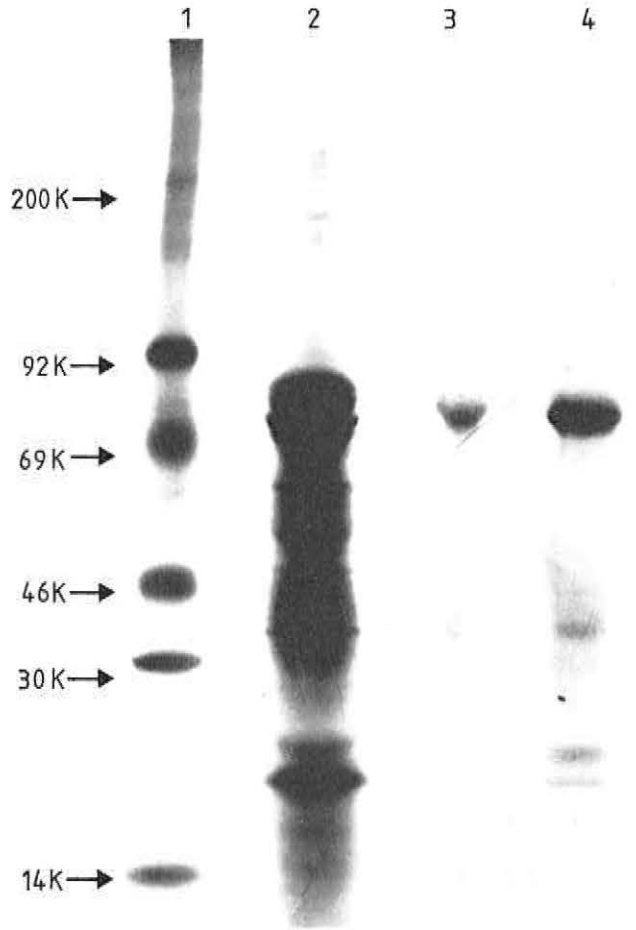


Figure 5.6 Dissociating electrophoresis of isolated BDP. Protein samples were separated on a gradient SDS-PAGE (4–15%); (1) arylphorin (18μg), (2) isolated BDP (20μg), (3) low mol. wt standards, (4) high mol. wt standards.

Staining by Sudan Black showed that BDP is lipidated, and the lipid content was determined to be 2% by weight. The presence of covalently-bound carbohydrates was shown by staining with periodate-Schiff reagent. The carbohydrate content was determined to be about 1% by the phenol sulphuric acid assay. The lack of binding of BDP to the plant lectin, concanavalin A, suggested that the carbohydrate moiety is not of the high mannose type.

Amino acid composition analysis of BDP showed a high content of aspartate (12 mol%) and glutamate (11 mol%), as well as the aromatic amino acids tyrosine (9 mol%) and phenylalanine (7 mol%). The high content of aromatic amino acids suggested that BDP might serve as a pool of amino acids used for the synthesis of adult protein structures.

Using rabbit antibodies against BDP, it was shown by both double radial-immunodiffusion and immunoblotting techniques that (a) both subunits react strongly with the antiserum, (b) no reaction occurs in haemolymph obtained from non-diapausing insects and (c) no cross-reactivity occurs with any other haemolymph proteins (Figure 5.7).

Figure 5.7. Immunoblotting using antibodies against BDP. Haemolymph samples from non-diapausing sixth-instar larvae (9μg, lane 1), isolated BDP (10μg, lane 2) and haemolymph from diapausing larvae (15μg, lane 3) were separated by gradient SDS-PAGE (4–15%) and then transferred onto nitrocellulose paper. Non-specific binding sites were blocked, the blots were incubated with anti-BDP and the antigen-antibody complexes visualized directly with Protein A coupled to colloidal gold particles.

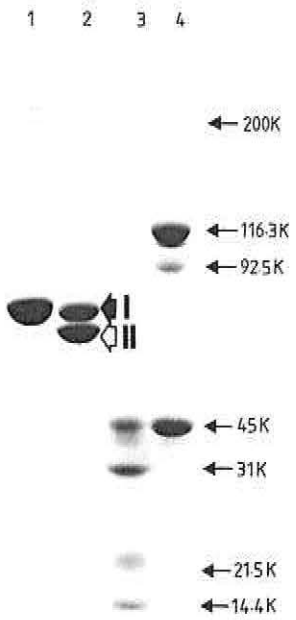


Figure 5.8 Fluorogram of fat body synthesized proteins, prepared as described in the text, (1) mol. wt markers, [^{14}C]-labelled, (2) total labelled fat body proteins, (3) immunoprecipitate of secreted proteins, (4) total fat body secreted proteins.

5.5.3 De novo *synthesis of BDP by the fat body*

The ability of the fat body tissue from diapausing insects to synthesize and release BDP was tested by incubating the tissue with [^{35}S]-methionine in lepidopteran saline. Immunoprecipitation using specific anti-BDP antibodies showed that BDP is one of the proteins secreted by this tissue (Figure 5.8).

5.6 SOLUBILIZED MEMBRANE-BOUND PROTEINS OF THE GUT OF *R. APPENDICULATUS* AND THEIR BIOLOGICAL EFFECTS

S. Essuman and E.N. Ole Sitayo

5.6.1 Fractionation studies

Crude Triton X-100 extracts of the gut wall of partially fed female *Rhipicephalus appendiculatus* ticks were used to immunise rabbits. It has been shown that this caused a reduction in the feeding performance of female and nymphal ticks subsequently fed on the rabbits, and in the ability of the engorged females to convert the blood meal into eggs. A Sephacryl S-200 fraction of this homogenate containing protein subunits with molecular weights ranging from 67–180 Kd was found to induce an immune response which caused about 30% reduction in the feeding performance.

To facilitate the isolation and purification of the active proteins, we explored the possibility of employing detergents with different lyophilicities to leach different groups of polypeptides selectively. The detergents used were Triton X-100, Triton X-114, Chapso and Octyl-ß-glucoside. Two concentrations of Triton X-100 and Triton X-114 (0.1% and 0.5%), and 10mM each of Chapso and Octyl-ß-glucoside were used. Solubilization was carried out sequentially. The fractions obtained were screened by the LTRP for the presence of protective antigens using rabbits as the host. Unfortunately no recognizable immune responses were observed, possibly because the emulsions formed were unstable. The chemical nature of the membrane proteins and the presence of residual detergent may have rendered the usual 1:1 ratio of antigen: adjuvant inappropriate. The experiment is now being repeated.

5.6.2 Quantification and characterization of immunological effects of gut wall

Modification of the physio-chemical properties of the gut wall is considered the key to the successful use of so-called "hidden antigens" to disrupt the physiological processes in the tick. Immunoglobulins to these antigens in the blood of the host need to cross into the tick haemolymph in significant amounts through the gut wall before they can exert any biological effect on their respective targets. Although there is indirect evidence of the disruption of the wall by anti-gut antigens, no quantification or characterization of this disruption has been undertaken in *R. appendiculatus*. We are measuring the passage of immunoglobulins through the gut wall of ticks fed on rabbits immunized with gut and whole tick membrane proteins. Data are being collected from

experiments based on two techniques: enzyme-linked immunosorbent assay (ELISA) for quantification of host immunoglobulins in the tick haemolymph, and radio-immunoassay using radio-labelled anti-rabbit immunoglobulins. Characterization of the ultrastructural gut damage is currently underway in collaboration with Cell Biology Research Unit.

5.7 WEEVIL REPELLENCY OF CONSTITUENTS OF PLANTS USED AS STORED PRODUCT PROTECTANTS

A. Chapya, A. Hassanali, W. Lwande, L. Moreka and E.N. Ole Sitayo

Plant materials and minerals have for long been in use as traditional protectants of stored food products. In East Africa, communities in different locations appear to have evolved their own grain protection strategies based on the specific situation in their area and the flora available around them. We have evaluated the efficacy of a number of plants used as traditional grain protectants. Our assay consists of undamaged maize seeds (10g) in vials (76 × 19 mm) to which are introduced 10 males and 10 females of the maize weevil *Sitophilus zeamais*, plus powdered plant material (1, 2.5 and 5%). These are then compared visually with controls for 8 weeks.

Two plant materials which showed protective properties were selected for detailed study: the leaves of the wild shrub *Ocimum suave* and the flower buds of *Eugenia caryophyllata* (cloves). Gas chromatographic and mass spectrometric examination of the essential oil of *O. suave* showed that in addition to mono- and sesquiterpenes it contained eugenol which has been shown to be the principal volatile component of oil of cloves.

A literature search for biological activity of the phenol indicated that it had not been previously reported as an allomone of any insect. On the contrary, eugenol has been reported to be a component of an attractant blend for the Japanese beetle, *Popillia japonica*, and the pure compound has been demonstrated to be an attractant for the housefly. Our observations with *S. zeamais* weevils, however, suggested that they might be repelled by the

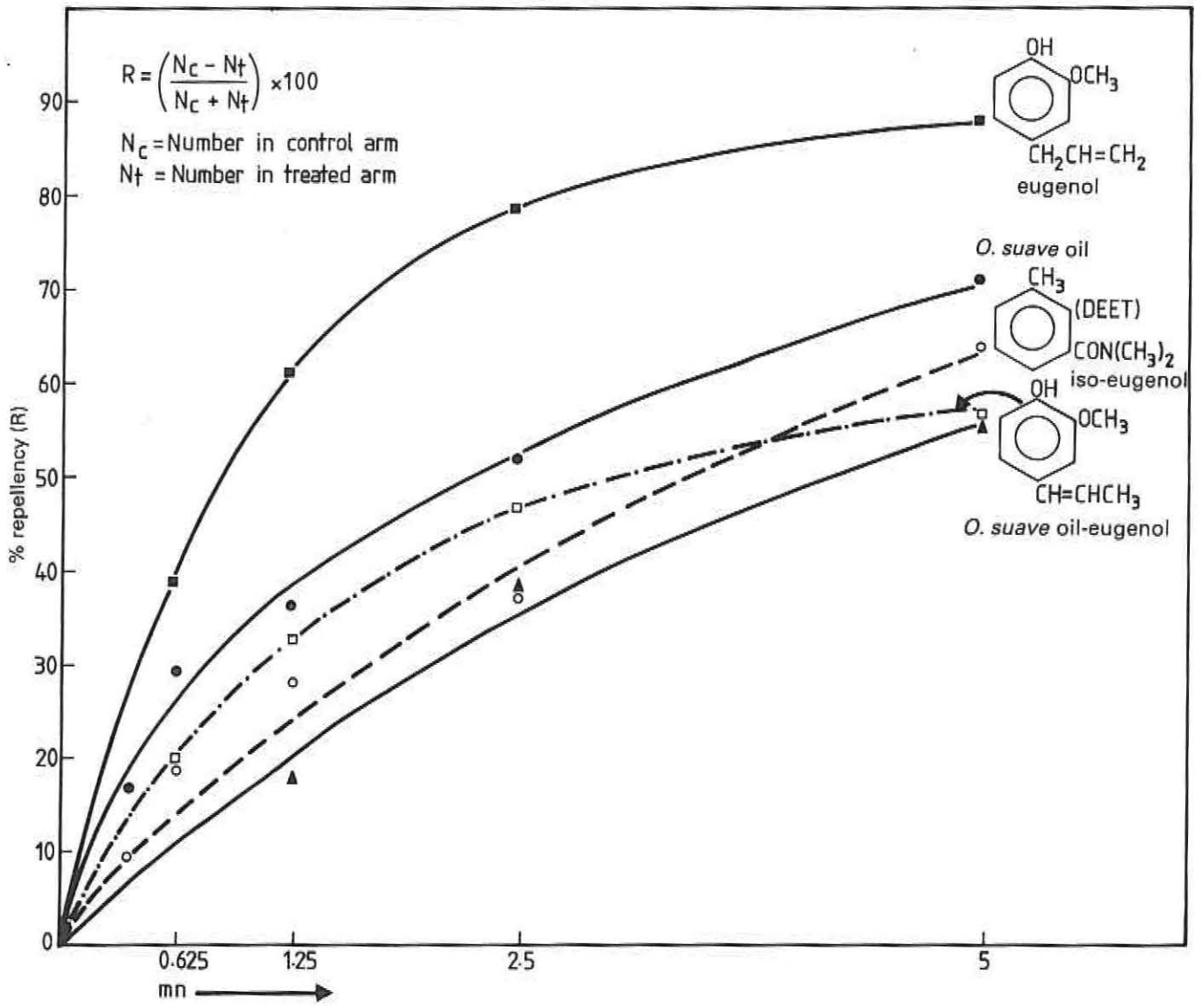
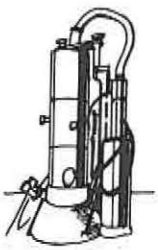


Figure 5.9 Dose-response curves for the repellency of eugenol, *O. suave* oil, DEET, iso-eugenol and *O. suave* oil minus eugenol.

phenol and it became imperative to demonstrate this quantitatively in an unequivocal fashion. For this purpose, a number of Y-shaped olfactometer designs were constructed and tested. Comparison of the behaviour of the maize weevil towards different doses of eugenol and DEET (N,N-diethyltoluamide, a synthetic insect repellent), clearly demonstrated the repellent property of the phenol towards this insect (Figure 5.9). Eugenol was found to be significantly more repellent than its isomer iso-eugenol. Contrary to our expectation, olfactometric tests with the oil of *O. suave,* and the oil minus eugenol showed that both the terpenoid and the phenolic fractions contributed to the repellent activity of the essential oil. The relative importance of the terpenoid components will become clear once their identification and assays on authentic samples are completed. In view of its milder (and some say more pleasant) odour than cloves, and the fact that it thrives under various conditions, *O. suave* (and other *Ocimum* species) is now our major focus for evaluation as a small-scale protectant of stored food products in rural homes.

CELL BIOLOGY RESEARCH UNIT



6 Cell Biology Research Unit

The primary role of the Cell Biology Research Unit is to assist and support the ICIPE core programmes by carrying out morphological and functional studies of target pest insects at organ and cellular level. Approximately 60% of the time is devoted to collaborative research activities and 40% is spent on basic studies initiated by the Unit.

The Unit received its first in-depth review in March 1988, when a panel of external scientists judged the work to be of a high level. Existing research areas on functional tissues and biological control agents were recommended for expansion, to include the characterization of common proteins in the accessory reproductive glands (ARG) and spermatophore of male tsetse, and the isolation, characterization and tissue culture propagation of a DNA virus discovered in tsetse. New areas of research will include:

- *Analysis of the distribution of regulatory neurohormones*
- *Determination of neurotransmitters present in sensory nerve terminals*
- *Examination of the infection process of tsetse by entomopathogenic fungi*
- *The effect of antibacterial factors on insect reproductive performance*
- *Application of cell biology expertise in the fields of biotechnology, biological control and insect pathology.*

The installation of an advanced Philips CM12 transmission electron microscope means that studies involving mechanisms of regulatory physiology under normal and injury conditions can be pursued with greater vigour.

Work continued on the morphological and functional maturation of the ARG in the male pupae of the tsetse Glossina morsitans.

The major focus of our collaborative research with the Biological Control Sub-Programme provided morphological details of the infection process in G. morsitans *by the fungi* Beauveria bassiana *and* Metarhizium anisopliae. *They develop at the cuticular surface forming massive hyphae which penetrate the epicuticle and enter the body cavity where they grow abundantly. The resulting high mortality in infected tsetse is attributed to liberation of mycotoxins and destruction of the host tissues by fungal mycelia.*

Studies on the repeated inoculation of female G. morsitans *with live bacteria* (Escherichia coli) *showed severe degeneration of the symbionts housed in the mycetocytes and a marked decrease in fecundity.*

6.1 ULTRASTRUCTURE OF THE ACCESSORY REPRODUCTIVE GLANDS IN THE MALE PUPA OF *G. MORSITANS*

E. D. Kokwaro and J. Murithi

Accessory reproductive glands (ARG) are present in the pupal and adult stages of the tsetse *Glossina morsitans*. They synthesise and export proteins and precursors of the spermatophore wall. We have found that the pupal ARGs remain small until 25–30 days before eclosion, but are already fully differentiated at the time of eclosion. Active protein synthesis and the accummulation of large quantities of proteinaceous secretion take place during the first 7 days of adult life, corresponding with the period of highest reproductive activity in the male flies. When analysed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) more than 27 protein bands were detected. These showed age-specific patterns by 5–7 days post eclosion which are strikingly similar to those of the spermatophore *(ICIPE 1987 Annual Report)*. Thirteen major protein bands were also observed by the use of isoelectric focusing of which 10 were in the acidic range.

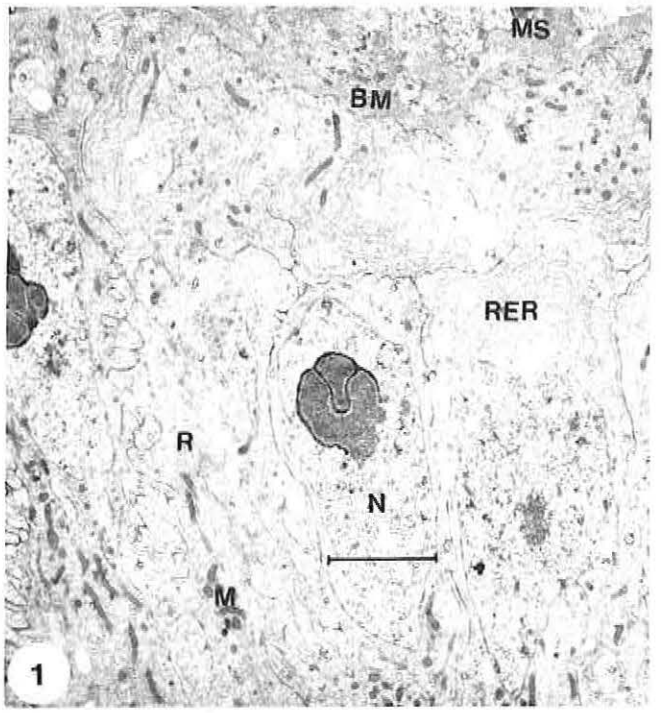


Figure 6.1 Electron micrograph of 30-day-old pupal *G. morsitans* ARG showing the characteristic columnar cells with cytoplasm containing whorls of rough endoplasmic reticulum (RER), mitochondria (M), free ribosomes (R) and nuclei (N). Note also the muscle sheath (MS) and basement membrane (BM); bar = 2 μm.

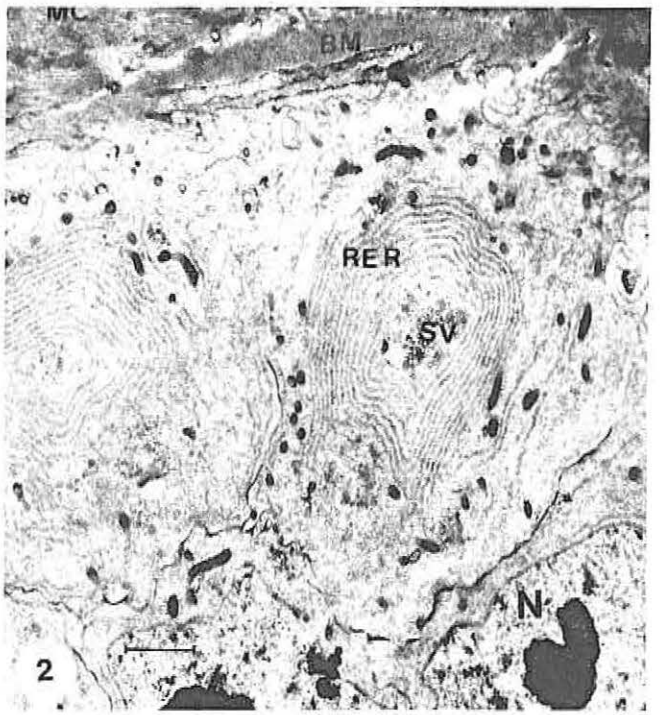


Figure 6.2 Electron micrograph of 28-day-old pupal ARG showing basally situated whorls of RER enclosing membrane-bound secretory vesicles (SV), as well as basement membrane (BM) and muscle cell (MC); bar = 1 μm.

Ultrastructural observations were made during the last part of the pupal stage (days 25–30). It was shown that the ARG consists of a single-layered columnar epithelium surrounded by several layers of muscle cells (Figure 6.1). The nuclei of the epithelial cells contain dispersed masses of chromatin and prominent nucleoli. The rough endoplasmic reticulum commonly occurs in whorls primarily in the perinuclear region at the basal cell surface, and surrounds a variety of membrane-bound vesicles and Golgi apparatus (Figure 6.2). An association between rough endoplasmic reticulum, Golgi apparatus and vesicles is present, and the flow of protein between the vesicles and endoplasmic reticulum cisternae can therefore be inferred. Unbound ribosomes are fairly abundant. The Golgi apparatus has both flattened and inflated lamellae which give rise to small electron-transparent vesicles. Many active mitochondria are scattered throughout the cytoplasm, although they are most numerous in the apical region. The microtubules spread in all directions within the cytoplasm. All of the cells appear to be secretory and the apical regions have sparse and irregular microvilli projecting into an electron-lucent sub-luminal region. Small membrane-bound vesicles occur closely around the apical border of the cell and some can be seen to be fusing with the apical plasma membrane and may be undergoing exocytosis. The pupal cells are morphologically similar to those of the adult but differ in shape, organelle organization and in the apical surface structure, especially the microvilli and the secretory material which they contain. The other major elements are the perinuclear whorls of rough endoplasmic reticulum enclosing vesicles and the Golgi system. In the adult, the secretory vesicles contain 4 different components of electron-dense secretory product similar to the secretion visible in the lumen. The Golgi lamellae also become more distended and give rise to condensing vacuoles containing several types of secretory material.

The following functional interpretations can be made. At 25–30 days before eclosion pupae are in a phase of cell specialization when maturation of the secretory machinery (including an increase in prominence of the nucleoli), proliferation of the rough endoplasmic reticulum, formation of secretory vesicles in the Golgi apparatus and accummulation of the vesicles in the apical portion of the secretory cell can be detected. Invaginations of the tracheae into the glandular epithelium are also very obvious at this stage. The cell lumina are, however, devoid of secretory components of the definitive adult morphology, except for the granule substructure and randomly dispersed filaments found at 30 days. These filaments seem to originate by pinching off microvilli (Figure 6.3).

Our ultrastructural studies have thus shown that the ARGs mature rapidly in late pre-adult stages and reach maximum development by the time of eclosion to give full secretory activity in young adults.

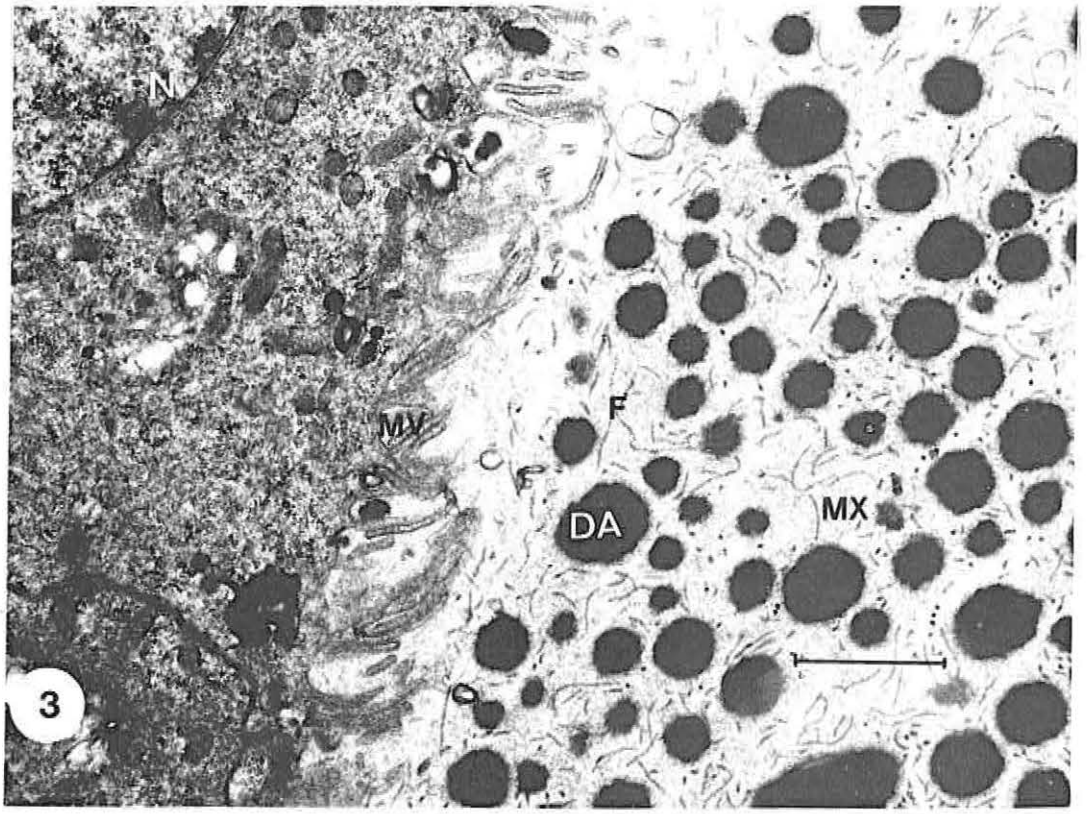


Figure 6.3 Ultrastructure of apical region of ARG of unmated, 6-day-old adult male *G. morsitans*. Note prominent microvilli (MV) projecting into lumen with filaments (F), matrix (MX) and dense aggregates of material (DA); bar = 1 μm.

6.2 SUBCELLULAR DEGENERATION OF MYCETOMAL ENDOCYTOBIONTS IN TSETSE *(G. MORSITANS)* INOCULATED WITH *E. COLI*

W. G. Z. O. Jura and G. P. Kaaya

Specimens of the mycetome, that portion of the anterior midgut harbouring intracellular bacterioids (endocytobionts), were obtained from both untreated female tsetse, *Glossina morsitans*, and those inoculated twice into the haemocoel with live bacteria, *Escherichia coli* (strain D31). They were then processed for routine electron microscopy and the endocytobionts examined for structural alterations.

In the untreated controls, mycetocytes contained intact bacterioids (Figures 6.4 and 6.5) with numerous, electron-dense ribosomal particles in the cytoplasmic matrix (Figure 6.6).

By comparison, females subjected to inoculations with *E. coli* had empty gut lumina, devoid of bacterioids (Figure 6.7). Elements of the cytosol of the endocytobionts in these females were extremely degenerative. The cytoplasm of the bacterioids showed advanced lysis, was markedly electron-lucent and had patchy granular aggregates (Figure 6.8). Fine fibrils were scattered throughout the cytoplasmic matrix.

Inoculation of *E. coli* into *G. morsitans* induces production of a potent, humoural antibacterial response which is due both to the release of stored lysozyme, and to the *de novo* synthesis of other classes of antibacterial

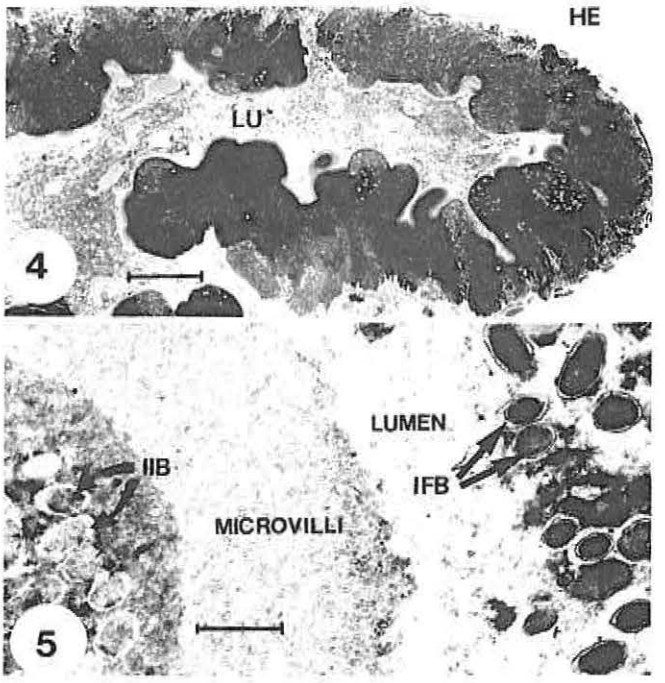


Figure 6.4 Photomicrograph of a section of mycetome from control, female *G. morsitans* showing numerous, densely staining, bacterioid particles free within the gut lumen (LU); haemolymph (HE).

Figure 6.5 Section of mycetome of control, female *G. morsitans* showing intact, intracellular bacterioids (IIB) and intact, free bacterioids (IFB) in the gut lumen.

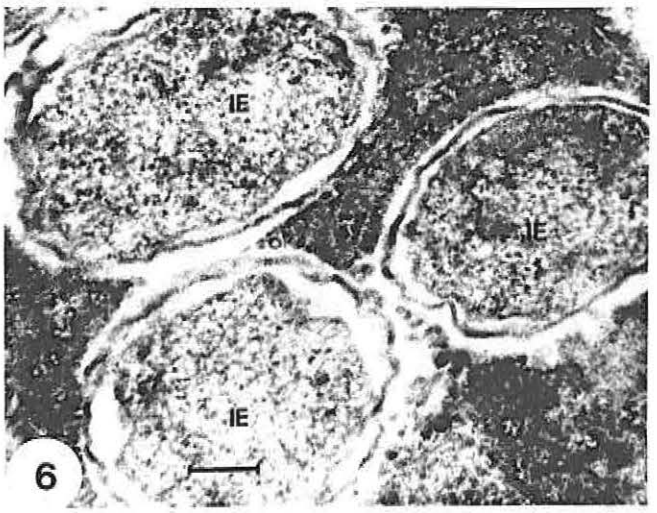


Figure 6.6 Section of mycetome of control, female *G. morsitans* showing intact endocytobionts (IE) with loosely scattered electron-dense ribosomal particles within the cytosol.

proteins, e.g. cecropins and attacin-like factors. This induced, multifactorial antibacterial activity is higher in females than in males. Lysozyme concentration in insect haemolymph increases up to 50-fold following microbial interaction which generally induces lysis of the microbes. The lesion in our study is a severe degenerative change in which elements of the cytoplasmic matrix of the intracellular symbiotic bacterioids show advanced lysis and rarefaction in treated flies.

We believe that the degradation observed in the bacterioids is attributable to the effects of induced lysozyme and the other antibacterial proteins whose production has been demonstrated previously in *G. morsitans*. In fact, administration of egg-white lysozyme to tsetse, either orally or by intrahaemocoelic injection, has been shown to damage the symbionts and lead to cessation of reproduction. It appears that lysozyme is the most important component of the humoural defence system in tsetse.

Extracellular bacterioids would be more accessible, and prone to more extensive degradation by humoural immune factors, than symbionts within mycetocytes. It is not surprising, therefore, that the gut lumina of females inoculated with *E. coli* were entirely free of bacterioids.

The massive degeneration of the endocytobionts greatly reduces their ability to provide their host with

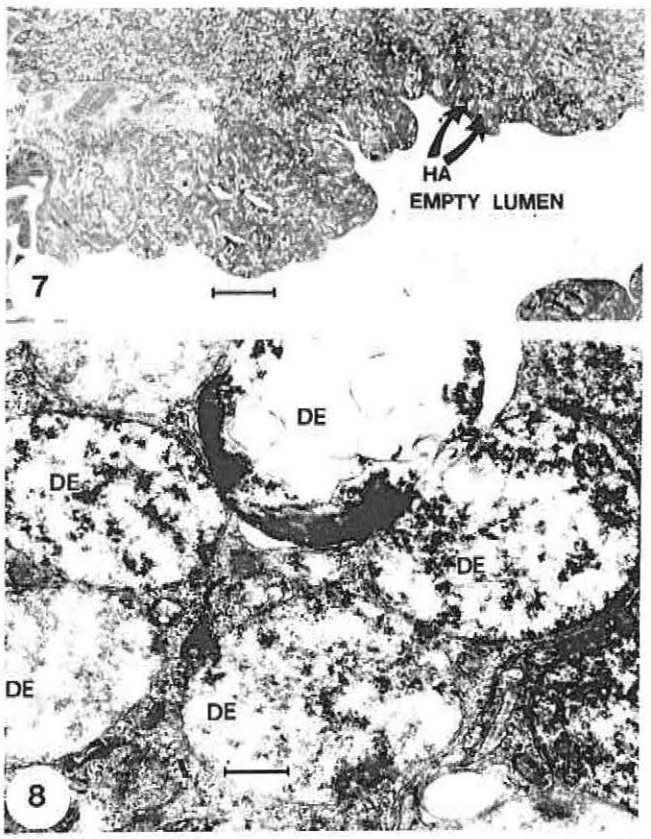


Figure 6.7 Photomicrograph section of mycetome from *E.coli*-inoculated, female *G. morsitans* showing the gut lumen with no free bacterioids. The staining reaction of the intracellular symbionts is so light that they are easily recognisable as numerous haloes (HA) within mycetocytes.

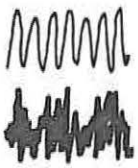
Figure 6.8 Section of mycetome of *E. coli*-inoculated, female *G. morsitans* showing degenerative endocytobionts (DE) with extremely electron-lucent cytoplasm, depicting lysis of subcellular structures.

B-group vitamins resulting in the impaired reproductive capacity of female tsetse inoculated with *E. coli*. It has been observed that the decrease in fecundity is proportional to the damage to the bacterioid population and culminates in sterility if there is aposymbiosis.

These observations imply that raising specific immune serum to these bacterioids in mammals living in areas where tsetse are endemic could contribute to tsetse control through elimination of their gut symbionts. Further work on the induced antibacterial factors may also lead to the production of a new group of antibiotics.

SENSORY PHYSIOLOGY RESEARCH UNIT

7



Sensory Physiology Research Unit

The main objectives of the Sensory Physiology Research Unit (SPRU) have been to study the factors and mechanisms which influence insect responses to various stimuli. These factors include various kairomones, pheromones or other host or non-host chemicals which may influence such behaviour of insects as host-finding, host-selection, feeding, mating and oviposition. Using a combination of behavioural assays and electrophysiological methods, studies were conducted to elucidate the mechanism of the stimulus-response activities which mediate these behaviours. Research focussed on the following topics:

- *Feeding behaviour of the maize stem borer,* Chilo partellus
- *Sensitivity of the tarsal and ovipositor sensilla of the cowpea pod borer,* Maruca testulalis
- *Olfactory sensitivity and behaviour of the tsetse flies,* Glossina morsitans morsitans *and* Glossina pallidipes *to various kairomones.*

7.1 FEEDING BEHAVIOUR PATTERNS OF *C. PARTELLUS* ON MAIZE

P. G. N. Njagi

Preliminary field and laboratory observations on the feeding behaviour patterns of the stem borer *Chilo partellus* were carried out at Mbita Point Field Station in October – December 1988. These observations are the prelude to experiments to investigate those host-plant characteristics (physical and chemical) that influence the feeding behaviour of the pest. The information obtained may be useful in breeding maize lines that are resistant to the stem borer. Several aspects of the feeding behaviour of the larvae have been noted.

1. In a laboratory bioassay of leaf choice, *C. partellus* larvae showed a preference for whorl leaves of young maize plants over lower, older leaves (Table 7.1). Since the experimental preparations were kept in darkness, this may indicate utilization of an olfactory component by newly hatched larvae of *C. partellus* for orientation to the feeding site. Further olfactometer experiments will be done to see whether there is an olfactory stimulant in the surface chemicals of young maize leaves.

2. Larvae of *C. partellus* feed on either the abaxial (outer) or adaxial (inner) surface of the leaf tissue of maize, leaving the cuticle of the undamaged side intact. In most cases, larvae feed on the adaxial surface of older leaves, but on the abaxial surface of the folded whorl leaves. Further investigations will be carried out to determine whether physical and/or chemical factors are utilized by the larvae to locate a suitable feeding site.

3. Observations on the feeding of larvae on different parts of maize plants at various infestation densities have shown that larvae invade leaf sheaths earlier at relatively high infestation levels (20 larvae/plant and more). There is also a likelihood of dead-hearts occurring when plants are infested with larvae at 3 weeks after germination.

These behaviour studies will continue, and in addition, electrophysiological studies are in progress to see whether *C. partellus* larvae have sensory receptors that are sensitive to specific chemicals which may be phagostimulatory. Appropriate methods of presenting the plant material to the larvae are also being developed.

Table 7.1 Percentages (mean ± s.e.) of *C. partellus* larvae responding in choice tests for orientation and feeding on maize leaves

Replicates	Total larvae	Older lower leaf	Whorl leaf
29	450	22.4 ± 2.7	47.8 ± 3.5*

* Difference between means significant (t = 2.0, df = 56; $P < 0.05$).

7.2 OVIPOSITOR AND TARSAL SENSILLA FUNCTIONS IN THE DRINKING AND OVIPOSITION BEHAVIOUR OF *M. TESTULALIS*

S. M. Waladde

Investigations on the sensitivity of *Maruca testulalis* tarsal and ovipositor sensilla were started with the objective of identifying some of the potential stimuli for these

sensilla, and establishing their effect on drinking and oviposition behaviour. Since these behaviour patterns are intertwined, it is appropriate that the two types of sensilla be studied together.

7.2.1 Tarsi

The tarsus of the first leg is equipped with 3 pairs of sensilla. One pair has receptor cells that are particularly sensitive to cations such as Na^+ and K^+. Stimulation of these sensilla with sugars such as sucrose evokes multispike responses. Experiments were carried out to see whether these responses can be associated with a definite pattern of behaviour. This was done by presenting separate cotton wool pads soaked in 3 different ingredients: 10% sucrose, 1% NaCl and water only. During the drinking period, which reaches its peak at about midnight, 99% of the moths landed and drank from the sucrose pads. As soon as a moth landed on the pad it extended the proboscis and remained in one position for prolonged periods, presumably imbibing fluid. Those moths landing on the pad with the salt solution did not extend the proboscis, but spent some time cleaning their antennae with the fore tarsi. They finally moved off the pad without drinking. It is likely that the mononeuronal responses evoked by the cation then produced rejection behaviour. If this is the case, then the polyneuronal responses evoked by sucrose caused movement arrest, extension of the proboscis and drinking. About 1% of the moths were observed drinking from the pads holding water; in most cases they extended the proboscis into the cotton pad but kept the tarsi away from direct contact with it. The frequency and duration of drinking will be recorded electronically and the receptors involved in this behaviour are to be characterized using spike analysis methods.

7.2.2 Ovipositor

The ovipositor has 3 types of receptors. There are several long bristles, possibly mechanoreceptors. There are 3 pairs of chemoreceptor sensilla with receptor cells specifically sensitive to sucrose. The function of the third type of sensilla remains to be identified, but it is possibly sensitive to odour or temperature/moisture stimuli. Observations of ovipositing females show that egg deposition is not a random behaviour. The ovipositor with its sensilla plays an active role in the choice of the oviposition site. Moths frequently oviposit at a site where other moths have oviposited. On the leaves of cowpea, eggs are invariably deposited adjacent to the leaf veins. On the stems, eggs are found in the shallow grooves.

Successful rearing of *M. testulalis* is currently hampered by the fact that it is not yet possible to get the moths to oviposit effectively on an artificial medium. Although there are some reports of an artificial oviposition medium, we still rely heavily on the availability of fresh cowpea leaves as a medium on which *M. testulalis* can oviposit. Work in progress will attempt to identify the relevant stimulus of the ovipositor sensilla. With this information, it should be possible to develop a reliable artificial medium on which *M. testulalis* can oviposit.

7.3 OLFACTORY SENSITIVITY OF TSETSE FLIES TO PHENOLIC KAIROMONES

R. K. Saini and A. Hassanali

Behavioural and electrophysiological studies were undertaken to determine the olfactory selectivity of the antennae of *Glossina morsitans morsitans* and *G. pallidipes* to phenolic kairomones. Results (given in Figure 7.1) indicated that 4-cresol and 3-n-propylphenol were important in conferring high activity to the buffinol mixture — phenols extracted from buffalo urine, a potent natural attractant for tsetse. Responses of both species to 4-alkylphenols increased as the alkyl chain decreased from C_3 to C_1, while a reverse trend was observed with 3-alkylphenols. Comparison of the responses indicated differences in the sensitivity of the chemoreceptor system of each species. Hence separate blends of the phenols may be required to attract different species.

The variations in activity of the phenols provide some insight into the nature of the interaction of these compounds with the complementary sites on the receptors. The models shown in Figure 7.2 are postulated to represent these sites for the two groups of phenols. For the 4-alkylphenols, a close-knit binding site which involves an electron-pair donor for H-binding, and two hydrophobic points of interaction (one each for the phenyl and methyl moieties) to a potential ligand would account for the relatively high stimulatory activity of 4-cresol, see Figure 7.2(a). The lower activity of the other two 4-alkylphenols is accounted for by steric factors and that of phenol by lack of the second hydrophobic interaction. For 3-alkylphenols, two additional hydrophobic points of interaction would account for the high activity of 3-propylphenol in *G. pallidipes,* although in *G.m. morsitans* this may be of less importance, see Figure 7.2(b). However, it must be pointed out that only one of a number of possible topological variants, corresponding to the different conformations of the propyl group is shown in Figure 7.2.

The inference is that although it would be difficult to improve upon the performance of 4-cresol, it might be worthwhile to evaluate some analogues of 3-propylphenol corresponding to different binding topologies, as well as longer alkyl chain lengths. In addition, it is possible to visualize a generalized receptor site that allows interaction with either of the two phenolic types and, kairomonal activity arising from the sharing of existing sites between the two. If so, a compound such as 3-n-propyl-4-cresol could have interesting properties. Studies on the responses of tsetse flies to such analogues of phenolic kairomones are now underway.

7.4 RESPONSES OF TSETSE FLIES TO ODOURS IN A FLIGHT TUNNEL

R. K. Saini

Investigations have been started on the way that various chemicals affect tsetse behaviour. Such studies are important because a fuller understanding of the types

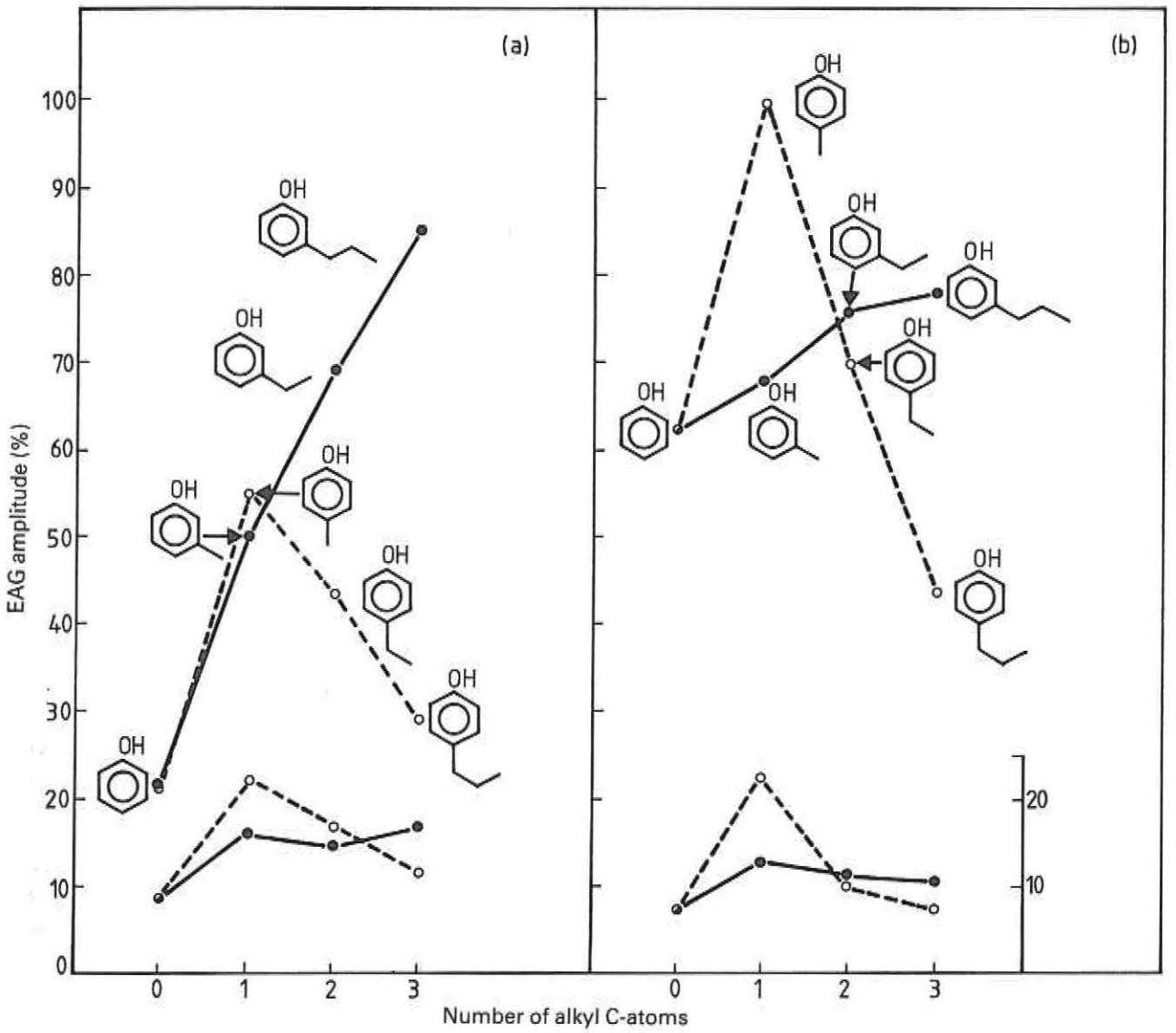


Figure 7.1 Antennal responses (lower part of each figure) and electroantennogram (EAG) responses of tsetse flies to 7 phenols: (a) *G.m. morsitans* (b) *G. pallidipes.*

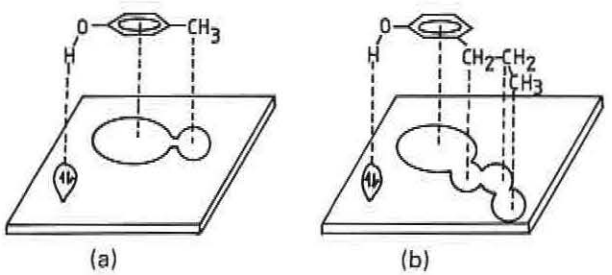


Figure 7.2 Models illustrating probable points of interaction of (a) 4-cresol and (b) 3-n-propylphenol at the binding sites on the receptor surface on the antennae of tsetse flies.

and interplay of responses to individual chemicals and their combinations may suggest means by which odours can be used more effectively against tsetse.

A wind tunnel has been developed to investigate the responses of flies to odours (Figure 7.3). Responses of teneral male *Glossina morsitans morsitans* (3 days old and 3 days hungry) were investigated on stimulation with 4 different doses of various phenolic kairomones, 1-octen-3-ol (octenol) and buffalo urine (undiluted and 10× diluted). As a control, responses were observed to blanks (filter paper loaded with an equivalent amount of paraffin oil or water). The time to, and occurrence of, the following behaviours were recorded: activation (flight or walking in the release cage without any upwind movement), upwind flight (initiation of upwind flight out of the release cage) and source contact behaviour (actual contact with the stimulus-release device).

Activation was not dependent on the dose of the compounds tested. All except 4-ethylphenol elicited activation behaviour that was significantly greater than the control (10%). Octenol, urine, 4-cresol, 3-cresol and 3-propylphenol elicited the most activation behaviour (more than 50%) but there was no significant difference in the number of flies being activated on stimulation with these compounds.

Octenol elicited significantly more upwind flight (50%) compared to urine (32%) or its constituent

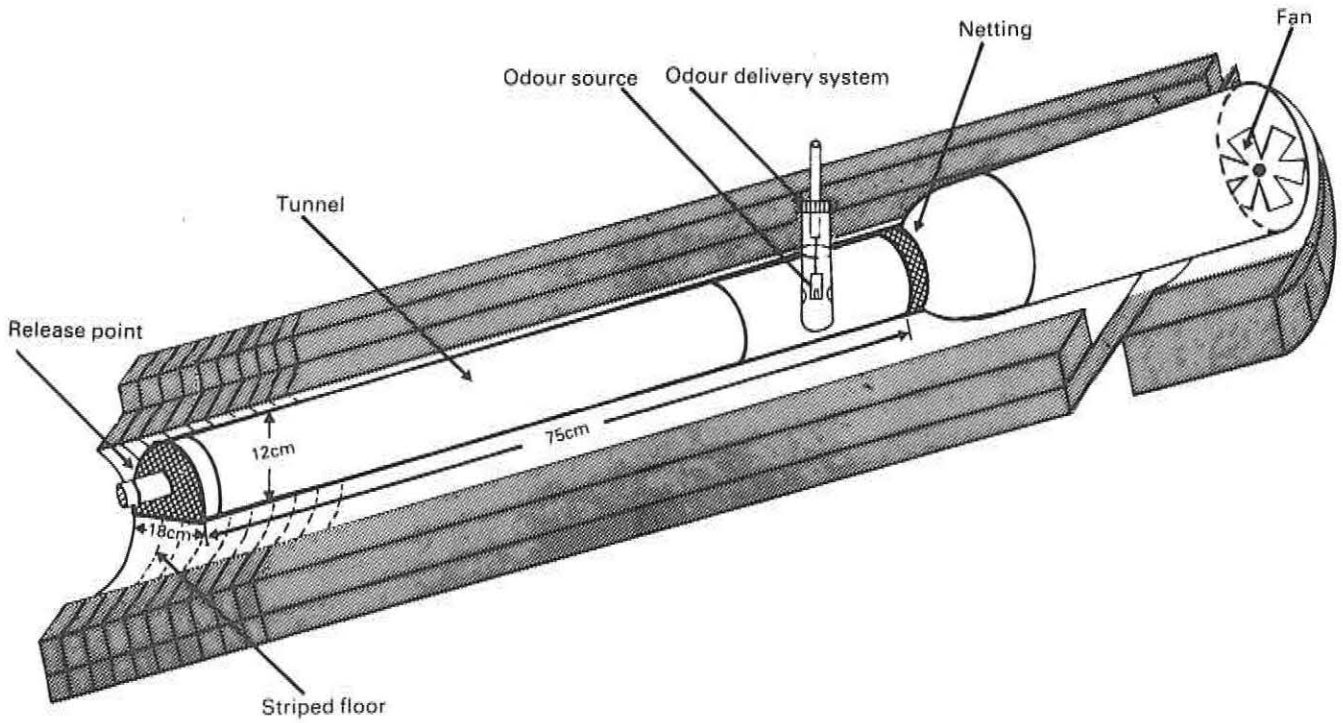


Figure 7.3 Diagram of the wind tunnel used to investigate the responses of tsetse to odours.

phenols. Only about 10% of control flies showed upwind flight.

Source contact behaviour was dependent on dose in the case of octenol and 4-cresol. However, it was interesting to note that although octenol caused more upwind flight than urine or its constituent phenols, there was no difference in the number of flies actually contacting the source after stimulation with these compounds. In fact urine itself elicited source contact behaviour that was not significantly different from the control.

The control flies took longest (75 sec) to initiate activation behaviour (latency to activation) while urine, octenol and 4-cresol were the quickest (about 50 sec). On stimulation with 3-propylphenol flies spent significantly more time (about 30 sec) during activation, compared to the rest of the compounds tested. In fact there was no significant difference in the time spent during activation with urine, various phenols, octenol or the control (about 15 sec). With 3-propylphenol, the flies spent a lot of time walking and flying in the release cage without initiating much upwind flight. Octenol made the flies move the furthest (more than 35 cm) compared to urine, its constituent phenols or the control. In fact there was no significant difference in the distance moved on stimulation with the latter compounds.

These results indicate that octenol acts over longer distances and urine over much shorter distances. Preliminary studies with *G. pallidipes* indicate that this species responds more strongly to various phenols than *G.m. morsitans*. Investigations of the responses of tsetse flies to various blends of chemicals are in progress.

INSTITUTIONAL BUILDING AND INTERACTIVE RESEARCH UNIT

8

Institutional Building and Interactive Research Unit

The Unit, now known as IBIRU (formerly Outreach and Training Unit), acquired its present name to reflect more clearly its function, long term objectives and philosophy of interactive collaboration. The activities of the Unit continued to be structured into two major components, firstly, International Cooperation and Linkages comprising (1) the African Regional Pest Management Research and Development Network (PESTNET), (2) collaboration with other institutions, (3) Research and Development and the Financial and Administrative Management of Research Projects in Eastern and Southern Africa (FAMESA); and, secondly, Training Programmes, comprising (4) the African Regional Postgraduate Programme in Insect Science (ARPPIS), (5) the Postgraduate Research Fellowship and Research Associate Schemes and (7) Staff Development Schemes.

Although effort was sustained on all the Unit's activities, special emphasis was placed on consolidating the PESTNET programme and strengthening graduate training. Resident PESTNET scientific research teams were effectively placed in Somalia and Zambia and arrangements completed to attach, by early 1989, a PESTNET research scientist to Rwanda's national pest management research programme at the Institut Scientifique et Agronomique du Rwanda (ISAR), Rubona. In Kenya, validation and test trials for integrated crop pest management were firmly established in several ecological zones. With regard to training, a firm structure was developed for the ICIPE's consolidated graduate training programme.

8.1 AFRICAN REGIONAL PEST MANAGEMENT RESEARCH AND DEVELOPMENT NETWORK (PESTNET)

E.O. Omolo

PESTNET, now in its fourth year, is firmly established. The objective of this project is to increase food production through the development and verification of integrated pest management (IPM) strategies for the control of crop and livestock pests.

During 1988, several missions and consultative meetings were undertaken in Kenya, Zambia, Somalia and Rwanda, mainly to discuss the plan of work and the administrative and technical logistics for effective implementation of PESTNET activities. The meetings also served to finalise arrangements for posting resident PESTNET scientists in three of the four countries where initial PESTNET activities were established.

Two resident scientists took up their duties in March in Somalia and Zambia, where each immediately embarked on the first part of his programme, which comprised a survey of the status of crop-borers, with particular emphasis on stalk-borers.

In Zambia, a survey undertaken in the low, medium and high rainfall areas confirmed the status of stalk-borers as major pests of maize on peasant farms throughout the country. Leafhoppers, responsible for transmitting maize streak virus, are widespread, and the disease resulted in maize yield losses ranging between 10–100%. Termites are also a serious problem at harvesting in all areas.

A survey undertaken in the major agricultural areas of Somalia, namely Jowher, Baidoa and Afgoi, during the "gu" season confirmed the economic importance of the stalk-borer problem on both maize and sorghum. Fortunately, Somalia already has a programme on evaluation of the two crops for resistance against these pests.

In Kenya, PESTNET survey work was specifically for assessing the incidence of stalk-borers and their parasitoids, and the borer profile, in the major maize growing areas of the country, with a view to testing some of the IPM components recently developed by ICIPE.

The survey revealed devastating infestations by stalk-borers, particularly *Chilo* species, in the majority of areas surveyed thus confirming that stalk-borers are a major constraint to maize production in Kenya.

The first Ph.D. candidate from Zambia under the PESTNET training programme joined the 1988 ARPPIS Class and has now embarked on the research project for her thesis. A second candidate has been accepted from Somalia to start his ARPPIS studies in 1989. Short term trainees from Kenya attended a six-week course on Insect Mass-Rearing Technology at the ICIPE's Mbita Point Field Station and a visitor from Uganda spent one month in the Crop Pests Research Programme (CPRP), familiarising himself with various pest management techniques. Two trainees from Somalia also undertook intensive training at Mbita Point Field Station, covering all aspects of the work of the CPRP.

PESTNET continued to promote information exchange by publishing the newsletter *PESTNET Today*, and successfully organised the second PESTNET Annual Conference in April, as well as the second and third meetings of the Steering Committee.

8.2 COLLABORATION WITH OTHER INSTITUTIONS

Z.M. Nyiira

The Centre maintained effective cooperation with various national research and extension institutions, centres of excellence, regional and international development agencies and member institutions of the Consultative Group for International Agricultural Research (CGIAR), and continued to emphasise technology evaluation and technology transfer linkages.

In Africa, a technology evaluation project on integrated management for tsetse fly in the Kagera Basin, to the S.W. of Lake Victoria, was initiated through a tripartite agreement between the Kagera Basin Organisation, United Nations Economic Commission for Africa (ECA) and the ICIPE. Technology evaluation projects (other than those through PESTNET) continued in Kenya (cereal insect management), Mozambique (maize resistance to stem-borers), Tanzania (banana weevil), Uganda (cowpea resistance to pod-borers) and Côte d'Ivoire (tsetse physiology).

Negotiations for cooperation in various pest management projects were undertaken with Sudan, Zimbabwe, Lesotho and Ethiopia. A cooperative agreement was subsequently signed with Ethiopia. Strategies for the implementation of the cooperative agreement with Philippines were developed. Requests continued to be received from national institutions in Central and Southern America for collaboration.

The Centre continued to have excellent relations and effective linkages with centres of excellence in Europe, North and South America, the Middle East and Africa. These included, Instituto Guido Donagani, Italy (naturally-occurring tropical pesticides); Imperial College, United Kingdom (blood meal analyses); University of Lund, Sweden (*Chilo* pheromones); University of Neuchâtel, Switzerland (host immunity to ticks and tick ecology); Universidade Federale de Minas Gerais, Brazil (phlebotomine immunotaxonomy); Addis Ababa University, Ethiopia (kala-azar epidemiology); Groupe d'Etude et de Recherche en Microscopie Electronique (GERME), Côte d'Ivoire (sensory physiology and fine structure).

Successful liaison was maintained with regional and international development agencies, particularly the United Nations Development Programme (UNDP), ECA, Food and Agriculture Organization of the United Nations (FAO), European Economic Community (EEC), United States Agency for International Development (USAID), International Atomic Energy Agency (IAEA), Overseas Development Natural Resources Institute (ODNRI), Commonwealth Agricultural Bureaux International (CABI), African Biosciences Network (ABN), African Development Bank (ADB) and the Scientific, Technical and Research Commission of the Organisation of African Unity (OAU/STRC).

The Centre continued to have effective collaborative programmes with the International Institute of Tropical Agriculture (IITA) (evaluation of cowpea germplasm against major pests in the West African cropping ecology); International Centre for Improvement of Wheat and Maize (CIMMYT) (evaluation of maize germplasm against major stem-borers); International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (testing sorghum germplasm against stem-borers and shootfly); International Rice Research Institute (IRRI) (leaffolder research) and International Laboratory for Research on Animal Diseases (ILRAD) (livestock disease vectors research).

8.3 FINANCIAL AND ADMINISTRATIVE MANAGEMENT OF RESEARCH PROJECTS IN EASTERN AND SOUTHERN AFRICA (FAMESA)

Z.M. Nyiira

The work programme comprises four sub-programmes, Institute Survey, Curricula Development, Delivery of Training Material and the Research and Development Management Information and Documentation Service; the major preoccupation during the year was completion of on-going projects, information exchange and fund raising.

Work on the development of training material was confined to refining the *Manual on R&D Institute-Constituency Relationship* and completion of the *Manual on Project Planning Monitoring and Evaluation.* Both publications went to press. *Inter-Institutional Relations and Linkages for Effective Research and Development* was completed and published and is now available from the FAMESA Secretariat.

Intensification of training and the delivery of training material to national systems were the major considerations during the year. One regional workshop was

organised to provide the forum for evaluation of the relevance and potential impact of the training material in *R&D Institute-Constituency Relationship*. A colloquium was also organised on training R&D management trainers. At the national level, only Malawi was able to convene, in February, a second national research management forum. Ethiopia rescheduled its national training workshop on Strategic and Project Planning and Budgeting. Instead, the Ethiopian Commission for Science and Technology organized a conference on National Science and Technology Policy, to which FAMESA was invited to discuss incorporation of a research management training strategy in the final recommendations. Meetings were undertaken with Zambia and Uganda to plan national R&D management training. In June, a two-day course for postgraduate students on Project Identification and Preparation of Research Projects was conducted jointly by FAMESA and the African Regional Postgraduate Programme in Insect Science (ARPPIS).

Activities of the R&D Management Information and Documentation Service were limited to the publication of the bulletin *Research Management Review*, with emphasis on improving the quality and substance of the publication.

During the year FAMESA expanded its collaboration with selected regional institutions to promote regionally centralised training in specific areas of science and technology R&D management. The FAMESA Secretariat therefore contacted such bodies as the UN Economic Commission for Africa (ECA), Food and Agriculture Organization of the UN (FAO), the Network of Deans, Directors and Coordinators of Graduate Studies in Eastern and Southern African Universities (NDGS), and the Commonwealth Regional Health Community Secretariat (CRHS). Discussions were started with the African Centre for Technology to seek a way of addressing research management training needs in the wider sector of science and technology.

Finally, the Regional Advisory Committee of the FAMESA project met in December to review progress, workplans and budget, and to evaluate the various activities of the project. It expressed satisfaction that the project is equipped to continue delivery of research management training courses, and felt that the major thrust in the coming year should be to make use of the training material so far developed by national systems and help establish an element of sustainability.

8.4 AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE (ARPPIS)

M.E. Smalley

The sixth ARPPIS Ph.D. Class began its studies in March, with ten students from five countries — Kenya, Nigeria, Sudan, Uganda and Zambia. Including the 1988 Class, ARPPIS has now registered 50 students from 12 countries.

The Ph.D. programme involves a compulsory six-month semester of six courses followed by a thirty-month research project. The 1988 teaching semester was presented, as usual, by ICIPE scientists and visiting lecturers from ARPPIS-participating universities. Professor R. Kumar, Rivers State University of Science and Technology (RSUST) and Dr. R.K. Bagine, National Museums of Kenya, taught Insect Taxonomy; Professor El Amin El Rayah, University of Khartoum, taught Insect Functional Morphology; Dr. J. Allotey, RSUST, and Dr. C. Mutero, ICIPE, taught Insect Ecology; Dr. B. Williams, ICIPE, taught Biostatistics; Dr. G.P. Kaaya, Dr. M.O. Odindo and Dr. M. Brownbridge all of ICIPE, taught Insect Pathology and Dr. M.F.B. Chaudhury, Dr. E. Osir and Dr. R.K. Saini taught Insect Physiology and Biochemistry. Each course was examined by a three-hour written paper; the papers and scripts were externally moderated by Dr. R.W. Mwangi, University of Nairobi.

During the year all seven members of the 1985 class joined the 16 members of the 1983 and 1984 Classes in having completed their studies, and by the end of the year, 16 of the 23 students had submitted a thesis to their registering university. Five more ARPPIS students were awarded the Ph.D. degree during 1988 (Table 8.1).

All ARPPIS graduates are actively engaged in either research or teaching in institutions within Africa. The International Foundation for Science awarded research grants to two former ARPPIS students in 1988: Dr. R.K. Bagine (1983) to work on the taxonomy of *Odontotermes* termites in Kenya and Dr. J.H.P. Nyeko (1983) for a study of the epidemiology of bovine trypanosomiasis in Uganda. As a further contribution to the work of its graduates, ARPPIS is administering the money for the recurrent expenditures of the IFS grant to Dr. Nyeko.

ARPPIS was host to the university supervisors of 14 Ph.D. students during the year: Professor C.W. Baliddawa, Makerere University, to Mr. M.W. Ogenga-Latigo (1984); Professor W.Z. Coker, University of Ghana, Mr. C. Kyorku (1985); Dr. B. Khaemba, Moi University, Mr. M. Macharia (1986); Professor H.Y. Kayumbo, University of Dar-es-Salaam, Mr. J. Nderitu (1984) and Miss E. Minja (1986); Professor R. Kumar, RSUST, Mr. J. Ogwang (1987); Dr. K. Mbata, University of Zambia, Mr. M. Mwangelwa (1987); Professor J.R. Mainoya, University of Dar-es-Salaam, Mr. M. Njau (1986); Professor H.G. Morgan, University of Sierra Leone, Mrs. R. Sang (1985), Mr. G. Tikubet (1985), Mr. H. Hassane (1987) and Mr. J.O. Davies-Cole (1987); and Professor J. Mueke, Kenyatta University, Mrs. M. Ndonga (1986) and Mr. M. Gethi (1986).

As usual, the ARPPIS Academic Board met twice during the year; in June and December. The December Board Meeting was preceded by a one-day scientific meeting at which all second and third year Ph.D. students presented a paper on an aspect of their work. The December meetings also included the third ARPPIS Lecture, which was given by His Excellency Mr. E. Fiil, the Ambassador of Denmark.

The ARPPIS M. Phil. degree programme for students specialising in biological control, sponsored by the Africa Wide Biological Control Programme (ABCP),

Table 8.1 ARPPIS Scholars awarded degrees in 1988

Name	Thesis title	Registering University
Ph.D. Scholars		
Dr. R.K. Bagine	Biosystematic studies of the termite genus *Odontotermes* with special reference to Kenya	Rivers State University of Science and Technology (RSUST)
Dr. S. Kyamanywa	Ecological factors governing insect pest populations in maize and cowpea crop mixtures with special reference to the bean flower thrips *Megalurothrips sjostedti*	Makerere University
Dr. J. Okeyo-Owuor	Population ecology of the legume pod-borer *Maruca testulalis* in relation to its natural enemies on cowpea in Western Kenya	University of Dar-es-Salaam
Dr. I.G. Aniedu	Ecology of malaria vectors in relation to an irrigation scheme in Baringo District, Kenya	RSUST
Dr. M. Basimike	Studies on the factors affecting the distribution and abundance of phlebotomine sandflies in a leishmaniasis endemic focus in Baringo District, Kenya	RSUST
M. Phil. Scholar		
Mr. P.N. Amifor	Biology and predation efficiency of an aphidophagous coccinellid (*Cheilomenes lunata)* on the cowpea aphid *(Aphis craccivora)*	RSUST

admitted its third and final class in 1987. The ABCP is unable to continue the funding but, in the four years of the project, ten students from six countries have been able to study for a masters degree. In 1988 Mr. P.N. Amifor (1986 Class) was awarded his degree (Table 8.1).

During the year ARPPIS moved to the new Duduville Headquarters complex. The move produced a significant improvement in the physical facilities, and ARPPIS now has, for the first time, a teaching laboratory, a microcomputer teaching room and a small lecture room.

Financial support for ARPPIS during 1988 came from the German Academic Exchange Programme (DAAD), the Netherlands Government, the Ford Foundation, the International Livestock Council for Africa (ILCA) and the International Fund for Agricultural Development (IFAD). DAAD also provided departmental support which enabled more equipment to be purchased for the ARPPIS teaching laboratory.

ARPPIS was a member of an ICIPE mission to Côte d'Ivoire, including the National University of Côte d'Ivoire; provided expert advice on training to a regional workshop held in Swaziland by the Southern Africa Centre for Cooperation in Agricultural Research (SACCAR) on strategies for integrated pest management; and participated in a meeting of Deans, Directors, and Coordinators of Graduate Studies in Eastern and Southern African Universities (NDGS) held in Botswana.

8.5 POSTDOCTORAL RESEARCH FELLOWSHIP PROGRAMME

M.E. Smalley

Nine postdoctoral scientists from eight countries worked in the ICIPE during 1988. Dr. M. Basimike from Zaire joined the Medical Vectors Research Programme and Dr. R. Ramachandran joined the ICIPE research team based at the International Rice Research Institute (IRRI). Four postdoctoral scientists continued with their research: Dr. M. Brownbridge from the United Kingdom and Dr. V. Okoth from Uganda, both in the Crop Pests Research Programme; Dr. S. Essuman, from Ghana, in the Chemistry and Biochemistry Research Unit; and Dr. E. Kamanga-Sollo, from Tanzania, in the Livestock Ticks Research Programme (LTRP). Three postdoctoral scientists completed their contracts: Dr. S.K. Firempong, from Ghana, with the Crop Pests Research Programme; Dr. R.S. Copeland, from USA, with the Tsetse Research Programme; and Dr. A. Latif, from Sudan, with LTRP. Dr. Latif was subsequently appointed as a research scientist in the same programme.

8.6 GROUP TRAINING COURSE IN PEST AND VECTOR MANAGEMENT SYSTEMS

J.F. Omange

The eleventh International Group Training Course on Components Essential for Ecologically Sound Pest and Vector Management Systems was held from 7–27

August, attended by 33 participants from 14 tropical developing countries. This brings to over 300 the number of trainees who have benefited from this course since the series was established in 1977.

8.7 STAFF DEVELOPMENT TRAINING

J.F. Omange

A comprehensive survey of the staff development training needs of the ICIPE was completed during the year. This resulted in the identification of priority training areas for the Centre over the period 1988–1990, and an approved list of staff members to benefit from this scheme was drawn up.

Meanwhile 16 staff members who were sponsored by the Centre completed their training during the year. They consisted of seven scientific staff, five technical and four administrative staff.

BIOMATHEMATICS RESEARCH UNIT

9

Biomathematics Research Unit

In addition to the research and scientific collaboration with other units and programmes, the Biomathematics Research Unit (BRU) provides a wide range of services within the ICIPE. Our main research interest lies in the application of geographical information systems (GIS), remote sensing and modelling to the study of the distribution and movement of insect pests. The services which we provide cover the following:

- *Maintenance of computer hardware and other electronic environmental monitoring equipment*
- *Acquiring and evaluating software packages*
- *Giving courses on computing, statistics and data analysis*
- *Running the electronic mail service (Email)*
- *Advice and implementation on accounting packages for the Finance Division.*

9.1 HARDWARE

There are at present about 60 microcomputers in the centre and several more are on order. Both dot matrix printers and laser printers are used for producing hard copies. Our GIS runs on a 286 AT class computer to which we have added an 80 Mb hard disk, a large format plotter and a digitizer. It is now possible to obtain locally assembled personal computers and we are hoping to obtain much of our equipment from local suppliers in the future. Multi-user systems are becoming available and we have this area under active consideration.

We have embarked on a preventative maintenance scheme, including lectures on the use and abuse of hardware in the hope of reducing costs to the centre and down time of the machines.

9.2 SOFTWARE

We now have a very extensive range of software packages in the unit which are available to ICIPE staff. These include statistics programs and also databases, spread sheets, graphics, word processing, GIS, mapping and a wide range of general utility programs. We provide advice as well as support to those who use the various packages.

9.3 ELECTRONIC MAIL

We have recently employed an information consultant who has upgraded our electronic mail system so that it operates four times as fast as before, resulting in a substantial saving in costs. The networks which we access most frequently are CGNET, (covering the Consultative Group for International Agricultural Research (CGIAR) and related institutions), GREENNET, PEACENET and BITNET (the American academic network). We have also been involved with discussions on AFRONET, an African network which is being developed. Our own internal network, linking Mbita Point Field Station to ICIPE Headquarters at Duduville, is called PESTNET and is functioning well.

9.4 FINANCIAL SYSTEMS

A new payroll system, developed to run on a personal computer by Information Processing and Consultation Company of Nairobi, has been tested and implemented by staff from BRU and Finance Division. We have been involved in discussions with Finance Division concerning a complete financial accounting system for the centre.

9.5 RESEARCH

Our own research effort is focussed on the application of GIS to the monitoring and control of insect pests in Africa. Eventually this will require substantial mathematical modelling and we have a particular interest in combining spatial-temporal models of insect movement and migration with GIS. Our GIS has evolved throughout 1988 and is still expanding to meet research needs and to make use of state-of-the-art technology.

9.5.1 Geographic information systems

The power of GIS systems resides in the ability to overlay a number of maps and then use the relational data base to carry out logical operations to create new maps. Figure 9.1 provides an overview of a typical GIS. The GIS is run on two AST 286 microcomputers connected to a Calcomp digitizing tablet and a Calcomp plotter.

There are two approaches to GIS, based either on vector mapping or on raster mapping (see below). Arc/Info is a vector-based system in which the primitive elements are points, lines and polygons. These elements can be accessed using INFO, which is a database management system. For example, the point data set might contain tsetse trap catches at particular field sites, the line data set might contain hydrological information about rivers, and the polygon data set might contain information about vegetation types. The ICIPE's vector-based GIS is pcARC/INFO.

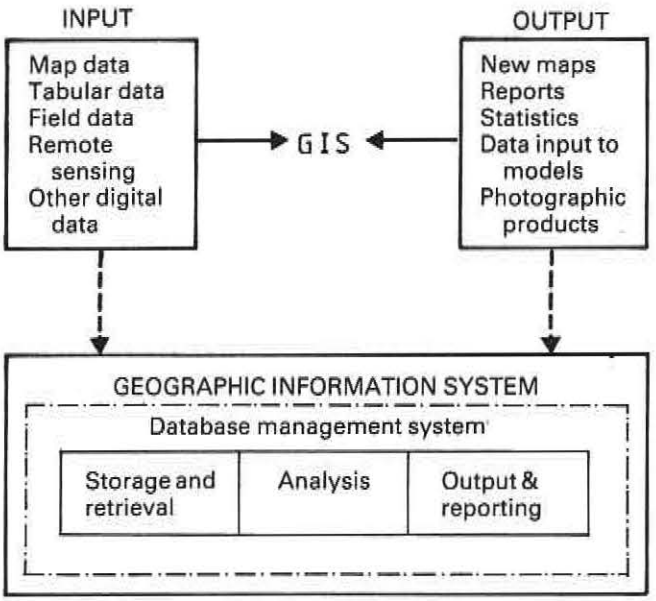


Figure 9.1 An overview of the capabilities of a typical geographic information system (GIS).

Raster GIS systems are designed to perform the same functions but the data are stored as a rectangular array of points and the operations are carried out on individual points. The raster system in use at the ICIPE is CRIES-GIS, a low cost but highly effective software package. One advantage of raster systems is their ability to process data like satellite images, in a digital format.

The Nguruman Tsetse Project has been the focus of GIS activities this year within BRU. The Nguruman database consists of baseline information digitized from Kenya Survey maps. The study area consists of 7 maps covering a total area of 5,488 km^2. Seven layers of information have been computerized for this area including elevations, vegetation types, rivers, tsetse trap locations, roads, lakes and political boundaries. Figure 9.2, for example, combines data from 4 of these layers.

The Nguruman tsetse fly trap catch data have been input into a database management system which is used to query the database selectively and output catch data to the GIS. CRIES-GIS has been successfully employed

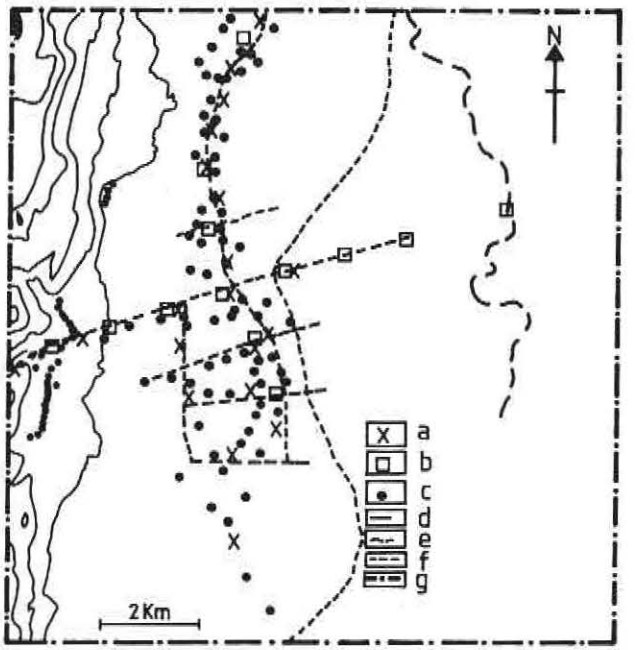


Figure 9.2 Tsetse trap locations and base map features at the Nguruman Tsetse Project, developed by use of GIS; (a) NGU traps (X-traps), (b) biconical traps (B-traps), (c) suppression traps, (d) 100-m contours, (e) rivers, (f) transect traps, (g) area boundary.

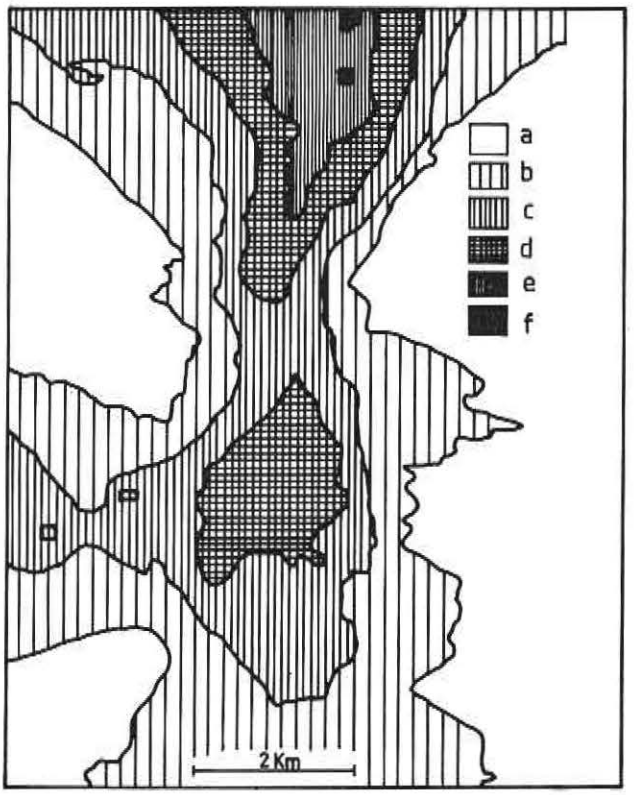


Figure 9.3 Distribution map of male *G. pallidipes* in November 1987 on the Nguruman Tsetse Project; fly densities per ha, (a) 0, (b) 1–3, (c) 4–10, (d) 11–35, (e) 36–120, (f) 121–4000.

to interpolate or "surface" the trap data to yield monthly average tsetse fly distribution maps from February 1987 to February 1988. Such distribution maps have helped us keep track of population changes over time, and also

to monitor the suppression zones. This analysis has been carried out separately for male and female flies of both *Glossina pallidipes* and *G. longipennis*. Figure 9.3 illustrates tsetse fly distribution at Nguruman.

9.5.2 Remote sensing

With the advent of cheap, yet powerful, computers and the development of GIS systems, remote sensing has become an essential tool for all aspects of environmental monitoring. Satellite data provides immediately accessible information about both vegetation and climate on a very large scale. Aerial surveys provide more detailed and complementary information.

We have begun to build up a substantial database of such information for Africa as a whole, and Kenya especially, covering those areas in which the ICIPE is carrying out field work. So far our greatest effort has been devoted to the Nguruman Tsetse Project, where we have used remote sensing data to map the vegetation cover of the area. In collaboration with the Regional Centre for Services in Surveying Mapping and Remote Sensing (RCSSMRS) we used Landsat multi-spectral scanner data to determine the different vegetation classes based on spectral reflectance. We have been able to establish collaboration with other international organizations such as Kenya Rangeland Ecological Monitoring Unit (KREMU), United Nations Environment Programme (UNEP), International Council for Research in Agroforestry (ICRAF) and United Nations Centre for Human Settlement (HABITAT), in order to develop the remote sensing side of the GIS Unit.

Work has begun on the development of models of tick and tsetse fly population dynamics and movement and these will be integrated with the GIS system over the next year. We hope also to develop models for the Crop Pests Research Programme (CPRP) which will assist in the prediction of pest outbreaks and these, too, will depend heavily on remote sensing data and the GIS system.

9.5.3 Standard indices

This activity is aimed at standardizing measures of resistance in crops and animals to pests and vectors, respectively. During the year an index was developed for the Livestock Ticks Research Programme (LTRP), which could be used to determine, fairly accurately, the level of resistance of cattle to *Rhipicephalus appendiculatus* when challenged with the Rusinga strain of the tick. The index is dependent on the number of engorged nymphs and females.

Further testing using 3 other strains including the Muguga (laboratory) strain of *R. appendiculatus* did not alter the relative rankings or indices of the Zebu cattle used in the study.

In the coming year, different breeds of cattle will be used to ascertain if the same functional form of the index expression can be applied. A similar study will be extended to cover the CPRP.

SOCIAL SCIENCE INTERFACE RESEARCH UNIT

10
Social Science Interface Research Unit

In 1988, the ICIPE established the Social Science Interface Research Unit (SSIRU) and incorporated socio-economic considerations in its core research activities. During the year the SSIRU consolidated its methodology and field research activities and strengthened its interaction with the biological science research programmes. It also developed a coherent definition of the interface between social and biological sciences towards a new science of understanding and achieving effective application of integrated pest management (IPM) packages suitable for application in resource-poor farm conditions.

The ICIPE's mandate is to develop IPM strategies which are technically effective, environmentally safe and sustainable, economically viable and socially acceptable to the resource-poor farmer (RPF) in the tropics. This provides a framework for both the rationale and objectives of social science interface research. The notion of economic viability entails deploying resources to more efficient use and ensuring that IPM strategies are economically affordable by RPFs. Social acceptability underscores the idea of compatibility between IPM and the RPF's total production systems, goals, priorities, and resource-base and indigenous knowledge-base. Issues surrounding farmers capacity to choose and adopt new and improved technologies and practices constitute the substance of SSIRU's work.

In pursuit of these objectives, the SSIRU adopts an interdisciplinary approach through which social and biological scientists work together and in collaboration with national agricultural systems and farmers. Farmers influence the form, content and direction of IPM in two ways. First, their indigenous knowledge-base forms the basis of IPM. Second, they are encouraged to participate actively and effectively in research, particularly in on-farm trials.

SSIRU has been working in collaboration with national governments and international organizations such as the Rockefeller Foundation, United Nations International Children's Emergency Fund (UNICEF), United Nations Economic Commission for Africa (ECA), the International Food Policy Research Institute and universities and research institutes worldwide. SSIRU works in collaboration with national agricultural systems through the African Regional Pest Management Research and Development Network for Integrated Control of Crop and Livestock Pests (PESTNET).

The SSIRU has continued to work jointly with the Crop Pests Research Programme and the Livestock Ticks Research Programme in the areas reported in 1987. The work has focussed attention on agricultural and livestock management, usages and agricultural practices of RPFs with the object of effectively evaluating their response to the ICIPE IPM packages and the ICIPE's responsibilities to the farming environment and their needs.

The emerging SSIRU vision and goal is to create a supportive environment between the technology-generating and the technology-user communities which will lead to a new science of the interface between biological and social sciences and will provide a realistic agenda for technological change and development.

The following reports provide summaries of how these objectives and orientations were pursued and developed during the year under review.

10.1 LIVESTOCK TICKS INTERFACE RESEARCH

J. W. Ssennyonga

Results from a 1986–87 study of tick populations on Rusinga Island showed a marked seasonal variation in adult ticks on cattle *(ICIPE 1987 Annual Report)*. After August, the tick population gradually builds up and peaks in December–February; thereafter it begins to decline appreciably and reaches its lowest level in July (see Chapter 2, this report). It has also been established that the variation is unrelated to climatological factors such as rainfall, relative humidity or temperature. It is now believed that the key causal factor may be host behaviour which in turn may be influenced by farm management methods in general, and animal husbandry practices in particular. Studies of herd management and land tenure and use were therefore undertaken in 1988 to provide the empirical socio-economic data with which to investigate the situation described above.

10.1.1 Herd management

A sample of 171 homesteads was selected for a study of herd management, especially grazing regimes, the time when the animals are taken out for grazing, the mode of grazing (e.g. tethering) and the regime for feeding calves. Data analysis is underway, but the first impression is that the duration of the animals' feeding time is greatly reduced for a variety of reasons. Farmers wait until the heavy dew dries up, for it is believed that it causes diarrhoea. Conflicts arising out of the farmers' commitment to several economic activities become more pronounced when there is a heavy demand for labour for cultivation. Men who fish at night wake up late in many cases and, as a result, animals can only be taken out after lower primary school children return from school between 1230 and 1300 hours.

10.1.2 Land tenure and use

There is a single cropping season in March when land is used on the basis of individual ownership. Even though 45% of available land is uncultivated, it is difficult to put it to optimal grazing use, for it does not form contiguous units. Because of the prevailing system of scattered holdings each homestead owns, on average, 4.7 plots of land — 38% along the lake shore, 19% along the main road and 43% in the uplands. Furthermore, 42% of the cropped area is made up of partially cultivated plots. The reasons for leaving a large proportion of the land uncultivated include lack of labour (18%) and lack of finance (27%) to hire animal draught power on which two-thirds of the farmers depend for field preparation. A further 17% of fields are deliberately left fallow to regain fertility.

These findings will not only shed light on the variation between farms in the tick loads observed on their livestock; they also reveal important factors which will have to be considered when developing effective methods of controlling ticks and tick-borne diseases.

10.2 SSIRU DATA MANAGEMENT AND ANALYSIS

J. Pittchar

The following tasks were undertaken, within the broader objectives of the SSIRU, in order to gain the necessary understanding of farmers' behaviour, and their capacity as well as opportunities for adopting innovations in pest management: (a) Design of data collection devices (b) Supervision of field staff (c) Analysis of data drawn from the farming community participating in the under-mentioned projects.

10.2.1 The ICIPE/Kenya Government/ECA Project

The essence of SSIRU research work in this project at Oyugis and Kendu Bay, funded by the United Nations Economic Commission for Africa (ECA), has been to continue monitoring changes in the way participating farmers evaluate and adopt integrated pest management (IPM) strategies. Farmers' operations have been monitored in terms of growing resistant cultivars, intercropping resistant maize and sorghum varieties with cowpea, post-harvest disposal of crop residues, and their knowledge base and responses to the proposed use of biological control agents as a component of IPM. Specific sections of these data have been arranged to evaluate the cost-effectiveness of the IPM package.

Two data collection devices have been designed in collaboration with the project scientists to provide information, using indices, to assess the uptake of technology among non-participating farmers. The first device is similar to that used to monitor participating farmers and forms a basis for comparing the performance of the two groups. This has been administered to a sample of 20 farmers drawn from a sampling frame of farmers interviewed during the pre-implementation phase of the project. The second is a questionnaire which seeks to illuminate the process of technology uptake by the non-participating farmers, and its rate of progress.

The evaluation of results from the project sites is in progress and will be reported during 1989.

10.3 THE ICIPE/ROCKEFELLER PROJECT ON FOOD SECURITY AND PRODUCTION CONSTRAINTS

A. Pala-Okeyo and E. O. Omolo

Following the scientist-managed experiments of 1986–87, those aspects of integrated pest management (IPM) have been monitored during the long rains and short rains of 1988, under the farmers' own management, which cover intercropping and plant resistance to pests. Farmers have continued to practise cotton-based intercropping, growing cotton as a cash crop with a wide range of other crops. Maize and sorghum are the main cereal staples; cowpea and green gram are the main legumes; and cassava and sweet potato are the main root crops.

Intercropping was the preferred choice, as a result of increased land fragmentation, and the need to vary crops by soil type. From their own experience, farmers intercropped to suppress weeds and insect pests, and to conserve land and labour. Planting the crops in mixtures at the same time smothered weeds to a significant extent (Table 10.1) and also gave the farmers time to destroy weeds before too much damage was done to crops. Shortage of labour compelled the farmers to stagger their planting and weeding. Improved crop varieties showed no better resistance than the land races to such serious weeds as striga and couch grass. Intercropping itself was found to minimize the amount and area covered by weeds, thereby cutting down the labour required for weeding. Similarly, the impact of stem-borers and boll-worms was reduced in maize and cotton, respectively, under intercropping (Table 10.2). The ICIPE's intercropping strategy seems to be useful to these farmers. Maize, sorghum and cowpea play a strong role in the local economy and sizes of land holdings are such that intercropping has many advantages.

Table 10.1 Percentage of area covered by weeds under different cropping patterns: Seme site

Cropping pattern	Days after germination		Mean
	28	56	
Maize	17.5	25.0	21.3[b*]
Maize/cotton	13.0	6.0	9.5[a]
Maize/beans/cotton	4.0	8.0	6.0[a]
Mean	11.5	13.0	12.3

* Means followed by the same letter do not differ significantly ($P > 0.05$).

Table 10.2 Incidence and damage by insect pests on maize and cotton under different cropping patterns: Seme site

Cropping pattern	Infestation, stem-borers/ plant (maize)	% plants damaged (maize)	% squares/bolls damaged by boll-worms (cotton)
Maize	0.10[b*]	17.5[b]	
Beans			
Cotton			20.3[b]
Cotton/maize	0.05[a]	12.5[a]	
Cotton/beans			17.0[b]
Cotton/maize/ beans	0.07[a]	10.6[a]	10.6[a]
Mean	0.07	13.5	16.0

* Within-column means followed by the same letter do not differ significantly ($P > 0.05$).

A further investigation is being carried out to assess the impact of legal ownership of land on women farmers' farming practices. Assessment focusses on how the land was acquired, the nature of ownership, how the land is used, how these women differ socio-economically from other women, and whether there are any perceived or real advantages of owning land.

10.4 ICIPE/UNICEF TIME BUDGET SURVEY, RUSINGA ISLAND

A. Pala-Okeyo, J. Pittchar and A. Khan

A time allocation survey was conducted with funding by the United Nations International Children's Emergency Fund (UNICEF) to find out how the population used their time during the different agricultural seasons, and to what extent lack of time was a constraint on adequate child care and farming.

Results from the first phase of the survey revealed that women were the major subsistence food producers, as well as providers of food and other needs for the family. Their time was therefore divided into many tasks: working in the fields for food production, taking care of the children and trading in fish to supplement family income. Most males aged between 20–40 years were away working, leaving women with the responsibility for most of the agricultural work; shortage of labour was a common problem. During the periods of land preparation, weeding and harvesting, most women stopped their fish trading in order to concentrate on the work on their farms.

The second phase of the survey generated data on the use of time over the 12-hour day, and also during the night, by members of several households. The data are being analysed and will be correlated with results from the first phase of the project.

10.5 THE ICIPE/KENYA GOVERNMENT/ECA FUNDED PROJECT

A. Pala-Okeyo and E.O. Omolo

Work on this project with the United Nations Economic Commission for Africa (ECA) to reduce food losses through insect pest management and the use of small-scale and low-cost farm equipment continued at Oyugis and Kendu Bay and made satisfactory progress. The SSIRU's work with the above project showed that the use of ox ploughs improved the timing of land preparation by participating farmers. This made it possible for planting to be done on schedule using the ICIPE calendar. There was improvement over 1987 in adopting line planting, thinning and plant population per hole and per unit area.

The germination rate of the different cultivars was satisfactory. However, V 37 (maize) did not do as well as expected on some farms. Due to timely pre-planting and weeding operations, most farmers had vigorous crop growth which enabled early harvesting. On most farms ICIPE's varieties performed better than the local varieties. KRN 1 was as good as the hybrids while V 37 had the advantage of maturing faster. There was a lot of rain and a few farms were water-logged; cowpea was much more affected than maize and sorghum. In intercrops, cowpea under sorghum LRB 8 was much more affected

by rain since this is a tall variety. Hail damage was greater on monocropped cowpea.

Compared to the previous year, there has been an overall improvement in efficiency of performing farm operations. Practices and recommendations giving positive gains are easily assimilated, while those with no immediate benefits tend to be overlooked. For instance, intercropping was found to be efficient as a land-saving technology and as security against adverse weather where early maturity presented a clear advantage.

Emphasis is being placed on the use of local resources such as organic manure available on the farms. Labour input for farming operations is drawn from the farm family, and hiring is done only during peak demand periods where necessary.

Collaboration has been initiated with the On-Farm Grain Storage Project sponsored by the US Agency for International Development. The object is to provide information on the post-harvest situation which may be of help to farmers participating in the ECA project. A training course will be organized in collaboration with the On-Farm Grain Storage Project on grain storage for ECA project farmers.

ADMINISTRATION AND INFORMATION DIVISION

11

Administration and Information Division

One of the highlights of the year in terms of human resources management was the revision of staff emoluments which had been authorized by the Governing Council during its 1987 meetings. The implementation of the new and restructured salary scales was to be in two stages: 50% with effect from July 1988 and the remaining 50% with effect from July 1989. Although the revision did not cover allowances and other benefits it went some way towards compensating the staff for the sharp rise in the cost of living since the last salary revision exercise in 1985.

Senior staff changes during the year included the appointment of Dr. Paul B. Capstick as Deputy Director, taking over from Professor Dean L. Haynes who had returned to Michigan State University, U.S.A., on completion of his two-year contract. Professor O.O. Dipeolu joined the Centre as Principal Research Scientist and Programme Leader, Livestock Ticks Research Programme, a post that was previously held by Dr. Capstick.

The launching of an ICIPE Alumni Association during the 18th Annual Research Conference was another notable highlight, as it will open an important new window onto the world for the ICIPE.

11.1 CAPITAL DEVELOPMENT

The year saw the completion of the Duduville Capital Development Programme Phase II Contract I, and the relocation of the headquarters from the Chiromo Campus to the new premises. This was the climax of years of concerted efforts to provide the Centre with physical facilities that match its status as a mature international research centre. The transfer of the laboratories and offices was completed in October 1988, when the new world headquarters became fully operational.

Although Contract I did not include administrative offices the management decided that everyone, except for the staff of the ICIPE Science Press, should move to Duduville. This meant that the office of the Director, the Finance Division, the Administration and Information Division, and the Institutional Building and Interactive Research Unit were to be temporarily accommodated within the Laboratory Complex, pending the completion of the Administration Block and, eventually, the construction of an IBIRU-cum-Training Building.

The new premises have provided ICIPE scientists with excellent custom-designed working space, which they have longed for ever since the early days of the Centre. In due course this is bound to lead to greater scientific productivity and higher morale among the staff.

Contract II, for the Administration Block, was awarded to SIETCO, a Chinese construction company, on a competitive basis in accordance with the World Bank procedures, and the foundation stone was laid by the Chinese Ambassador to Kenya, H.E. Professor Xue Mouhoug, on 25th August 1988.

11.2 COMMUNICATION SERVICES

11.2.1 18th Annual Research Conference

The Annual Research Conference held at the new headquarters premises at Duduville was once again a resounding success and was attended by a broad cross-section of both the Kenyan and the international scientific community. This time the conference focussed on the Livestock Ticks Research Programme, the Plant Resistance to Insect Pests section of the Crop Pests Research Programme, the Cell Biology Research Unit and the Sensory Physiology Research Unit. In addition there were short presentations on the Social Science Interface Research Unit, the Biostatistics and Computer Service Unit, and the African Regional Pest Management Research and Development Network (PESTNET).

The special guest lecture was delivered by Professor Gian Scarascia Mugnozza of the University of Tuscia, Italy, on the theme: "Genetic Resources and Modern

Agriculture", the text of which will be published by the ICIPE Science Press in the course of next year.

An ICIPE Alumni Association was launched prior to the opening of the conference and was enthusiastically received by those present.

The 1988 ICIPE Medal for Innovative Research was awarded jointly to Dr. L.H. Otieno of the Tsetse Research Programme for his studies on rearing the tsetse *Glossina pallidipes,* and Dr. Wilber Lwande of the Chemistry and Biochemistry Research Unit for his work on airborne volatile compounds of sorghum and cowpeas.

11.2.2 International Study Workshops, Conferences and Seminars

Owing to other commitments the Centre hosted fewer meetings under this category than usual during 1988. However, a Fungicide Resistance Workshop held at Duduville 6–16 February, a Regional Seminar on the Role of Chemistry in Improving Food Supplies in Africa (co-sponsored by the ICIPE, the African Academy of Sciences, CHEMRAWN Committee, the International Union of Pure and Applied Chemistry and the American Chemical Society) and the Consultation on the Management of Science for Development in Africa (sponsored by the Canadian International Development Agency and hosted jointly by the ICIPE and the African Academy of Sciences) are to be noted.

11.2.3 Visitors

Once again the ICIPE received a very large number of visitors from the international community as well as the host country, particularly donor representatives, government officials, and collaborating scientists. Among the notable ones were the following:

- Dr. Callisto Madavo, Director of Programmes for Eastern Africa, the World Bank, Washington, D.C.
- H.E. Mr. E. O. Z. Chipamunga, Zimbabwe's High Commissioner to Kenya.
- H.E. Mr. A. M. Chabane, High Commissioner of Lesotho to Kenya.
- Dr. Robert W. Herdt, Director for Agriculture, the Rockefeller Foundation, New York, U.S.A.
- Dr. Jorgen Lissner, Regional Project Officer, United Nations Development Programme, New York, U.S.A.
- Professor Mahmud Musa Mahmud, Vice Chancellor, University of Juba, Sudan.
- Mr. Hans Wyss, Director of African Projects, The World Bank, Washington, D.C., U.S.A.
- Professor Stuart Gage, Professor of Entomology, Michigan State University.
- Dr. Ismail Mahmoud El Ramly, Senior Water Resources Specialist, Desert Research Institute, Egypt.
- Dr. O. Bruinsma and Mr. J.M. Krosskhell, the Netherlands Universities Foundation for International Cooperation (NUFFIC).
- Mr. E. Rodriguez and Mr. M.A. Caceres, Embassy of Mexico, Kenya.
- Mr. Stewart Marples, Senior Livestock Specialist, the World Bank, Washington, D.C.
- Mr. James Adams and Mr. Peter Eigen, the World Bank, Eastern Africa Region, Nairobi.
- Hon. Maina Wanjigi, Minister for Livestock Development, Kenya.
- Professor Walter A. Rosenblith, Professor Emeritus, Massachusetts Institute of Technology (MIT).
- Professor Lars Skattebol, Department of Chemistry, University of Oslo, Norway.
- Dr. D. A. Lindqvist, International Atomic Energy Agency (IAEA), Vienna, Austria.
- Mr. Peter C. Goldmark Jr., President, the Rockefeller Foundation, New York, U.S.A.
- Hon. Rodrigo Zeledon, Minister for Science and Technology, Costa Rica.
- H.E. Mr. Franz von Mentzingen, Ambassador of the Federal Republic of Germany to Kenya.
- Hon. Mr. G.M. Ndotto, Minister for Research, Science and Technology, Kenya.
- Mr. H. Jeffrey Leonard, World Resources Institute, Washington, D.C., U.S.A.
- Mr. I. Nagame, First Secretary, Embassy of Japan, Kenya.
- Dr. S. Carr, Principal Agriculturalist, the World Bank, Washington, D.C., U.S.A.
- Ms. Judy Cheng-Hopkins, Deputy Resident Representative, UNDP, Kenya.
- Dr. Leif Christofferson, Chief of the Environmental Programme, the World Bank, Washington, D.C.
- Dr. Abdoulaye Niang', Project Officer, UN Economic Commission for Africa, Addis Ababa, Ethiopia.

11.3 PUBLISHING AND DOCUMENTATION

11.3.1 ICIPE Science Press

A number of new publications were produced by the Press, including the *1987 Annual Report* and some occasional monographs, conference proceedings, brochures, leaflets, posters, etc.

As usual the Graphics and Typesetting Section was kept busy producing a constant flow of scientific illustrations, slides, posters and other graphic materials required by the scientists for their presentations.

During the year the Press commissioned a small offset printing machine and a guillotine, thus giving itself the capacity to print small publications and stationery in-house. The facilities will be expanded as funds become available.

Staffing constraints, particularly in the editorial section, led to delays in processing scientific papers for submission to journals and in the production of some regular publications, including the quarterly newsletter, *Dudu*. Steps have now been taken to increase the editorial capacity of the Press with new appointments during the 1989/90 budget year.

11.3.1 Scientific Editorial Unit

The ICIPE Science Press has continued to produce, publish and distribute the bimonthly journal *Insect Science and Its Application.* This year, Volume 9 contained 768 pages. A special issue of the journal (No.6) based on the Proceedings of the ICIPE/ECA Regional Study Workshop on "On-farm and Post-Harvest Losses of Cereal Crops in Africa due to Pests and Diseases" was published.

The Unit has assisted in promotion campaigns by sending out brochures on the journal and the book series, "Current Themes in Tropical Science" to potential subscribers by direct mail.

Plans are at an advanced stage for the Unit to set the journal in Kenya with a desktop unit, preferably a Macintosh. To achieve this goal, the Scientific Editor of the journal was attached to Balaban Publishers in Israel for a one-month hands-on-training programme in Desktop Publishing and Publications Management. During this training Volume 10 No.1 was set on the Macintosh.

Volumes 1–3 of the book series "Current Themes in Tropical Science" are still on sale at ICIPE, and Volume 4 is in preparation.

11.3.2 ICIPE Library and Documentation Service

The central concerns of the Library and Documentation Service are to facilitate ICIPE's research, training and institutional building activities through the collection, organisation, storage and dissemination of appropriate information, and to collaborate with other institutions for optimum use of research results. Two major factors affected operations during 1988. The first was the relocation of the main library from Chiromo to the new ICIPE Headquarters in Duduville, and the second was initiatives taken towards the development of a documentation facility as part of the implementation of PESTNET (see Section 8.1).

The Relocation to Duduville. At the end of August as the scientific programmes and units transferred to Duduville, the main library had also to relocate from ICIPE House in Chiromo in order to continue serving the scientists effectively. Because new library accommodation was not yet available, the facility was moved temporarily into laboratory space.

Pest Management Documentation and Information System Service. PMDISS is a special collaborative project between the ICIPE Library and Documentation Service and PESTNET. The main goal of PMDISS is to create an authoritative centre for information and documentation on pest and vector management, for all categories of potential users in research and development, management and field work in the tropical developing world, especially Africa.

Work was started during the year to develop an extensive collection of pest management information with initial emphasis on crop-borers. The information gathered was mainly documentary but the database is planned to include non-documentary data as well. In this regard two briefing sessions were given at separate PESTNET workshops that were held with the Kenya National Programme scientists, one in Nairobi and another at Mbita Point Field Station.

Acquisition of reading materials. Now that the library has moved to Duduville it is no longer close to a number of interlibrary partners, especially the University of Nairobi Biological Sciences Library which is now about 15 km distant. The ICIPE Library has therefore had to acquire a number of reference and other materials for which it had hitherto relied on friendly neighbours. The necessary scope of the library's acquisition was also extended by the needs of PMDISS.

The demands for acquisitions, from these and other regular ICIPE programme needs was, however, to be tempered by the availability of funds, currency exchange rate adjustments and price inflation in the book trade. In addition, the library had to take over the subscription to 10 journals previously donated by the former Netherlands Literature Programme (NLP). The Library was, however, fortunate to receive a book grant of Dfl 10,000 from Direct Aid to Educational Establishments in Developing Countries (DSO), the programme that succeeded NLP. Books ordered on this grant will arrive in 1989.

Acquisitions amounted to 404 books and about 500 reprints, including those by non-ICIPE staff collected for PMDISS. About 150 periodical titles were currently received, of which about 120 were subscriptions.

Archives. Although the move to Duduville rejoined the Library with the archives project which had been relocated in 1987, the pace of activity continued to be slow. In view of the limited human and other resources that were available, the project was given a lower priority in order to increase activity in other areas.

Services. The Library was compelled to close for about a month in order to effect the relocation. Otherwise the emphasis of its services continued to be put on current awareness. The *Library and Documentation Bulletin* was issued quarterly, and in-house selective dissemination of information (SDI) as well as the subscription to SDI from the Commonwealth Agricultural Bureaux International (CABI) database continued. In addition, 13 retrospective computer literature searches were done against specific requests, and 1,860 document supply requests were fulfilled.

Cooperation. Interlibrary partners continued to play a very important role in the delivery of documents to ICIPE users. After the move to Duduville there was a reduction in direct usage of other libraries by ICIPE scientists. This, however, increased the readers' dependence on the ICIPE Library to obtain documents for them and the percentage supplied by interlibrary partners through the Library rose to about 35%.

In other areas of cooperation the following may be highlighted:

- One student from the Kenya Polytechnic was attached to the library for 6 weeks.
- The ICIPE, through the agency of the Library, hosted the SATCRIS Travelling Workshop for Kenya on 14 November. (SATCRIS is the Semi-Arid Tropics Crops Information Service of ICRISAT — The International Crops Research Institute for the Semi-Arid Tropics.)

• The Library contributed its periodicals' data to the International Agricultural Research Centres' Union List Project based at ICRISAT. This project was agreed upon at the Information and Documentation Services Meeting of the Consultative Group for International Agricultural Research, held at Lima, Peru in 1987.

• The Senior Librarian continued to participate in meetings with interlibrary partners, especially those of the Nairobi Information Group which comprises information workers from Nairobi-based international organizations.

Her Excellency Ms. A. Raynell Andreychuk, Canada's High Commissioner to Kenya, arriving at Duduville on Sunday, 24th April 1988, to officially open the 18th Annual Research Conference. On hand to receive her are Professor H.C. Weltzien, Chairman of the ICIPE Governing Council, and Professor Thomas R. Odhiambo, the ICIPE Director

1988 Seminars Hosted by ICIPE

SPEAKER	TITLE
Dr. E. O. Osir, Chemistry and Biochemistry Research Unit, ICIPE, Nairobi	Methods for Determining Primary Structures of Carbohydrate Chains on Proteins
Dr. J. L. Peterson, Gorgas Memorial Laboratory, Panama, Panama	Identification by Enzyme Electrophoresis of Mesoamerican *Leishmania* spp. and their Phlebotomine Vectors
Professor K. Maramorosch, Department of Entomology, Cook College, New Brunswick, NJ, USA	Vectors, Plant Diseases and History
Dr. J. O. Midiwo, Department of Chemistry, University of Nairobi, Kenya	Phytochemistry of Polygonaceae and Myrsinaceae
Dr. P. A. O. Majiwa, International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya	The Potential Contribution of Recombinant DNA Technology to the Epidemiology of the Animal Trypanosomiases
Dr. J. A. Van Vegten, Former Professor of Medical and Veterinary Entomology, University of Garyounis, Benghazi, Libya	Human Parasitism
Dr. O. Zethner, Former FAO Expert, Rome, Italy	Integrated Pest Management Project for Basic Food Crops in the Sahel
Professor O. O. Dipeolu, School of Veterinary Medicine, Tuskegee University, Alabama, USA	Control of Ticks Through Genetic Methods: A New Approach
Mr. J. P. Kithinji, University of Leeds, Leeds , England	Chromatography and Extraction with Supercritical Fluids
Dr. M. A. Nyindo, Livestock Ticks Research Programme, ICIPE, Nairobi	Fighting Ticks: Man Versus Nature
Hon. Dr. R. Zeledon, Minister for Science and Technology, Costa Rica	Ecology of *Trypanosoma cruzi* in the Insect Vector
Professor P. Campbell, University College, London, England	1. Targeting of Proteins in Eukaryotic Cells 2. Recombinant DNA Techniques to Elucidate the Origin and Function of Homologous Proteins

Dr. D. Ben-Yakir Hebrew University, Jerusalem, Israel	Developing a Vaccine Against Haematophagous Arthropods
Mr. P. Rosales, Consultant, Centro Internacional de Mejoramiento de Maiz Y Trigo (CIMMYT) El Batan, Mexico	1. File Restructuring: Assessing Your Computer 2. Electronic Mail 3. Electronic Mail: Preparing Messages for Transmission
Professor M. P. Pener, Hebrew University, Jerusalem, Israel	The Moult-Inhibitory Effect of Azadirachtin in Insects
Dr. S. Mihok, Tsetse Research Programme, ICIPE, Nairobi	A New Immune Cell from the Vole *Microtus:* A Potential New Approach to Therapeutic Drug Development for Trypanosomiasis

1988 Conferences Attended by ICIPE Staff

18th International Congress of Entomology: Vancouver, Canada, July 3–9.
A. M. Alghali, G. C. Unnithan, Z. R. Khan, S. Nokoe and B. Williams

International Congress on Dryland Farming: Amarillo/Bushland, Texas, U.S.A., August 15–19.
A. M. Alghali

Workshop on Bacterial Control of Agricultural Insect Pests and the Vectors of Human Diseases: En Gedi, Israel, February 29–March 3.
M. Brownbridge

7th Annual Conference of the Association of African Insect Scientists: Dakar, Senegal, December 7–10.
S. K. Firempong

21st Annual Meeting and Conference Society for Invertebrate Pathology: University of California, San Diego, USA, August 14–18.
M. O. Odindo

2nd Kenya National Seminar on Agroforestry: ICRAF, Nairobi, Kenya, November 7–16.
E. O. Omolo, K. N. Saxena, K. V. Seshu Reddy, M. O. Odindo and G. C. Unnithan

The 16th International Congress of Genetics: Toronto, Canada, August 20–27.
R. S. Pathak

FAO/IAEA Workshop on Improvement of Grain Legume Production using Induced Mutations: IAEA, Vienna.
R. S. Pathak

2nd PESTNET Methodology Workshop: Mbita Point Field Station, May 23–26.
K. N. Saxena, K. V. Seshu Reddy, G. C. Unnithan and S. Nokoe

Some Biological Observations on the Banana Weevil in Western Kenya. Workshop: Bujumbura, Burundi, 1987 December 7–11.
K. V. Seshu Reddy

EARSAM 6th Regional Sorghum and Millet Workshop: Mogadishu, Somalia, July 20–27.
K. V. Seshu Reddy and K. N. Saxena

The 3rd Chemical Congress of North America: Toronto, Canada, June 5–11.
A. Hassanali

CHEMRAWN II Regional Seminar on "The Role of Chemistry in Improving Food Supplies in Africa": Nairobi, Kenya, July 17–21.
S. A. Essuman, A. Hassanali, W. Lwande, M. A. Okech and E.O. Osir.

International Congress of Biochemistry: Prague, Czechoslovakia, July 10–15.
E. O. Osir

"Estimation and Analysis of Insect Population": Laramie. Wyoming, USA, January 24–30.
S. Nokoe

Kenya Veterinary Association and the Nairobi Cluster: KETRI, Nairobi, Kenya.
S. Nokoe

Biomathematics Workshop, University of Ibadan, September 1988.
S. Nokoe

3rd International Conference on Science and Technology Management: Sao Paulo, Brazil, October 24–26.
Z.M. Nyiira

Consultative Meeting on Long Term Strategy for Locust Management: Nairobi, Kenya September 21–26.
Z. M. Nyiira

SACCAR Workshop on Strategies for Integrated Pest Management and Weed Control for Smallholder Farmers: Mbabane, Swaziland, August 1–4.
M.E. Smalley and Z.M. Nyiira

18th International Congress of Entomology: Vancouver, Canada, July 3–9.
J. B. Kaddu, M.J. Mutinga and R.M. Musyoki

7th Annual Joint Scientific Conference: Arusha, Tanzania, February 1988.
M. J. Mutinga

12th International Congress on Tropical Medicine and Malaria: Amsterdam, September 18–23.
M. J. Mutinga, C. M. Mutero and C. C. Kamau

Meeting of American Society of Parasitologists: Winston, Salem, USA, August 1988.
M. J. Mutinga

China-IRRI Collaborative Planning Workshop: Los Baños, Laguna, Philippines, August 23–30.
Z. R. Khan

International Rice Research Conference: IRRI, Los Baños, Laguna, Philippines, November 7–11.
Z. R. Khan

Botanical Pest Control in Rice-Based Cropping Systems: IRRI, Los Baños, Laguna, Philippines, December 12–16.
Z. R. Khan

National Seminar on Theileriosis, Samora Machel College of Veterinary Medicine: University of Zambia, Lusaka, Zambia, October 19–21.
O. O. Dipeolu

Symposium on Tick Biology, Integrated Control and Disease Transmission: College Station, Texas, USA, June 30–July 1.
R. M. Newson.

7th Joint Scientific Conference, Arusha, Tanzania, March 3–4.
L.H. Otieno

14th Executive Committee Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC): Nairobi, Kenya, March 9–10.
L. H. Otieno.

16th Institutional Tsetse Research Committee Meeting: Tanga, Tanzania, August 31.
L. H. Otieno

12th International Congress on Tropical Medicine and Malaria: Amsterdam, Holland, September 18–23.
L. H. Otieno

Fifth Session of the Commission on African Animal Trypanosomiasis: Accra, Ghana, November 10–11.
L. H. Otieno

6th Tanzania Veterinary Association Scientific and 20th Anniversary: Arusha, Tanzania, December 6–8.
L. H. Otieno

Workshop on Modelling Sleeping Sickness Epidemiology and Control: Antwerp, Belgium, January 25–29.
R. D. Dransfield

The Role of Women in the Development of Science and Technology in the Third World, October 3–7.
M. L. A. Owaga

18th International Congress of Entomology: Vancouver, Canada, July 3–9.
G. P. Kaaya

Inauguration of the IITA Biological Control Programme: Benin, Peoples Republic of Cotonou, Benin, December 5–9.
G. P. Kaaya

1988 Publications by ICIPE Staff

Agatsuma T. and Otieno L.H. Isoenzyme studies on two field populations of *Glossina pallidipes* Austen (Diptera, Glossinidae) in Kenya. *Insect Science and its Application* 9(4), 527–530.

Alghali A.M. Oviposition on sorghum by the stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). *Malaysian Journal of Plant Protection in the Tropics* 5(1), 45–50.

Alghali A.M. and Saxena K.N. Larval movement, feeding and development of *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) on two sorghum cultivars. *Insect Science and its Application* 9(1), 7–11.

Ampofo J.K.O. Some observations on *Chilo partellus* (Lepidoptera: Pyralidae) development biology under field and laboratory conditions. *Insect Science and its Application* 9(2), 271–274.

Ampofo J.K.O. Assessment of on-farm losses in maize production due to insect pests. *Insect Science and its Application* 9(6), 687–690.

Arshad M.A., Schnitzer M. and Preston C.M. Characterization of humic acids from termite mounds and surrounding soils, Kenya. *Geoderma* 42, 213–225.

Bartkowski J., Odindo M.O. and Otieno W.A. Some fungal pathogens of the cassava green spider mites *Mononychellus* spp. (Tetranychidae) in Kenya. *Insect Science and its Application* 9(4), 457–459.

Bentley M.D., Rajab M.S., Alford A.R., Mendel M.J. and Hassanali A. Structure-activity studies of modified citrus limonoids as antifeedants for Colorado potato beetle larvae, *Leptinotarsa decemlineata*. *Entomologia experimentalis et applicata* 49(3), 189–193.

Brydson R., Vvedensky D.D., Engel W., Sauer H., Williams B.G., Zeitler E. and Thomas J.M. Chemical information from electron energy loss near-edge structure. Core hole effects in the beryllium and boron K-edges in rhodizite. *Journal of Physical Chemistry* 92, 962–980.

Brydson R., Williams B.G., Engel W., Sauer H., Zeitler E. and Thomas J.M. Electron energy-loss spectroscopy and the crystal chemistry of rhodizite. II. Near-edge structures. *Journal of the Chemical Society, Faraday Transactions* 84, 631–646.

Caballero P., Shin D.H., Khan Z.R., Saxena R.C., Juliano B.O. and Zapata F.J. Use of tissue culture to evaluate rice resistance to lepidopterous pests. *International Rice Research Newsletter* 13(5), 14–15.

Chigusa Y. and Otieno L.H. Longevity and feeding behaviour of *Glossina morsitans morsitans* infected with *Trypanosoma brucei brucei*. *Japan Society of Sanitary Zoology* 39(1), 71–75.

Darlington J.P.E.C. Mutilation of the primary reproductives in termites of the genus *Macrotermes*. *Insect Science and its Application* 9(1), 81–83.

Darlington J.P.E.C. The structure of mature mounds of the termite *Macrotermes herus* in Kenya. *Insect Science and its Application* 9(3), 339–345.

Engel W., Sauer H., Brydson R., Williams B.G., Zeitler E. and Thomas J.M. Electron energy-loss spectroscopy and the crystal chemistry of rhodizite. I. Chemical analysis. *Journal of the Chemical Society, Faraday Transactions.* 84, 617–629.

Firempong S. Components of resistance to *Aphis craccivora* in some cowpea cultivars. *Entomologia experimentalis et applicata* 48(3), 241–246.

Gettinby G., Newson R.M., Calpin M.M. and Paton G. A simulation model for genetic resistance to acaricides in the African brown tick, *Rhipicephalus appendiculatus* (Acarina: Ixodidae). *Preventive Veterinary Medicine* 6(2), 183–197.

Griffiths D.C., Hassanali A., Mudd A., Merritt L.A., Pickett J.A., Shah S.J., Smart L.E., Wadhams L.J. and Woodcock C.M. Highly active antifeedants against coleopteran pests. Proceedings of a conference on crop protection against pests and diseases, Brighton, U.K., pp. 1041–1046.

Jura W.G.Z.O., Odhiambo T.R., Otieno L.H. and Tabu N.O. Gonadal lesions in virus infected male and female tsetse, *Glossina pallidipes* (Diptera: Glossinidae). *Journal of Invertebrate Pathology* 52(1), 1–8.

Jura W.G.Z.O., Otieno L.H. and Chimtawi M.B. Ultrastructural evidence for transovum transmission of the DNA virus of tsetse, *Glossina pallidipes* (Diptera: Glossinidae). *Current Microbiology* 18(1), 1–4.

Kaaya G.P. and Darji N. The humoral defense system in tsetse: Differences in response due to age, sex and antigen types. *Developmental and Comparative Immunology* 12(2), 255–268.

Kaddu J.B. and Musyoki R.M. Detection of *Leishmania donovani* in live experimental hamsters. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 82(2), 229–230.

Kaddu J.B. and Mutinga M.J. Some concepts of the interaction of *Trypanosoma (Nannomonas) congolense* and *Glossina pallidipes*. *Annals of Tropical Medicine and Parasitology* 82(3), 229–234.

Kaddu J.B., Mutinga M.J., Chimtawi B.M., Okot-Kotber B.M., Nyamori M.P.and Musyoki R. *Leishmania* in Kenyan phlebotomine sandflies –V. *Leishmania aethiopica* in the oesophagus of *Phlebotomus pedifer*. *Insect Science and its Application* 9(1), 117–121.

Kawooya J.K., Osir E.O. and Law J.H. Uptake of the major hemolymph lipoprotein and its transformation in the insect egg. *Journal of Biological Chemistry* 263(18), 8740–8747.

Khan Z.R. Artificial diet for rearing rice leaffolder. *International Rice Research Newsletter* 12(6), 30–31.

Khan Z.R., Barrion A.T., Litsinger J.A., Castilla N.P. and Joshi R.C. A bibliography of rice leaffolders (Lepidoptera: Pyralidae). *Insect Science and its Application* 9(2), 129–174.

Khan Z.R. and Saxena R.C. Responses of rice-infesting and grass-infesting populations of brown planthopper to rice plants and *Leersia* grass. *Journal of Economic Entomology* 81(4), 1081–1088.

Khan Z.R. and Saxena R.C. Probing behavior of three biotypes of *Nilaparvata lugens* on different resistant and susceptible rice varieties. *Journal of Economic Entomology* 81(5), 1338–1345.

King D., Gettinby G. and Newson R.M. A climate-based model for the development of the ixodid tick, *Rhipicephalus appendiculatus* in East Coast Fever zones. *Veterinary Parasitology* 29(1), 41–51.

Kokwaro E.D. and Murithi J.K. Ultrastructural characteristics of the ejaculatory duct of the male tsetse, *Glossina morsitans morsitans* Westwood. *Insect Science and its Application* 9(4), 475–482.

Kongoro J.A. and Odhiambo T.R. Functional ultrastructure of Malpighian tubules of tsetse, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Insect Science and its Application* 9(4), 563–571.

Kumar H. Effect of stalk damage on growth and yield of certain maize cultivars by the maize stalk borer *Chilo partellus*. *Entomologia experimentalis et applicata* 46(2), 149–153.

Kumar H. Ovipositional responses of *Chilo partellus* (Swinhoe) to certain locally grown maize cultivars in Kenya. *Insect Science and its Application* 9(3), 303–307.

Kyamanywa S. and Ampofo J.K.O. Effect of cowpea/maize mixed cropping on the incident light at the cowpea canopy and flower thrips (Thysanoptera: Thripidae) population density. *Crop Protection* 7(3), 186–189.

Latif A.A., Dhadialla T.S. and Newson R.M. Abnormal development of *Amblyomma variegatum* (Acarina: Ixodidae). *Journal of Medical Entomology* 25(2), 142–143.

Latif A.A., Newson R.M. and Dhadialla T.S. Feeding performance of *Amblyomma variegatum* (Acarina: Ixodidae) fed repeatedly on rabbits. *Experimental and Applied Acarology* 5(1), 88–92.

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Lyko G. and Mutinga M.J. Tumbu fly myiasis in a child. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 82(2), 345.

McDowell P.G., Lwande W., Deans S.G. and Waterman P.G. Volatile resin exudate from stem bark of *Commiphora rostrata:* Potential role in plant defence. *Phytochemistry* 27(8), 2519–2521.

Mutayoba B.M., Gombe S., Kaaya G.P. and Waindi E.N. Effect of chronic experimental *Trypanosoma congolense* infection on the ovaries, pituitary, thyroid and adrenal glands in female goats. *Research in Veterinary Science* 44(2), 140–146.

Mutayoba B.M., Gombe S., Kaaya G.P. and Waindi E.N. Trypanosome-induced ovarian dysfunction: Evidence of higher residual fertility in trypanotolerant small East African goats. *Acta Tropica* 45(3), 225–237.

Mutayoba B.M., Gombe S., Waindi, E.N. and Kaaya G. P. Depression of ovarian function and plasma progesterone and estradiol-17B in female goats chronically infected with *Trypanosoma congolense*. *Acta endocrinologica (Copenhagen)* 117, 477–484.

Mutero C.M., Mutinga M.J. and Omogo D. Development of *Leishmania* sp. in mosquitoes –I. Experimental infection of *Aedes aegypti* with *Leishmania donovani* promastigotes. *Insect Science and its Application* 9(4), 453–456.

Mutinga M.J. Leishmaniasis in Kenya. Proceedings of an international workshop on research and control strategies for leishmaniases, Ottawa, Canada, 1–4 June, 1987. International Development Research Centre, Ottawa, IDRC-MR 184, 24–37.

Mutinga M.J., Mutero C.M., Ngindu A. and Amimo F. The isolation of leishmanial parasites from domestic goats and wild hosts in Kenya and possible role of goats as reservoirs of leishmaniases. *Insect Science and its Application* 9(3), 347–349.

Njau B.C., Nyindo M. and Mutani A. Acquired resistance in rabbits to immature stages of *Rhipicephalus evertsi evertsi*. *Veterinary Research Communications* 12(4/5), 363–373.

Nokoe S. and Rogo L.M. A discriminant function for the short- and long-setaed forms of the *Mononychellus* (Acari: Tetranychidae) species complex. *Insect Science and its Application* 9(4), 429–432.

Nyeko J.H.P., Golder T.K. and Otieno L.H. Selection for drug resistance in *Trypanosoma congolense* during cyclic transmissions through *Glossina morsitans morsitans* and drug treated rabbits. *Acta Tropica* 45(1), 21–26.

Odhiambo T.R. New directions for agricultural research (increasing productivity). *African Farmer* 1, 28–33.

Odindo M.O. *Glossina pallidipes* virus: Its potential for use in biological control of tsetse. *Insect Science and its Application* 9(3), 399–403.

Okech M.A. and Kotengo M.O. Culture, isolation and microscopic studies on *Termitomyces* species from the fungus-comb of *Macrotermes michaelseni* (Isoptera: Macrotermitidae). *Mushroom Journal for the Tropics* 8(2), 53–57.

Okuda T. Effect of artificial wetting and rainfall on the larval diapause of a stem borer, *Busseola fusca,* in Western Kenya. *Entomologia experimentalis et applicata* 48(3), 263–267.

Opiyo E.A., Kinoti G.K. and Otieno L.H. Adaptation of the pig parasite *Trypanosoma simiae* to the laboratory rat. *Annals of Tropical Medicine and Parasitology* 82(4), 397–398.

Osir E.O. and Riddiford L.M. Nuclear binding sites for juvenile hormone and its analogues in the epidermis of the tobacco hornworm. *Journal of Biological Chemistry* 263, 13812–13818.

Otieno W.A., Mutinga M.J. and Laurence B.R. Response of an East African mosquito, *Culex pipiens fatigans* Wied to synthetic oviposition attractant pheromone in the laboratory at Mbita Point, Kenya, East Africa. *Insect Science and its Application* 9(2), 261–262.

Otieno W.A., Onyango T.O., Pile M.M., Laurence B.R., Dawson G.W., Wadhams L.J. and Pickett J.A. A field trial of the synthetic oviposition pheromone with *Culex quinquefasciatus* (Diptera: Culicidae) in Kenya. *Bulletin of Entomological Research* 78(3), 463–478.

Owaga M.L.A., Hassanali A. and McDowell P.G. The role of 4-cresol and 3-n-propylphenol in the attraction of tsetse flies to buffalo urine. *Insect Science and its Application* 9(1), 95–100.

Pathak R.S. Genetics of resistance to aphid in cowpea. *Crop Science* 28(3), 474–476.

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Rajab M.S., Bentley M.D., Hassanali A. and Chapya A. A new limonoid from *Turraea robusta*. *Phytochemistry* 27(7), 2353–2355.

Rogo L.M., Khamala C.P.M. and Mutinga M.J. Biochemical identification of *Phlebotomus (Larroussius) pedifer* and *Phlebotomus (Larroussius) elegonensis. Biochemical Systematics and Ecology* 16(7/8), 655–659.

Rogo L.M., Oloo G.W., Nokoe S. and Magalit H. A study of the *Mononychellus* (Acari: Tetranychidae) species complex from selected cassava growing areas of Africa using principal component analysis. *Insect Science and its Application* 9(5), 593–599.

Saini R.K. Odour attractants for tsetse flies, *Glossina* sp. In *Medical and Veterinary Dipterology* (Edited by Olejniček J.), pp.206–210. Proceedings of an international conference on medical and veterinary dipterology, České Budejovice, Czechoslovakia, 30 November–4 December 1987.

Seshu Reddy K.V. Assessment of on-farm yield losses in sorghum due to insect pests. *Insect Science and its Application* 9(6), 679–685.

Snow W.F., Tarimo S.A., Staak C. and Butler L. The feeding habits of the tsetse, *Glossina pallidipes* Austen on the South Kenya Coast, in the context of its host range and trypanosome infection rates in the other parts of East Africa. *Acta Tropica* 45(4), 339–349.

Tayo T.O. Flower and pod development in three cowpea *(Vigna unguiculata* [L.] Walp.) varieties with varying susceptibility to the pod-borer, *Maruca testulalis* (Geyer). *Insect Science and its Application* 9(2), 249–253.

Villanueva F.F.D. and Khan Z.R. Mode of feeding on selected wild rices and weight gain of first instar larvae of rice leaffolder (LF). *International Rice Research Newsletter* 13(6), 17.

ADDITIONAL 1987 PUBLICATIONS BY ICIPE STAFF

Darlington J.P.E.C. and Dransfield R.D. Size relationships in nest populations and mound parameters in the termite *Macrotermes michaelseni* in Kenya. *Insectes Sociaux* 34(3), 165–180.

Kawooya J.K., Osir E.O. and Law J.H. Vitellogenesis in *Manduca sexta. Memorias do Instituto Oswaldo Cruz* 82(3), 83–88.

Saxena R.C. and Khan Z.R. New bioactive products: Growth regulators, antifeedants, pheromones and other attractants. *Academia Nacionale de la Scienze, Rome.* Series V, 11(2), 303–317.

1988 Personnel*

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Mr. D. Chege, *cleaner/messenger*
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Mrs. Y. Obiero, *secretary*

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Dr. V. A O. Okoth, *postdoctoral research fellow*
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Mr. F. D. O. Odawa, *technician*
Mr. E. L. Kidiavai, *technician*
Mr. M. O. Arwa, *technical assistant*
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Dr. G. C. Unnithan, *senior research scientist*
Dr. B. Somrith, *visiting scientist*
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Mr. J. O. Ondijo, *field assistant*
Mr. I. O. Odhul, *field assistant*
Miss J. A. Oduol, *field assistant/typist*
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Mr. J. O. Awendo, *field assistant*
Mrs. T. A. Odero, *field assistant*
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Mr. J. M. Kagoiya, *principal technician*
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Dr. A. A. Latif, *research scientist*
Dr. A. O. J. Amoo, *research scientist*
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Mr. P. Muteria, *junior technician*
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Mr. L. O. Otieno, *driver*
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Mr. E. G. Kabiru, *farm foreman*
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Mr. S. O. Odero, *farm assistant*
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Mr. P. O. Ouma, *farm assistant*
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Mr. T. O. Akelo, *groundsman*
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Mr. V. O. Nyangute, *groundsman*
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Mr. S. M. Aritho, *accounts clerical assistant*
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Mrs. J. A. O. Musiga, *housekeeper*
Mr. A. Lweya, *senior cook*
Mr. G. Gichuru, *cook*
Mr. J. M. Mwakisha, *cook*
Mr. D. K. Yaem, *stores assistant*
Mr. P. A. Omollo, *barman/waiter*
Mr. L. M. Mulae, *room steward*
Mrs. P. A. Ochola, *room steward*
Mr. H. M. Kibisu, *senior launder*
Miss H. N. Githinji, *assistant secretary*
Mr. J. O. Mukhobi, *janitorial assistant*
Mrs. R. M. Wekesa, *telephonist/receptionist*
Mrs. M. A. Asetto, *telephonist/receptionist*
Mr. A. O. Were, *messenger/cleaner*
Mr. H. Wara, *room steward*
Mrs. T. A. Ogongo, *room steward*
Mr. P. Mungithya, *messenger/cleaner*
Mrs. J. A. Awich, *messenger/cleaner*
Mr. S. O. Araka, *driver*
Mr. M. O. Ombech, *driver*

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Mr. J. O. Koyaa, *assistant cook*
Mr. L. A. Nyolo, *kitchen assistant*
Mr. A. O. Nyarima, *kitchen assistant*
Mr. F. O. Orwa, *kitchen assistant*
Mr. E. J. Odero, *bar assistant*
Mr. C. O. Nyagaya, *room steward*
Mr. H. O. Omalo, *room steward*
Mr. F. N. Omutsembi, *assistant launder*
Miss M. A. Nalo, *laundry assistant*
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Mr. Y. M. Koko, *teacher*
Mr. D. P. Makachola, *teacher*
Mrs. C. O.M. Ndiege, *teacher*
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Mr. C. O. Nyanjom, *pharmaceutical technologist*
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Mr. A. O. Olwoko, *senior driver*
Mrs. L. A. Abuya, *janitorial assistant*

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Mrs. C. E. Okoth, *nurse*
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Miss J. W. Mwaniki, *secretary*

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