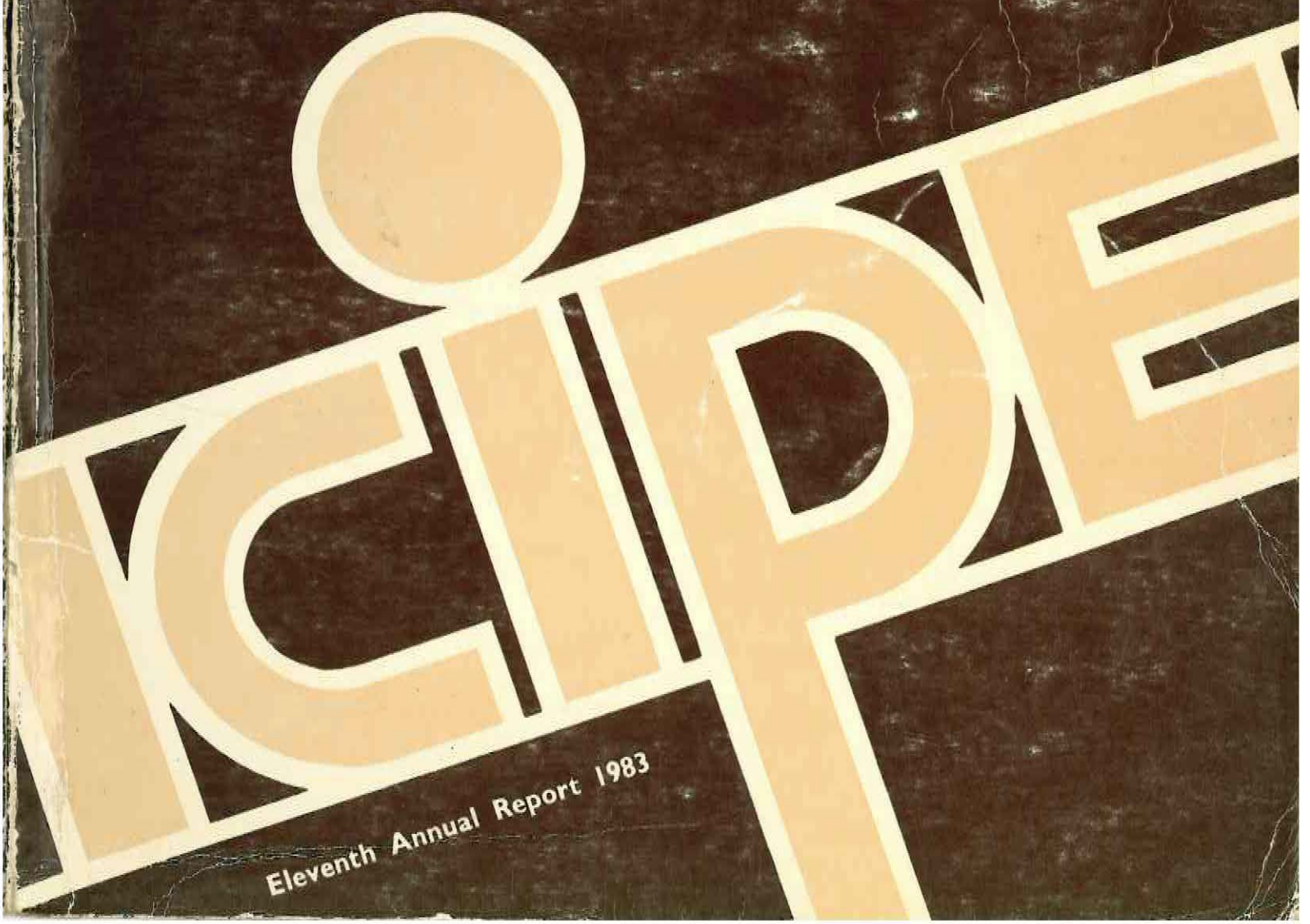
R. M. Newton

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

ICIPE



Eleventh Annual Report 1983

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

Eleventh Annual Report 1983

Nairobi, March 1984

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C = ICIPE Company nominee
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K = Kenya Government nominee
S = Sponsoring Group for the ICIPE (SGI) nominee
GB = Governing Board, PC = Programme committee
*** = Member of the Executive Committee**
**** = Member of the Programme Committee**
Each term lasts 3 years; I = first term, II = second term

Foreword

The ICIPE sharpens its focus and its management

The International Centre of Insect Physiology and Ecology (ICIPE), and its Governing Board, took a bold policy decision when they agreed that the Sponsoring Group for the ICIPE (SGI) should mount an external review of the Centre, by appointing an international task force of respected scientists and science administrators to review the progress the ICIPE has made so far within the context of its mandate, in the following words:

> "The overall objectives of the review would be, on behalf of the ICIPE's donors, to assess the content, quality and potential impact of the research programme of the Centre and to examine whether the work being funded is being done according to the policies of the Board and to the high levels of scientific excellence expected of such an institute".

The Review Team was instructed to pay particular attention to several matters, but especially to the mandate of the Centre (to assess the relevance of the present mandate to the future work of the (ICIPE); its management (especially the efficiency and quality of the management from both a scientific and financial point of view); the quality and relevance of the research (particularly the quality and performance of the research staff in relation to advancing knowledge and technology); and the potential impact and usefulness of the centre's activities (for instance, the size and quality of the training programmes including their comparative advantages and impact, its influence on research in national programmes, and the potential value of its new technology).

The external review was not especially new in respect of advanced research institutions elsewhere in the world, but it was innovative in the sense that the review dealt with both the scientific work of the centre as well as its management and governance. The external review was mounted in the first half of 1983, and the final report was considered by both the Governing Board of the ICIPE and the SGI, and finally accepted by the latter in October 1983.

The distinguished team of this First Triennial Review made many recommendations for sharpening the centre's mandate, its programme strategy and content, and its management style. These are now being implemented. It is most encouraging, however, that the overall and concluding recommendation was the following, "The team strongly recommends to SGI that stable funding for ICIPE be given a high priority." We sincerely hope this Triennial Review has given ICIPE's supporters confidence in the productivity and relevance of this enterprise.

We look forward to having this system of external reviews every three years to provide the ICIPE (and its donors) with periodical and substantive land-mark occasions for taking stock of the ICIPE's Research and Development programme activities, in close relation to its mandate to the nations of the tropical world.

THOMAS R. ODHIAMBO
Director ICIPE

Nairobi
3rd March 1984

CORE PROGRAMMES

Programme on Bases of Plant Resistance to Insect Attack

The Programme on Bases of Plant Resistance to Insect Attack aims to compare susceptibility or resistance levels of different cultivars of certain crop plants to their key insect pests; to elucidate the principles determining this susceptibility/resistance by studying the different biological responses of the pests to the plants and the role of plant characters and other factors determining these responses in the pests. The programme is also undertaking studies to manipulate the susceptibility/resistance-imparting principles for better management of the pests. For the last few years, the following crop plants and their respective insect pests have been under investigations.

Sorghum and maize — stem borers including **Chilo partellus, Busseola fusca, Sesamia calamistis**

Rice — mainly the brown plant hopper, **Nilaparvata lugens**

Cowpea — the pod borer, **Maruca testulalis,** *and aphids.*

The relative susceptibility or resistance levels of different cultivars of these crop plants to the target pests have been compared previously at ICRISAT, CIMMYT, IRRI, IITA and checked at ICIPE. However, the principles determining differences in the susceptibility/resistance of the crops, particularly sorghum, maize and cowpea, to the above pests have not been clearly understood. A detailed study of these principles has, therefore, been initiated this year at MPFS, with reference to **Chilo partellus** *vis-a-vis sorghum and maize and* **Maruca testulalis** *vis-a-vis cowpea. Significant information on the relationship between certain biological responses of the insects and the susceptibility or resistance of the host plants has been gained. Work on the rice pests, particularly on* **Nilaparvata lugens** *has continued at IRRI. Investigations on the mass cultivars of the target pests are continuing in the Mass Rearing Unit with a view of improving their biological preferences so as to make them suitable for other studies in this programme.*

Introduction

The susceptibility or resistance of different plants to an insect is determined by their suitability to sustain the population of the insect. The population of the insect on a plant is, in turn, determined by the following main responses of the insect in six main stages of its establishment: (1) Orientation, involving attraction or repulsion of the insect and resulting in its arrival on or avoidance of different plants; (2) Feeding, involving stimulation or inhibition of food intake by different plants; (3) Metabolism of ingested food, involving its utilization by the insect and determining its nutrition; (4) Development of the insect, if in the larval stage; (5) Egg production in the insect, if in the adult stage; and (6) Oviposition, which may be stimulated or inhibited by different plants. Of these responses, those of the first, second and sixth categories are behavioural which determine the initial selection or rejection of a plant by the

insect. The nutritional factors are involved subsequently and, together with the food intake, determine the insect's development and egg production.

The study of the above-mentioned aspects has been taken up this year with reference to *Chilo partellus* infesting sorghum and maize, and *Maruca testulalis* infesting cowpea, to compare these biological responses and examine the role of different plant characteristics and other factors in determining these responses.

Responses of *C. partellus* to sorghum plants in relation to their susceptibility or resistance

K.N. Saxena, K.V. Seshu Reddy, A.M. Alghali, H. Kumar

Two cultivars of sorghum, one being relatively resistant (IS-2146) and the other relatively susceptible (IS-18363), have been used to compare the six main types of responses of *Chilo partellus*. The observations made thus far are presented below.

Ovipositional responses

The selection of a suitable oviposition plant by a lepidopterous insect is done mainly by adult females. Hence, various aspects of *C. partellus* oviposition behaviour have been examined first.

INSECT AGE, MATING AND DIURNAL CYCLE

Aspects of ovipostion in relation to insect age, mating and diurnal cycle, were studied in order to ascertain the most appropriate stage of the insect at which to measure ovipositional responses to the test plants and other substrates. The percentage of males and females mating was maximum (83 ± s.e. 3%) on the first day after their emergence, declined to 7 ± 3% on the second day and to 3 ± 3% on the third day. The insects started mating mostly after midnight, the percentages of mating pairs increasing from 3 ± 3% between 01.00 and 03.00 hrs, to 23 ± 3% between 03.00 and 05.00 hrs and, finally, to a maximum of 67 ± 3% during 05.00 and 0.7.00 hrs. Oviposition by the females was highest 1 day after mating, the percentage of eggs laid being 58 ± 7% and it declined to 28 ± 5%, 4 ± 1%, 5 ± 3%, 2 ± 2% and 1 ± 1% on subsequent days after mating. Oviposition occurred between 04.00 hrs and 08.00 hrs, the percentages of eggs laid being 44 ± 10 during the 16.00 to 20.00 hrs period, 37 ± 11 during 20.00 to 24.00 hrs, 15 ± 15% during midnight to 04.00 hrs and 4 ± 3% during the 04.00 to 08.00 hrs period.

In order, therefore, to reduce the variability in egg-laying due to the internal state of insects themselves, their ovipositional responses to different plants and other substrates were compared using the females which were mated 1 day after emergence, testing their oviposition 1 day later.

DIFFERENT SURFACES

To study the role of plant characters and other factors determining oviposition by the insects, it is necessary to have a non-plant material which can incorporate the desired plant characters and serve as a suitable substrate for egg-laying. A number of different non-plant substrates were tested, using methods so designed that the insects would remain in constant contact with one or the other of the materials during the entire test period (24 h).

On giving the insect a choice between a glass surface and another surface, the percentage of eggs laid on the glass surface was about 3 times higher than that on wax paper, parafilm membrane or filter paper. But 99-100% eggs were laid on the glass surface when it was offered against muslin cloth, nylon sheet, nylon net or wirenet. However, when these surfaces were given alone, the percentage of females laying eggs on glass, wax paper and nylon sheet was 100 each, that on muslin was 90, showing that glass surface would be the best, wax paper and parafilm, the next in suitability as substrates for studying various factors governing *C. partellus* ovipositional responses.

PLANT AGE

Tests in the fields revealed that egg-laying by *C. partellus* on sorghum plants was much greater 4 to 10 weeks after germination than before or after that period. The relationship between the number of egg-batches laid and the plant age was given by the equation:
$Y = -64.6 + 52.3X - 306X^2$ ($r = 0.91$; $p < 0.05$).
In view of the above, 4- to 6-week-old plants were tested for different responses by *C. partellus*.

DIFFERENT SORGHUM CULTIVARS

Oviposition responses to different sorghum cultivars were tested in the screen house in a chamber (150 X 130 X 120 cm) made of nylon net (6 meshes/ cm) on which the moths do not lay eggs. The tests were conducted under 2-choice as well as no-choice situations. In the 2-choice tests, a row of six plants of the susceptible IS—18363 cultivar was presented inside the chamber along one end-wall and a similar row of the resistant cultivar IS-2146 along the opposite end-wall. In the no-choice tests, one or the other of the two cultivars was presented inside the chamber along one end-wall whereas the blank end-wall was provided with 4 wax papers (15 X 15 cm each) to serve as ovipositional substrates. Ovipositing females at the

stage mentioned earlier were released in the centre of the chamber and were given water on wet towel paper. After 2 nights, the numbers of eggs on different plants as well as on the wax papers at the blank end wall were recorded. On the basis of the total number of eggs laid, the percentages of those laid on the susceptible and resistant cultivars in 2-choice tests were calculated.

In 2-choice tests (*a* in table 1), the percentage of eggs laid on the susceptible cultivar was almost twice that of the resistant cultivar. In no-choice tests, of the total number of eggs laid within the chamber, the percentage of those laid on the plants was greater for the susceptible than for the resistant cultivars. This clearly suggests that *C. partellus* showed greater ovipositional response to the susceptible than to the resistant sorghum cultivar in 2-choice as well as no-choice situations.

The above-mentioned differences in the percentages of eggs laid on the resistant and susceptible cultivars could be due to the following responses: orientation of the insects, arriving on one in a greater percentage than on the other; actual egg-deposition after arrival or both. In order to ascertain this, following aspects were studied.

DISTANCE STIMULI FROM THE PLANTS

Resistant (IS-2146) or susceptible (IS-18363) plants were kept just outside one end wall of a nylon net test chamber and the other end wall was left blank. Each end wall had, along its inner surface, 4 wax paper sheets (15 x 15 cm) to provide suitable substrates for the insects to lay eggs. Ten females were released inside the chamber. They were able to visit the net wall facing the plants but could not come in contact with the latter. The percentage of eggs on the wax paper stuck on the net wall facing the resistant or susceptible plants was much greater than that on the opposite end wall. This showed that certain distance-operating signals from both resistant and susceptible sorghum cultivars could attract the insects to the corresponding wall where the eggs were laid on wax papers. When both the resistant and susceptible plants were presented together on the outside of the net wall of a specially designed chamber, the percentage of eggs laid on the wall facing the susceptible plants was much greater than that on the wall facing the resistant plants (*b* in table 1).

Evidently, the insects were more attracted by the susceptible plants than by the resistant plants. In other words, the susceptible cultivar had greater ovipositional attractancy than the resistant one.

CONTACT STIMULI FROM THE PLANTS

Tests for this were conducted in specially designed chambers in which the insects would remain in contact with one or the other of the test plant leaves or another test substrate throughout the test period (24 h) without the need for orientation. When the leaves of susceptible or resistant plants were presented as a choice against the glass surface which elicits a high oviposition response, the percentage of eggs laid on both the susceptible and resistant plant leaves was much greater than that on the glass. Thus, the leaves of both cultivars had some characteristics, which, on contact with the females, elicited egg laying.

Table 1. Orientation-cum-ovipositional responses of *Chilo partellus* to a susceptible and a resistant cultivar of sorghum

	Experiment	Test materials		% eggs laid (mean ± s.e.)	
		A			
a.	Responses to whole plants	IS—18363	IS—2146	68.0 ± 4.0	32.0 ± 4.0
		IS—18363	nil	66.0 ± 14.0	34.0 ± 14.0
		nil	IS—2146	48 ± 11.0	52.0 ± 11.0
b.	Responses to distance perceivable Characters of plants	IS—18363	IS—2146	67.0 ± 1.5	25.0 ± 2.1
		IS—18363	nil	60.0 ± 2.6	40.0 ± 2.6
		nil	IS—2146	48.0 ± 2.4	52.0 ± 2.4
c.	Responses to contact — perceivable Characters of plants	IS—18363	IS—2146	52.0 ± 10.0	52.0 ± 2.4
		IS—18363	glass	92.0 ± 8.0	8.0 ± 8.0
		glass	IS—2146	16.0 ± 5.0	84.0 ± 8.0

On presenting to the moths a leaf each of the resistant and susceptible cultivar side by side the percentages of eggs laid on both were more or less equal (*c* in table 1), showing that, on close contact with the leaves the insects do not demonstrate preference for any of the cultivars over the other. These observations suggest that differences, if any, between the two cultivars in their mechanical or chemical characters do not have any major significance for oviposition after the insects have come into contact with the plants. At the same time, the fact that the insects allowed contact with the leaves preferred the two cultivars over the otherwise suitable glass surface (*c* in table 1) suggests that both the cultivars have some common characteristics which elicit oviposition by *C. partellus* and there is no ovipositional deterrence in the resistant cultivar.

VOLATILES OF SORGHUM CULTIVARS

This aspect was studied in specially designed clear plastic chambers having a perforated wax-paper bottom and a wire-net removable top. The wax-paper bottom was supported on a similar chamber divided into two compartments of equal size. One compartment was filled with freshly excised leaves of one or the other sorghum cultivar and the other compartment was left vacant or filled with the leaves of the same or another cultivar. The volatiles of the leaves could pass through the perforations in the wax paper bottom into the upper compartment where a single ovipositing female was released. On the basis of tests with 30 females in 3 replicates of 10 each, the percentages of eggs laid on the wax paper above the two lower compartments were compared. The results (table 2) show that egg-laying on the wax paper above the susceptible plant leaves was much greater than above the resistant plants leaves, when both were offered together in equal amounts. When compared with an empty compartment the egg-laying on the wax paper above the susceptible leaf-bearing compartment was almost 5 times as much as the empty one.

When the compartment contained resistant plant leaves, the egg-laying above it was only about 1½ times that above the empty compartment. These observations suggest that the volatiles of both the susceptible and resistant cultivars of sorghum can elicit oviposition by *C. partellus* but those of the susceptible cultivars are much more effective than those of the resistant one. Since the insects could not come in contact with the leaves in these tests, their responses to the volatiles, perceivable at a distance, would involve positive orientation (attractant) to the zone of the test chamber bearing the volatile.

It was also observed (table 2) that the egg-laying was much greater in the zone bearing the leaves of the susceptible or resistant cultivar in a quantity 4 times (by weight) that of the same cultivar in the other zone. The observations suggest that the quantitative differences in the volatiles, caused by those in the production or release by the two cultivars, may be responsible for differences in the egg-laying by the insect.

Larval orientation

After the adult females lay eggs on one or the other cultivar of sorghum plants, the larvae emerging on the plants may move about and stay on them or move out of them according to their orientation responses. A comparison of these responses of the 1st-instar larvae released on the susceptible (IS-18363) and the resistant (IS-2146) cultivars was, therefore, made. The percentages of the larvae staying on or moving out of the plants on different days after their release was more or less identical for the susceptible and the resistant cultivars (table 3). Thus,

Table 2. Role of volatiles of a susceptible and a resistant cultivar of sorghum in determining orientation/oviposition responses of *Chilo partellus*.

Source of volatiles		% eggs laid (mean ± s.e.)	
A	B	A	B
IS—18363 (1 part)	IS—2146 (1 part)	79.0 ± 5.8	21.0 ± 5.8
IS—18363	nil	83.0 ± 5.8	17.0 ± 5.8
IS—18363 (1 part)	IS—18363 (4 parts)	15.0 ± 5.2	85.0 ± 5.2
IS—2146	nil	60.0 ± 3.9	40.0 ± 3.9
IS—2146 (1 part)	IS—2146 (4 parts)	20.0 ± 5.2	80.0 ± 5.2

the two cultivars did not differ in their attractancy, repellency or deterrence for the larvae.

Table 3. Orientation of *Chilo partellus* larvae on a susceptible and a resistant cultivar of sorghum plants.

Days after release of neonate 1st-instar larvae on plants	% larvae present on plants	
	IS—18363	IS—2146
2	63.0 ± 10.0	65.0 ± 12.0
7	68.5 ± 13.0	69.0 ± 9.0
14	55.1 ± 9.8	45.4 ± 9.8
21	60.0 ± 10.6	67.0 ± 10.9

Larval feeding responses

Techniques were developed to compare the feeding responses of *C. partellus* larvae to the susceptible and resistant cultivars in terms of area of leaves consumed. First instar larvae consumed almost twice as much leaf area of the susceptible cultivar as that of the resistant cultivar (table 4), showing that the susceptible cultivar is more suitable for larval feeding than the resistant one. The factors responsible for this are under study.

Table 4. Feeding response of *Chilo partellus* larvae to a susceptible and a resistant cultivar of sorghum plants

Cultivar	Area (mm^2) of leaves consumed by 10 1st-instar larvae in 48 h
IS—18363	89.0 ± 11.0
IS—2146	47.0 ± 5.0

Larval development

Studies were carried out to compare the suitability of the susceptible and the resistant cultivar for supporting larval development. Neonate 1st-instar larvae were released one each on a plant, and percentage survival at different stages of development was recorded 3 weeks later (table 5). Larval development on the susceptible cultivar was only a little better than on the resistant cultivar. Thus, although larvae have been shown to feed better on the susceptible cultivar than on the resistant one, the difference in larval development on the two cultivars was not that great. This is possibly due to their nutritional efficiency.

Our conclusion is that the difference in the populations of *C. partellus* establishing on the susceptible and resistant sorghum cultivars is caused mainly by those in the volatiles eliciting orientation/oviposition and in larval feeding responses.

Responses of *C. partellus* to maize plants in relation to their susceptibility or resistance

J.K.O. Ampofo, H. Kumar, K.N. Saxena

The response of *C. partellus* to two cultivars of maize, one relatively susceptible (Inbred A) and another relatively resistant (CMT 324), were examined. For some aspects, a tolerant cultivar (CMT 33) was also included in the present study. The aspects investigated and the procedures followed were basically the same as those described above for sorghum.

Ovipositional responses

Plant age and some plant characteristics.

Three maize cultivars CMT 324, CMT 33 and Inbred A were planted over from dates under choice and non-choice conditions in the field. The dates were scattered such that nearly all stages of the plants overlapped. Oviposition was monitored twice weekly (at 3-day intervals) and weekly totals were compared.

The pattern of oviposition was similar for all cultivars and all stages of the plant were oviposited on. A major peak was observed at the 3—4 weeks old stage with a minor one at the 7—8 weeks old stage irrespective of the planting date. These periods correspond with the early whorl and tasselling stages of the plants respectively.

The choice of oviposition sites was also similar among the cultivars. On young plants (3-4 weeks old) the lower leaves 2 to 4 attracted about 67% of the total oviposition at this stage. The lower leaf surface was preferred for ovipo-

Table 5. Development of *Chilo partellus* larvae on a susceptible and a resistant cultivar of sorghum

Cultivar	% larvae in different stages after 3 weeks					
	1st instar	2nd instar	3rd instar	4th instar	5th instar	Pupal
IS—18363	0	5.0	35.0	37.5	20.0	2.5
IS—2146	0	0	43.5	43.5	13.0	0

Table 6. Percent *Chilo partellus* oviposition distribution on three maize cultivars over different growth stages

Maize cultivar	Plant growth stage: Pre-flowering (2-5 WAP)		flowering (6–9 WAP)		Post-flowering (10–12 WAP)		Season's total	
	Egg masses	No. of eggs	Egg masses	No. of eggs	Egg masses	No. of eggs	Egg masses	No. of eggs
Inb A	39.62 ± 3.91	40.84 ± 6.72	45.46 ± 10.28	39.89 ± 3.58	39.55 ± 8.97	41.33 ± 8.31	41.14 ± 3.95	39.58 ± 3.78
CMT 33	32.09 ± 3.50	30.11 ± 4.06	22.79 ± 6.54	20.83 ± 3.16	24.43 ± 3.16	24.43 ± 3.16	28.13 ± 1.84	25.10 ± 4.20
CMT 324	28.30 ± 3.40	28.81 ± 3.86	32.11 ± 3.26	36.17 ± 3.52	32.13 ± 3.52	34.09 ± 8.12	31.23 ± 2.47	34.87 ± 2.70

WAP — Weeks after planting

sition over the upper surface. This surface had 66% of the egg masses in Inbred A, 62% in CMT 324 and 60% in CMT 33. As the plants grew older and bigger, there was a shift from the lower to the higher leaves. There was also an increase in the proportion of egg masses that were laid on the upper leaf surface (from 35% at 2 WAP to 40% at 9 WAP). At the upper leaf surface of older leaves, the concavity within the midrib was the preferred site.

Both the choice and non-choice tests showed Inbred A plants to be the most preferred for oviposition and CMT 33 the least (table 6). This pattern was observed in the number of egg masses as well as the number of eggs. Some basic differences were observed among the cultivars and among the different leaves of each plant. The most prominent among these were: (a) plant colour and (b) trichomes: types and density. All three cultivars developed a purple pigmentation on the plant exterior at the seedling to whorl stages. The intensity was deepest in CMT 324 and highest in Inbred A. Three types of trichomes were observed on leaf surfaces under the binocular microscope. Type 1 trichomes were small spines arranged along the veins and in the spaces between the veins. Type 2 trichomes were long filaments emerging from the veins. Type 3 trichomes were very fine hairy structures scattered randomly over the upper leaf surface. The lower leaf surfaces of all cultivars were generally free of trichomes. With the exception of type 1 trichomes all trichomes showed a tendency to shed with age and older leaves were usually free of types 2 and 3 trichomes.

CMT 33 and CMT 324 had a higher density of type 2 trichomes than Inbred A. However Inbred A had a higher density of type 1 trichomes. The density of type 2 trichomes decreased as leaves were counted downwards (table 7), possibly as a result of shedding. The relationship between the trichomes and oviposition by *C. partellus* is being investigated.

Ovipositional responses to different cultivars

On the basis of the information obtained from the above-mentioned experiments, more detailed studies were taken up under controlled experi-

Table 7. Trichome density on the upper surfaces of different leaves on three maize cultivars. (Plant age 3—4 weeks after planting)

Cultivar	Trichome	No of trichomes/cm^2 (x ± sd). Leaf number: 3	5	7	9
	Type				
Inbred A	1	7317 ± 981	10675 ± 320	8017 ± 1604	—
	2	0	0	65.9 ± 10.6	—
CMT 33	1	7274 ± 2534	8017 0	101.7 ± 12.1	107.6 ± 3.8
CMT 324	1	7717.3± ±300	7036 ± 1729	7865 ± 726	—
	2	0	10.2 ± 10.2	128.6 ± 10.7	—

mental conditions. On presenting the susceptible cultivar (Inbred A) at one end and the resistant one (CMT 324) at the other, inside a test chamber with wire-net walls (125 cm x 100 x 80 cm), the percentage of eggs laid on both was almost identical when the wind blew at right angles. But, when the wind blew from one cultivar to the other, the egg-laying on the cultivar in the up-wind direction, whether it was the susceptible or resistant one, was greater than on the one in the opposite direction. This way the ovipositional responses of *C. partellus* to these two cultivars could not be readily distinguished one from the other.

DISTANCE-PERCEIVABLE STIMULI

The plants of one or the other cultivar were presented outside the wire-net wall at one end of the test chamber (100 x 100 x 80 cm) and the other end was left blank. Wax papers were stuck inside each end wall to provide a suitable surface for laying eggs. The test chamber was so placed as to have the wind blow from the direction of the plants through the chamber towards its blank end. With plants of the susceptible cultivar, Inbred A, the percentage of eggs laid on the end wall facing these plants (67% ± 9.9) was greater than that on the blank end wall (33% ± 3.7). This suggests that certain distance perceivable signals from the susceptible plants were responsible for eliciting the orientation/oviposition response of the females. But the resistant cultivar, under similar conditions, was not as effective, the percentage of eggs laid on the end wall facing these plants being 58% ± 9 and that on the blank end wall being 42% ± 3.9.

Further investigations are in progress to explain how the egg-laying on the two cultivars is almost the same when whole plants are accessible to the insects, whereas the distance-perceivable signals of the susceptible plants are preferred over those of the resistant ones.

Larval movement

This experiment was designed to examine the effect of relative resistance of the oviposited (infested) and surrounding plants on larval movement between them. Three cultivars, Inbred A, CMT 33 and CMT 324, were used in this study. Each cultivar was planted in the central row of a plot of each of the other two cultivars and the position of each plant was mapped on square paper. This central row was infested by fixing an egg batch on leaf 2 or 3 from the ground. All other egg batches were removed every other day before and after the artificial infestation. Larval movement was monitored 6 days after egg hatch.

A high degree of dispersal from CMT 324 plants into the surrounding plants of the other cultivars was observed. There was greater migration from the other cultivars into Inbred A plants than from the latter to others. Generally, the extent of migration was influenced by the relative resistance of the two cultivars in a particular plot. In each plot there was a greater migration from the more resistant cultivar to the more susceptible one and less migration the other way round.

There was a negative correlation between larval establishment in the infested plants and larval loss ($r = 0.89$; $p<02$), and also between their establishment in infested plants and recovery from surrounding ones ($r = 0.77$; $p< 10$). There was a positive correlation between larval recovery from surrounding plants and loss ($r = 0.95$; $p<05$). The results demonstrate an apparent ability of young *C. partellus* larvae to migrate in search of more acceptable plants than those on which they find themselves. The increased larval loss was apparently the result of increased exposure during migration to predation and other mortality factors.

This phenomenon has potential in a pest management strategy using the susceptible cultivar (e.g. Inbred A) as a trap crop, both for moth oviposition and larval trapping. This would result in a lower larval establishment and damage in the main crop (the resistant cultivar).

Larval feeding

The feeding responses of the 1st instar larvae to the susceptible and resistant cultivars of maize were compared by the same method as described above for sorghum. The consumption of leaves of the susceptible cultivar (Inbred A) was much greater than that of the resistant cultivar (CMT 324)

Further studies on these and other responses of *C. partellus* to maize plants in relation to their characters are in progress.

Insect mass rearing

R. S. Ochieng

One of the important activities under the Programme on Bases of Plant Resistance to Insect Attack has been to develop and improve techniques, including diets, for mass rearing of target insects. During the current year, emphasis was laid on improving the techniques for mass-rearing the cowpea pod borer *M. testulalis* and developing an artificial diet for the stem-borer *E. saccharina* which infests mainly sugar cane and occasionally sorghum.

In order to improve the techniques for rearing *M. testulalis*, it was felt necessary to

study its mating behaviour so as to improve mating in the insects in our culture. Our observations, made at Ibadan (Nigeria), revealed that only 40% adults mated under natural conditions. Mating started 2 days after emergence, the period of mating being between 09.00 and 18.00 hrs. Diffused light was found to be necessary for mating activity. With reference to *E. saccharina*, an artificial diet (D 282) having the following composition was developed:

Ingredients	Quantities
Water	2140.0 ml
Brewer's yeast	64.0 g
Agar	25.0 g
Bean (Resecoco)	213.0 g
Wesson's salt mixture	10.0 g
Wheat germ	30.0 g
Van der Zant vitamin mixture	10.0 g
Streptomycin	150.0 mg
Casein	17.0 g
Methyl parahydroxybenzoate	4.0 g
Sorbic acid	2.0 g
Penicillin	150.0 mg
Formalin	4.0 ml
Ascorbic acid	6.4 g
Benlate	10.8 g

The period of larval development on this diet (33.3 days) was almost the same as that on the stem of sorghum plant (33.2 days). But the number of larval instars on the diet was less (5) than that on sorghum stem (7). The percentage of larvae completing development on the artificial diet was also quite high (80%). When the moths emerged, their fecundity and longevity on water and sucrose was studied.

The longevity of the females fed on sucrose was significantly greater (4.1) than that on water (3.1 days) but their fecundity on both these remained identical. In view of this, the adult moths may be kept on water rather than on sucrose so as to reduce the cost of rearing them on a large scale.

ICIPE/IRRI collaborative project on insect pests of rice

R. C. Saxena

The ICIPE collaborative research project with IRRI was initiated in August 1977 to study resistance/susceptibility of rice plant to the brown plant hopper *Nilaparvata lugens* (Stal). However, during 1983, investigations were directed not only at the brown plant hopper but also at a few other rice pests, e.g., *Sogatella furcifera*, *Nephotettix virescens*, *Spodoptera mauritia acronynctoides*. The aspects studied and the highlights of the observations made are summarized below.

Several techniques were developed for a more efficient evaluation of varietal resistance to rice plant hopper and leaf hoppers. A no-choice seedling bulk test was found to be more effective than the routinely used free-choice seedling bulk test for evaluating resistance of rice varieties to *S. furcifera*. Phloem or xylem feeding by leaf hopper and plant hoppers in rice plants was demonstrated by the difference in the colour of their excreta on seedlings treated with safranine — a lignin-specific dye that is selectively translocated through roots in xylem vessels. An electronic device was used for recording the feeding behaviour of plant hoppers and leaf hoppers for reliable and rapid evaluation of hopper resistance in rice germplasm.

A seedling dip bioassay technique was utilized to monitor the resistance factors in extracts of rice plants at the maximum tillering stage. Extraction and solubility fractionation of fresh or frozen rice plants and their steam distillates confirmed the presence of resistance factor(s) in all rice samples in the relatively non-polar fractions eluted by hexane and diethyl ether.

Investigations of cytological variations among *N. lugens* biotype 1, biotype 2, biotype 3, and the grass-specific biotypes were made and the intraspecific hybridization between rice-infesting and grass-specific biotypes was studied. Studies were also initiated on enzyme polymorphism to determine the amount of genetic variation in *N. lugens* biotypes. Cytology of *S. furcifera* was investigated.

Dry and wet season voyages were made this year along new sea routes to trap airborne insects and to monitor hopper migration in the Philippine archipelago. Wing morphism was studied in *S. furcifera*.

Further laboratory and field trials were made using neem seed oil, custard-apple oil, and their mixtures. Effects of neem oil and custard-apple oil mixtures on *N. virescens* and its transmission of the rice tungro virus were studied. Biological effects of neem oil were also tested against the rice armyworm and the ear-cutting caterpillar. Morphogenetic effects of a water-soluble, methanolic neem seed extract on rice leaf hoppers and plant hoppers were evaluated. Action of a purified fraction of the indigenous plant extract was tested against *N. virescens*. Inhibitory effects of selected insecticides on entomogenous fungi were evaluated.

Crop Borers Research Programme

The major goal of the Crop Borers Research Programme is to develop environmentally safe and economically feasible integrated pest management systems for control of major pests of maize, sorghum, rice and cowpea for subsistence farmers. Research on major crop borer species include the sorghum shootfly, Atherigona soccata; *maize and sorghum stem borers,* Chilo partellus, Busseola fusca, Eldana saccharina *and* Sesamia calamistis; *rice stem borer,* Maliarpha separatella *and cowpea pod borer,* Maruca testulalis. *All research is carried out at the Mbita Point Field Station (MPFS) and in the nearby farmers' fields. The programme collaborates with international agriculture research centres such as ICRISAT*, CIMMYT*, IITA*, IRRI* and WARDA*, and with national agriculture research programmes in Kenya. Some of the important research activities during the year include; Research on survival of shootfly in off-season; shootfly oviposition deterrent pheromone; incidence and period of peak activity of sorghum stem borers; identification of some parasitoids and predators of stem borers; screening of sorghum cultivars for resistance to stem borers; stem borer damage in maize; crop loss assessment in rice; genetics of sorghum resistance to stem borers and shootfly, and intercropping and pest management.*

Sorghum shootfly

G.C. Unnithan

Surveys conducted in farmers' fields in four different locations in Mbita Division revealed that during the off-season, a small but active population of the sorghum shootfly survives on tillers produced by cultivated sorghum stubbles after harvest, and on the wild sorghum, *Sorghum arundinaceum.* Up to 35% of the tillers on stubbles had shootfly eggs during certain periods of the off-season. As the sorghum stubbles and wild sorghum are responsible for the off-season survival and seasonal carry-over of the pest, removal and destruction of these, after sorghum harvest, could disrupt the carry-over of the pest and hence may prove to be an effective cultural method for its control.

Studies on the influence of temperature on reproduction in the sorghum shootfly indicated that mean and median fecundity were highest at 25°C. Although lower temperatures tend to produce heavier pupae and adults, there was no correlation between mean pupal weight and mean fecundity. However, at 20°C, there was positive correlation between pupal weight and number of eggs laid by the fly (r=0.57). Egg mortality increased with increased maternal age and the rate of increase in mortality was faster at 30°C. Temperatures between 25 and 30°C appear to be most suitable for shootfly reproduction.

On the shootfly oviposition deterrent pheromone attempts are being made to fractionate the active component(s) from the shootfly egg-wash. The active component(s) is extractable into ethyl acetate. It appears that the compound produced by the shootfly and deposited at the time of oviposition is a "pro-oviposition deterrent" which is slowly converted into the ultimate oviposition deterrent itself.

This seems to be an interesting slow release mechanism which ensures the persistence of the deterrent effect for several days after egg-laying.

Shootfly host selection or oviposition attractants are also being investigated. Initial experiments show that CSH-1 (a highly susceptible sorghum hybrid from India) seedlings possess an oviposition stimulant. Shootflies laid more eggs on maize seedlings (a non-host plant) when sprayed with aqueous extract or steam distillate of CSH-1 seedlings. In laboratory bioassays, maize seedlings sprayed with aqueous extract of CSH-1 seedlings received, on an average, 66% of the total number of eggs laid; the rest were laid on maize seedlings sprayed with distilled water (control). While maize seedlings sprayed with distilled water, volatile and non-volatile distillate of CSH-1 seedlings received, on an average, 21.5%, 23.1% and 55.3% of the total eggs laid by the shootflies respectively, it appears that the stimulant is probably not volatile.

Sorghum stem borers

K. V. Seshu Reddy

Studies on the incidence and period of peak activity of stem borers on a local cultivar 'Serena' indicated that infestation with *C. partellus* and *S. calamistis* at MPFS started at 4th and 5th week after planting, respectively, while *B. fusca* and *E. saccharina* started at 8th and 10th week, respectively. On the contrary *B. fusca* was the first to infest plants at 5th week after planting in the farmers' fields, while *C. partellus* and *S. calamistis* started infesting plants at 9th or 10th week after planting. Infestation by *E. saccharina* was recorded at MPFS but not on farmers' fields and this might be due to the presence of sugar cane at MPFS. Although the extent of infestation by the stem borer complex varied from season to season, in general, the level of attack increased with the age of the crop and at harvest the infestation reached a peak.

During the course of studies the following parasitoids and predators were recorded: *Trichogramma* sp., a very effective egg parasite of *C. partellus* (60% of eggs were found to be parasitized); *Apanteles sesamiae*, a gregarious larval endoparasite of *C. partellus*, *B. fusca*, *S. calamistis* and *E. saccharina; Hyperchalcidia soudanensis* and *Dentichasmias busseolae*, solitary pupal endoparasites of *C. partellus* and *B. fusca*. All these larval and pupal parasites are important and some of them can survive in a number of ecological conditions and therefore could be utilized as biological control agents for large-scale trials. Common predators recorded were earwigs, *Diaperasticus erythrocephala* on eggs and early larval instars of *C. partellus*, *B. fusca*, *S. calamistis* and *E. saccharina* (found on 55% of the sorghum plants); spiders on the early larval instars of *C. partellus*, *S. calamistis*, *B. fusca*, and *E. saccharina* (12% of the plants had spiders in the leaf whorls), black ants *(Camponotus rufoglaucus* on eggs and larvae of *C. partellus*, *B. fusca*, *E. saccharina*, *S. calamistis;* and ladybird beetles on eggs and larvae of *C. partellus*.

Based on the screening carried out under natural infestation so far, several sorghum lines such as, IS No. 1044, 1082, 1096, 1151, 2122, 2123, 2146, 2195, 2205, 2295, 3962, 4213, 4437, 4660, 4881, 5092, 5253, 5480, 5613, 10280, 10711, 18427, 18479, 18480, 18551, 18676, 18677, and S-92, S-98 and S-178, were identified as sources of resistance to both *C. partellus* and *B. fusca*. Also some multiple sources of sorghum resistance to shootfly and stem borers *(C. partellus* and *B. fusca)* were identified (IS No. 1082, 1096, 2122, 2123, 2146, 2195, 2205, 2295, 3962, 4660, 5092, 5480, 5613, 18323, 18326 and 18551).

Maize stem borers

Mary Botchey

Research on damage caused by *C. partellus* on maize plant has shown that newly hatched larvae of *C. partellus* remain at the site of hatching for sometime and then migrate upwards over the surface of the maize plant to the funnel. They then crawl down into the funnel and feed on the tender, rolled up young leaves. These larvae eat their way downwards towards the growing point. When the attack coincides with tasselling, the tassel is extensively damaged and it breaks off. When this occurs before the pollen grains mature, a reduction in the overall field pollen grain output results.

Rice stem borers

Dang Thanh Ho

Previous studies under caged conditions indicated that yield loss due to the white borer, *Maliarpha separatella* was mainly attributable to the incomplete development of grains. Visible damage, such as "dead hearts" and "white head", seldom occurred on rice infested with *M. separatella*. Grain yield loss could reach as high as 10%. Further studies on yield loss estimation for irrigated rice at Ahero Irrigation Scheme indicated that the proportion of tillers infested by *M. separatella* was significantly related to yield loss.

A survey conducted in two growing seasons in Ahero revealed that about 20% of *M. separatella* eggs were parasitized. Three percent

of pupae were parasitized by *Cassarina* sp. Larvae at older and prepupal stages were infested by *Entomophthora* sp. (5%) and *Bacillus* sp. (0.5%). The incidence of the pathogens was higher during the long rainy season than in the short rainy season.

The stalk-eyed fly *(Diopsis* sp.) population in Kenya is composed mainly of two species *Diopsis thoracica* and *D. apicalis* of which the former is predominant. Damage caused by *Diopsis* is mainly in the form of "deadheart" which occur at early vegetative to pre-flowering stages. Damage simulation trial to assess yield loss revealed that 10 to 50% of damaged tillers (DAT=35) recovered and produced grains. However, tillers that were damaged at maximum tillering stage (DAT=55) or later, might recover but did not produce normal panicles. Damage inflicted at pre-flowering stage (DAT-75) caused the highest yield loss as compared to damage at earlier or later stages.

Preliminary studies on intercrop of upland rice and finger millet, *Eleusine coracana* indicated that intercrop suffered higher infestation of stem borers than the mixed-row intercrop. *Striga* was more abundant in pure rice crop than in the intercrop. Weed growth was less in the intercrop than in the pure rice crop.

Products extracted from the kernel of the neem tree *(Azadirachta indica)* were used for control of stem borers in irrigated rice. Neem oil was sprayed at one-month intervals. Neem cake and urea-neem cake mixture were incorporated into soil during land preparation at DAT=50. Results showed that at the early vegetative phase, neem oil produced better protection than neem cake or urea-neem cake mixture. However, at the later growth stages, stem borer infestation was less on crop treated with neem cake or the mixture. Crops treated with neem cake or urea-neem cake mixture produced higher yield than untreated or neem-oil treated crops.

Cowpea pod borer

J. B. Suh

Studies on cowpea phenology and yield loss assessment were conducted on two cowpea cultivars, TVu 1509 and Ex-Luanda, in the screenhouse and the field, in long and short rainy seasons. The observations indicate that cowpea plants shed off about 50 to 80% of the buds and flowers. As a result, grain yield is derived from 20 to 30% of buds that survive as mature harvestable pods. Obviously these must be protected from insect pest damage if any grain harvest is expected. However, a programme to increase grain yield by reducing reproductive loss through the use of insecticide would be ill advised. Such loss is best stemmed through breeding and selection programmes coupled with adoption of agronomic practices geared toward minimizing physiological stress in the field crop.

Damage simulation studies on field cowpea showed that the crop compensated adequately for injury to foliage, flowers, and pods especially under favourable conditions. Moderate (50%) leaf loss in the screenhouse apparently stimulated pod production in both varieties. More severe damage, however, decreased pod production markedly in Ex-Luanda than in TVu 1509, particularly during pre-flowering and flowering stages. Shoot removal stimulated pod development in both Ex-Luanda and TVu 1509 in the long rainy season. On the contrary, leaf damage caused instant and often drastic shortfalls in available photosynthates which during critical growth stages (e.g. flowering) result in considerable yield decline. Protection at this stage is advantageous for grain yields.

Experiments were carried out to determine damage thresholds of *Maruca* on field cowpea using caged and non-caged crop. Plants (at the flowering stage) were artificially infested with *Maruca* eggs at densities of 5, 10 and 15 per plant. Grain yield and seed damage were compared with those of a field cowpea crop where *Maruca* infestation had developed naturally. Grain yields varied greatly among crops and between varieties and were often unrelated with infestation levels. Seed damage was higher on the naturally infested than artificially infested crop. In either case, damage by all pests including *Maruca* was invariably greater on malformed than wholesome pods. Also seed damage by *Maruca* alone was usually lower than that by other pests (lycaenids, agromyzids, bugs, weevils etc) combined.

Genetics of host plant resistance

R. S. Pathak

SORGHUM RESISTANCE TO STEM BORERS

Inheritance studies of sorghum resistance to stem borers (mainly *Chilo partellus)* were conducted in crosses involving six cultivars — Serena (a local tolerant cultivar), Swarna (susceptible) IS 18363 (susceptible), IS 18427 (resistant) IS 18489 (resistant) and IS 2146 (resistant). The results indicated that resistance to stem borers is polygenically inherited. F_1 hybrids did not differ significantly from mid-parental values suggesting intermediate inheritance. However, the resistance was partially dominant to susceptibility. Serena showed a high degree of tolerance to stem borers and produced highest grain yield plants. Combining ability analysis showed that resistance to primary damage (deadheart) was governed by both additive and non-additive genes while

secondary damage (stem tunnelling) was governed predominantly by additive genes.

It was noted that the inheritance patterns of primary and secondary damages were different. Tunnel lengths showed positive correlation with the number of larvae per plant and negative association with plant height, but had no correlation with grain yield per plant. The cultivars such as IS 2146, IS 18427 and IS 18489 could be used in a resistance breeding programme in transferring resistance to susceptible cultivars and also to improve the tolerance mechanism of Serena. The cultivar IS 2146 was found to be the best source of resistance to stem borers and has been crossed with a number of cultivars to study in detail the nature of gene action in F_1, F_2 and F_3 progenies.

SORGHUM RESISTANCE TO THE SHOOTFLY

Genetic analysis of sorghum resistance to shootfly was performed in crosses involving susceptible and resistant cultivars. IS 2146, IS 18487, IS 1044, IS 18479 and IS 1082 showed notable resistance to shootfly while CSH-1, Serena, IS 8595, IS 18363 and IS 18361 indicated susceptibility in that order. Preliminary results indicated that resistance to shootfly is governed by additive genes. However, the degree of resistance in F_1 varies with the level of infestation. Under high infestation level, susceptibility appears to be dominant while resistance is dominant under low shootfly infestation. The "reversal of dominance" appears to be dependent on degree of insect plant interaction. Further analysis to study this phenomenon is in progress.

Intercropping and pest management

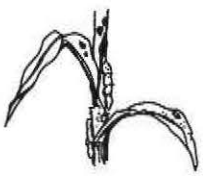
E.O. Omolo

Intercropping experiments conducted at Mbita Point Field Station and nearby farmers' fields between 1980 and 1982 indicated that there was a trend in pest population fluctuation. Early and late infestation, colonization, build-up and establishment of pests were related to different maize/sorghum/cowpea combinations. It was therefore decided that during the year 1983, the best combination (sorghum/cowpea, maize/cowpea/sorghum); the bad combination (sorghum/maize), and the controls of monocultures of maize, sorghum and cowpea; be planted in three different ecological zones, viz. MPFS (intensive pest population), Ogongo (one reliable rainfall) and Rongo (lower highlands with both reliable seasons).

Data from Mbita Point Field Station and Ogongo highly supported the previous finding that infestation started later in the major season. The colonization and build-up of stem borers was also much faster in monocultures of sorghum or maize and the two planted together. At MPFS infestation and build-up was much faster due to the intensive pest population at the station. At Rongo, however, the picture did not conform to the expected pattern. This could have been due to a number of factors among which; low level of stem borer pupulation in that region during the major season, continuous rainfall which tended to wash off most of the egg batches and the apparent inactivity of *B. fusca* that happens to be the major stem borer in the area. In the case of *M. testulalis*, in spite of the fact that attack on cowpea at Rongo was extremely low, probably for similar reasons as those given for reduced attack on maize or sorghum by *B. fusca*, most of the damage was still in the cowpea monoculture.

***ACRONYMS**

ICRISAT	— International Crops Research Institute for the Semi-Arid Tropics
CIMMYT	— Centro Internacional de Mejoramiento de Maize Y Trigo
IITA	— International Institute for Tropical Agriculture
IRRI	— International Rice Research Institute
WARDA	— West African Rice Development Association



Insect Pathology and Pest Management Programme

The primary objective of the Insect Pathology and Pest Management Programme is to develop an appropriate integrated pest management (IPM) programme for subsistence farming systems in the tropical region, the ultimate goal being to improve crop and livestock production as well as rural health in this region. To achieve this goal, our approach emphasizes the use of pest and vector management techniques that are feasible and can be implemented at subsistence level, and which take into account the limited resources at the disposal of the subsistence farmer. Our pest management strategy therefore gives priority to host resistance, cultural practices and biological control as major components of an IPM system we have set out to develop . We started off by conducting field surveys to identify biological agents that are associated with natural populations of the target pests, Chilo partellus *and* Busseola fusca *on maize and sorghum; and* Maruca testulalis *on cowpea at Mbita Point Field Station and nearby farmers' fields. The surveys started in 1982 continued this year. This time we were monitoring more closely the incidence and activity of insect pathogens and parasitoids in relation to pest population changes throughout the season on maize, sorghum and cowpea monocrops. Similar observations were made on maize-sorghum-cowpea mixed cropping systems on two farmers' fields; one in a two-season area and the other in one-season area. The Insect Pathology Unit set up some bioassay facilities to process field-collected cadavers to confirm whether the observed mortality was due to pathogens and, if so, to assess their potential pathogenicity or killing power.*

Seasonal occurrence of parasitoids

G. W. Oloo

The incidence of parasitoids on maize and sorghum monocrops (cowpea is being handled by an ARPPIS* Ph.D. student) was monitored by taking weekly samples from germination to harvest. The experimental field was stratified, samples obtained by random numbers and plants dissected and examined to record all immature stages of the target pests (stem borers). The insect material was thereafter maintained in the laboratory to recover parasites. Those showing obvious disease symptoms were passed on to the Insect Pathology Unit for further tests and characterization.

Chilo partellus was the most common stem borer on the station. As was the case last season, *Chilo* infestation was observed within a week and peak larval density and damage occurred a month after germination. However, the two most common parasites, *Dentichasmias busseolae* Heinrich (Ichneumonidae) and *Pediobius furvus* (Gah.) (Eulophidae), were recovered about 2 months after germination (i.e. maturity and hard dough stage for maize and sorghum, respectively). The former is a solitary pupal endoparasite, the latter, a gregarious pupal endoparasite. *D. busseolae* was responsible for 18% pupal parasitism on *Chilo* at the station. From the farmer's field 11% parasitism was recorded. *P. furvus* gave 11% parasitism at the

Table 1. Mortality due to pathogenic infection on the stem borer *C. partellus* on sorghum sampled progressively from planting to harvest at Mbita Point, western Kenya.

Time post-planting (weeks)	Stage of plant growth	Mean larval No. per plant	No. of larvae examined	Percent Disease incidence (%)
1 — 4	Vegetative	0.5	273	0.6
4 — 8	Reproductive	1.6	436	2.7
8 — 12	Maturity	2.9	520	6.3
12 — 16	Harvest	5.3	792	6.6

station and 2% in the farmer's field. Thus, the impact on pest population was relatively low. It is also noteworthy that the two parasites set in when most of the crop damage (by first generation larvae) was already done. However, *P. furvus*, as a gregarious parasite holds promise for mass culture and use for the control of stem borer complex in this agroecosystem on a long-term basis.

No egg parasite has been recovered so far from *Chilo* although *Trichogramma* has been recorded previously by workers in the Crop Borers Research Programme.

Apanteles sesamiae Cam. and *Bracon* sp. (Braconidae), larval parasites, and *Hyperchalcidia soudanensis* Steff (Chalcididae), a pupal parasite, were also recovered, but all the three are extremely rare and would not make much of an impact on the stem borer population in this area.

Incidence of pathogens

W. A. Otieno, M. O. Odindo

A systematic survey for pathogens of the stem borers only started this year, the two scientists having been pre-occupied with pathogenic micro-organisms affecting the *Anopheles* mosquito and the tsetse fly under the Medical Vectors Research Programme. Regular samples were collected throughout the season from sorghum and cowpea monocrops at the station and from maize-sorghum-cowpea mixed crops in both the station and the farmer's field. These samples were supplemented with dead borers obtained from the parasite survey work mentioned above.

The plants were dissected to recover both live and dead larvae. Live larvae were maintained in the laboratory to record mortality due to possible chronic pathogen infection. Field-collected cadavers were prepared into an inoculum (a suspension of 5 crushed dead larvae in 10 ml sterile distilled water). Fresh sorghum stems were dipped in the inoculum and offered to 10 laboratory-reared 3rd-instar *Chilo* larvae per petri dish. The cultures were checked and mortality recorded every 2 days. The material was discarded after 14 days. Larvae reared on stems treated with pure distilled water served as control.

Larval mortality due to pathogens in field populations of *C. partellus* on sorghum monocrop was first observed about one month after germination, coinciding with peak larval density on the crop. However, the level of mortality was low throughout the season, the highest recorded being approximately 7% (table 1). The rate of mortality for the inocula varied (table 2), but 41 out of 151 inocula assayed will be tested further to get a clearer picture.

Bacteria appeared to be the most common mortality factor among the pathogens observed attacking *C. partellus;* fungi and nematodes were also recorded. The protozoan, *Nosema* sp. was observed on *Maruca testulalis*. The pathogens tentatively identified so far are given in table 3.

As with parasitoids, mortality due to pathogens was relatively low and occurred too late in the cropping season to give adequate protection to the crop, unless manipulated in some way. The stem borer infestation was low in the two-season area (where pathogens were surveyed in the mixed crop). In the coming year, we intend to investigate species diversity and status of the natural enemies (parasitoids and pathogens) in selected mixed cropping systems. We also hope to perfect our bioassay techniques for evaluating the killing potential of the pathogens, when laboratory facilities become available. Any promising pathogens would then be isolated and purified for proper identification.

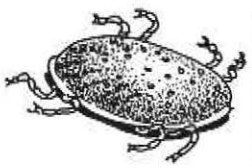
Table 2. Preliminary bioassays on the stem borer *C. partellus* using inocula prepared from field-collected dead *Chilo* larvae.

Test No.	Inocula tested	Inocula with +ve results	Highest recorded mortality (%) 7 days post-treatment	14 days post-treatment	Control
1 — 4	39	10	70	90	20
5 — 8	54	14	60	80	30
9 — 11	58	17	80	90	30

Table 3. **Pathogens tentatively identified from field populations of *C. partellus* and *B. fusca* on maize and sorghum, and *M. testulalis* on cowpea at Mbita Point.**

Pathogen	Group	Insect host
Serratia sp.	Bacteria	*B. fusca*
Bacillus sp.	Bacteria	*C. partellus*, *M. testulalis B. fusca*
Entomophthora sp.	Fungus	*C. partellus*, *M. testulalis*
Cordiceps sp.	Fungus	*B. fusca*, *C. partellus*
Beauvaria sp.	Fungus	*B. fusca*
Nosema sp.	Protozoa	*M. testulalis*

***ARPPIS — African Regional Postgraduate Programme in Insect Science**



Livestock Ticks Research Programme

In previous annual reports, it has been repeatedly pointed out that the application of the methods being developed at ICIPE for the control of **Rhipicephalus appendiculatus** *on cattle was dependent on the availability of a curative drug and a method of vaccination against East Coast Fever (ECF). It has also been repeatedly stated that both a curative drug and a method of vaccination would shortly become available. The first curative drug for ECF (Parvaquone) was registered in Kenya at the end of 1983. It was identified in 1975 and developed by colleagues at the Kenya Agricultural Research Institute (KARI) in collaboration with an international drug company. It is now being used efficaciously by veterinarians to give a better than 80% recovery rate for a disease, which untreated, will kill more than 90% of affected cattle.*

The method of vaccination against ECF (known as the infection and treatment method) which was developed by a UNDP/FAO project working at KARI during the early 1970's has been used in field trials during 1983 by colleagues at KARI and ILRAD in cooperation with Kenya government veterinary services. Trials have been carried out in western Kenya, Laikipia and several locations in Coastal Province with virtually 100% success. In addition, a DANIDA/FAO project in cooperation with the government veterinary services is currently carrying out vaccine field trials in Malawi. Thus, as a result of investigations carried out during the last 5 years, the ICIPE Livestock Ticks Research Programme is now ready to carry out field trials for the integrated control of ECF and its vector, **R. appendiculatus.**

Hypersensitivity induced by repeated infestation with *R. appendiculatus*

A. O. Mongi, M.P. Cunningham

Hypersensitivity reactions appearing in susceptible rabbits infested with *Rhipicephalus appendiculatus* larvae, nymphs and adults were investigated. During feeding, ixodid ticks transfer several antigenic substances to the host animal. Some of these antigens appear to engender allergic hypersensitivity responses, which provide the immunological basis for resistance to tick infestation.

Larval behaviour on naive rabbits

A total of 10 larvae from four different egg batches were enclosed in 20 x 20 mm plastic capsules and applied to shaved rabbit ears. The rabbits were restrained in a modified rabbit cage to minimize their body movements as much as possible. The behaviour of the larvae was observed through a dissecting microscope.

A schematic presentation of the attachment process is presented in figure 1. In all cases, the larvae and nymphs showed an initial searching period for the first 3 to 4 h of application, followed by a resting period when the ticks were observed at the bases of the rabbit

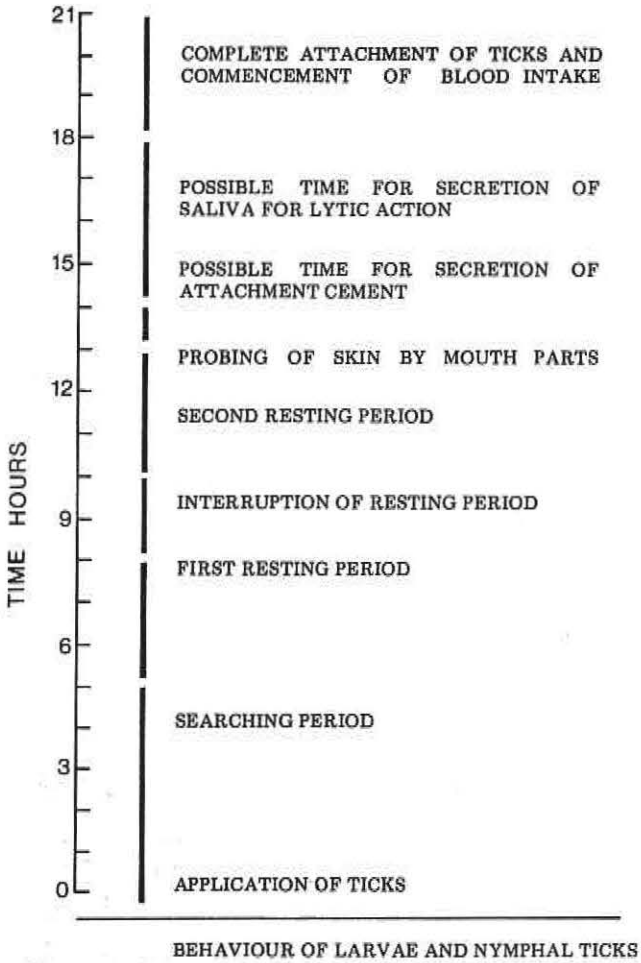


Figure 1: Schematic presentation of behaviour displayed by the larvae and nymphs of *R. appendiculatus* in the process of attachment on naive rabbits.

hair shafts. The leg appendages, especially the fore ones, were extended at an angle giving the false impression that the ticks were firmly attached. However, a gentle touch with a fine camel-hair brush caused them to wander randomly for about 20—30 min. By the end of 30 min the majority of ticks had settled again. Occasionally the ticks moved abruptly from their original site of attachment (site marked by a fine felt pen) to new sites. On further disturbance (i.e. by mild air currents, torch light, or by slight movement of the rabbit body itself), the ticks took 2 to 3 h before resettling and assuming a probing position. Complete attachment was attained after 18—24 h. Attempts then to pull them from their attached site showed that they were firmly attached to the rabbit skin. Cement deposition appeared also to have been formed by this time as the area around the inserted mouth parts of the tick appeared to be swollen. The ticks remained attached for between 24 to 36 h which signified intake of blood from the host. In some cases, fully engorged larvae were observed by the end of 48 h of infestation. Some of the nymphs were fully engorged by 60 h; at the end of 72 h most of them had engorged and dropped off the host.

Induction of resistance in rabbits by larvae, nymphs and adults.

Three groups of rabbits, each consisting of four rabbits of either sex and weighing 2.5 — 3.0 kg, were prepared for tick infestations by clipping short the hair on the ears. The rabbits were made resistant by repeated artificial infestations. Group one was infested with 1000 one-month-old larvae on each ear while groups two and three were infested with 500 nymphs, 75 adult males and 75 adult females of *R. appendiculatus* on each ear as well. Ticks not attached by 24 h were counted *in situ* and left undisturbed to continue their search for attachment.

Four successive infestations were carried out at 4-week intervals, and local skin reactions observed macroscopically during the course of the infestations. The prolonged interval was necessary to allow sufficient time for the ears of the rabbits to recover from the tick bites. Resistance was measured by the rabbits' ability to prevent the ticks from obtaining a normal blood meal. This was assessed by monitoring attachment patterns, engorgement weights and drop off patterns of the ticks during primary and subsequent infestations. The development of engorged ticks to other instars was also observed. The rabbits were bled from the ear vein 7 days after the final tick infestations to obtain sera which were stored at -20^0 C.

The mean percentages of successful infestations with tick larvae, nymphs and adults on four groups of rabbits are shown in tables 1,2 and 3, respectively. The number of successfully engorging larvae and nymphs decreased with each infestation. Partially engorged ticks were a consistent feature in the third and fourth infestations. The fourth infestation showed considerable reduction in the number of feeding ticks and a considerable fall in the number attaching. Increasing mortalities and numbers of partially engorged larvae and nymphs were observed in each successive infestation (tables 1 and 2 respectively). A one-way analysis of variance for all the parameters observed for both the larvae and nymphs showed that there was a significant difference ($P<0.001$) between the initial and subsequent infestations (tables 1 and 2). Adult female and male ticks were also repeatedly applied to naive rabbits for four infestations. In this study, only the female engorgement weights were recorded at the completion of engorgement, after each infestation (table 3). Female engorgement weights were observed to decrease from 335 mg at initial infestation to 186 mg, 179 mg and 45 mg during the second, third and fourth infestations respectively. A one-way analysis of variance of

the engorgement weights showed significant difference ($P<0.01$) between the initial and succeeding infestations (table 3).

Table 1: Per cent (mean ± S.E.) *R. appendiculatus* larvae fully engorging in the course of four successive infestations.

Number of infestations	Rabbits				Group Mean
	R_1	R_2	R_3	R_4	± S.E.
1	96.4	96.2	98.3	97.4	97.1 ± 0.6
2	83.7	80.9	84.5	90.6	84.9 ± 1.9
3	46.8	39.6	20.9	29.1	34.1 ± 5.2
4	18.0	9.3	13.9	11.0	13.0 ± 1.7

Analysis of variance:

Source	df	SS	MS	F value
Groups	3	19873	6624	174.3***
Residuals	13	192	38	
Total	16	20365		

Note: 1000 larvae were applied on each of the rabbit's ears in each infestation for tables 1, 2 and 3.

Table 2. Per cent number (mean ± S.E.) of *R. appendiculatus* nymphs able to fully engorge, during the course of four successive infestations. 500 nymphs were applied on each ear of the rabbits in each infestation.

Number of infestations	Rabbits				Group Mean
	R_1	R_2	R_3	R_4	± S.E.
1	92.0	95.0	97.0	93.0	94.3 ± 1.1
2	43.5	54.5	54.0	62.0	53.5 ± 3.5
3	53.0	52.5	53.0	47.5	51.5 ± 1.2
4	39.5	41.5	27.0	32.5	35.13± 3.0

Analysis of variance:

Source	df	SS	MS	F value
Groups	3	7995	2665	92.0
Residuals	13	378	29	
Total	16	8373		

Table 3. Mean weight measurements of *R. appendiculatus* adults on rabbits in four successive infestations. 75 males and 75 females were fed on each rabbit during each infestation.

Number of infestations	(Measurements in (mg±S.E.)				Group Mean
	R_1	R_2	R_3	R_4	± S.E.
1	344 ± 29	352 ± 37	314 ± 36	331 ± 27	335 ± 8
2	192 ± 6	195 ± 21	175 ± 13	183 ± 19	186 ± 5
3	113 ± 7	116 ± 10	119 ± 17	123 ± 22	179 ± 2
4	45 ± 3	47 ± 4	44 ± 4	46 ± 3	45 ± 1

Analysis of variance:

Source	df	SS	MS	F value
Groups	3	183368	61123	728 ***
Residuals	13	1095	84	
Total	16	184463		

Attachment process during successive infestation

In the first and second successive infestation, attachment took place between 18 and 24 h of application. In the third and fourth infestations, the attachment process was prolonged: by the end of 36 h complete attachment was not yet attained. Some of the ticks appeared poorly attached others detached and then reattached at a new site, sometimes along the periphery of the earlier attachment sites, especially during the third and fourth infestations. The larvae that were unable to attach and those that spontaneously detached but were unable to reattach were found dead. Larvae or nymphs that failed to complete engorgement died still attached. Some ticks also were observed trapped in the serous exudate.

The ticks feeding on inflamed areas did not take as much blood as those feeding on uninflamed areas. Those that managed to feed to completion were either yellow or pink in colour. They appeared to have fed on tissue fluids rather than on red blood cells from the host. However, when they were kept at 85% rh and 25°C, they were able to moult to nymphs and adults.

Successive tick infestation on resistant rabbits also affected post-feeding events. Slightly prolonged periods for development of larvae into nymphs were observed. Mean development periods to moult into either male or female adults was prolonged in nymphs fed on the resistant rabbits. The mean period from oviposition to egg hatching was also prolonged.

Skin reactions from the bites of larvae, nymphs and adults of *R. appendiculatus* on rabbits previously exposed to the same tick species were observed macroscopically. Mild skin reactions were evident as early as the second tick infestation; but became more severe in the third and fourth infestations. In each case the reaction initially showed erythema limited to the bitten area. This was followed by the development of a diffuse erythematous papule with substantial oedema. The ears appeared thickened and demonstrated increased temperature. Scratching of the reaction sites caused some of the observed secondary bacterial infections. These reactions were more pronounced in rabbits infested with larvae than in those infested with either nymphs or adults. Some serous exudate was observed accompanied by sloughing of tissues from the injured site. A microscopic smear of the exudate stained with Giemsa showed the presence of phagocytic cells, chiefly neutrophils and monocytes. The lesions were created below the tick mouthparts. Where the ticks had fallen off the host, they left behind crater-shaped small wounds of different sizes surrounded by a light yellowish hard crust of exudate. Some of the reactions formed plaques which persisted for almost 1 month before subsiding.

Immediate and delayed hypersensitivity

Salivary gland antigen was prepared from adult females that had been allowed to engorge on rabbits for 4 days. The ticks were dissected while partially embedded in paraffin wax and covered with PBS in a petri dish placed on ice. An incision was carried out around the entire scutum of the ticks at the dorsoventral junction. The salivary glands were then dissected out using a fine watchmaker's forceps, with the aid of a dissecting microscope. One hundred glands were suspended in 2 ml PBS and homogenized by hand in a glass tissue grinder. Purified sand (40-100, mesh BDH Chemicals Ltd, Poole, England) was used to assist in thorough disruption. The homogenate was centrifuged for 10,000 g for 30 min at 40°C. The resulting supernatant was collected and held at 40°C until used in the protein described below.

Fractionation of salivary gland homogenate

Salivary gland homogenates were fractionated on a column of Sephadex G75 (Pharmacia Fine Chemicals, Uppsala, Sweden), (2.3 x 100 cm diameter). A sample volume of 4 ml of the salivary gland extract in eluting buffer (0.1M Tris-HCl, pH 8.0, 0.01M $CaCl_2$) was added drop by drop to the centre of the bed. This was performed with a hydrostatic pressure pump at 8 ml per h. The protein concentration of the eluate was monitored on an ultraviolet monitor, ISCO, Model UA-5. The fractions were collected with an ISCO Model 328 Fraction Collector. Pooled fractions of the eluate were used for skin testing on naive and resistant rabbits. Total protein concentration was estimated by the Folin-Ciocalteau method.

Results of the gel fractionation of Sephadex G75 of protein of a salivary gland extract are shown in figs 2 and 3. Two major peaks of protein were obtained with the sample extract fractionated. Outchterlony immunodiffusion reactions were performed with the protein fractions and the serum from rabbits obtained 2 weeks after cessation of the fourth infestation with larval ticks. With the first peak, 1-2 precipitin lines were formed. There were no precipitin lines formed with the second peak and no precipitin lines formed between either peak and control serum collected from non-infested rabbits.

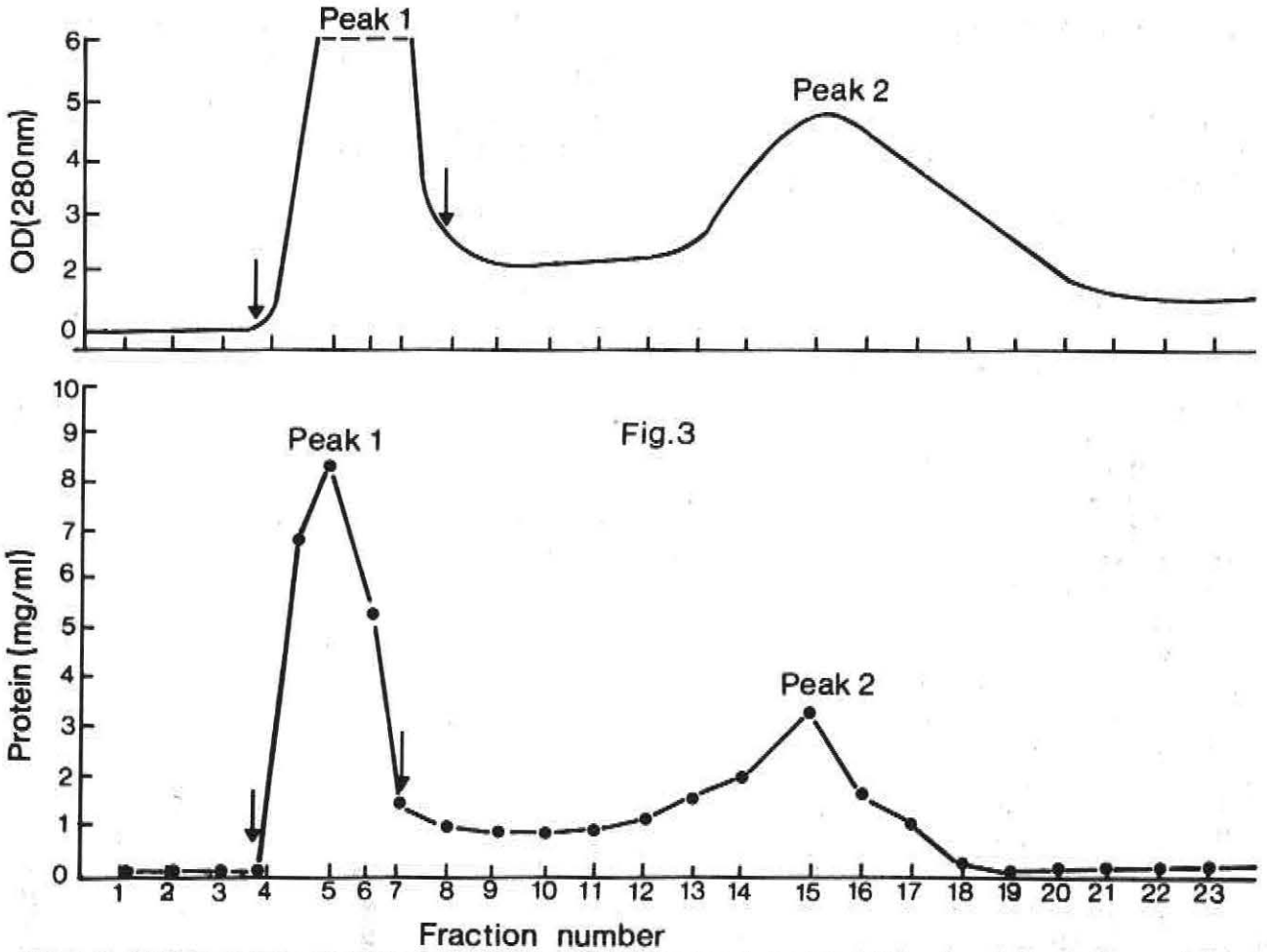


Fig.3

Figure 2: Elution pattern of salivary gland antigen extract on a Sephadex G 75, using eluting buffer containing 0.1M Tris-Hcl 0.01M $CaCl_2$, PH 8.0.

Figure 3: Protein determination for each of the eluted fractions performed using the method of Lowry et al.

The test sites on the rabbits were each inoculated intradermally with 70 μg per ml of protein obtained from the pooled fractions of peak 1 (figs 2—3). The reactions were immediate, within the first two hours of test followed by a gradual increase in skin thickness, indicating delayed reaction. The swellings reached a peak by 48 h. Within 48 h the skin thickness increased from 1.0 to 4.9 mm in the larvae-resistant rabbits, and from 1.0 to 4.3 mm in the nymph-resistant rabbits. The difference in skin thickness at 24, 48 and 72 h for the tick-resistant rabbits compared to non-resistant control rabbits was highly significant ($P<0.001$). Some of the swellings developed a necrotic centre. The reactions started to subside after 72 h and by 108 h the skin test sites had returned to pre-skin-test thickness. Normal saline inoculation along side the test sites as controls did not produce observed hypersensitivity reactions. Rabbits naive to tick infestations showed mild immediate skin reactions, erythema appearing within 30-50 min and disappearing by the end of 1½ h without a delayed response.

The results of this study have clearly shown that rabbits acquire resistance to repeated infestations with the tick *R. appendiculatus*. The immunological mechanisms involved in this resistance phenomenon appear to be both immediate and delayed hypersensitivity reactions.

Use of irradiated *R. appendiculatus* to induce resistance to infestation.

R.M. Newson, J.J. de Castro

Type I resistance to tick infestation in cattle or rabbits is induced by the action of tick saliva, which is injected either by natural feeding of ticks on cattle in the field, or by controlled application, usually in the laboratory. However, in certain experiments we may need to purposely infest cattle with large numbers of adult ticks in the field. The almost inevitable escape of even a few egg-laying females would be undesirable. We are therefore investigating the effect of gamma radiation on *R. appendiculatus*, to see if breeding can be prevented without losing the ability to induce resistance in the host. We are irradiating males and females as we intend in practice to take ticks from culture

without having to separate the sexes. The ticks are maintained at 28° C and 85% rh.

Optimum radiation dose

In order to establish the optimum dose of radiation, unfed adult ticks were exposed to doses of 0, 150, 300, 1200 and 2400 rads. The ticks were then applied to the ears of tick-naive rabbits to feed, using two rabbits for each dose level. On one ear 20 irradiated males and 20 irradiated females were fed, while the same number of untreated controls were fed on the other. Engorged weight and duration of feeding were measured for all the female ticks. The mean production of live larvae per female applied to feed was estimated from the performance of subsamples of 5 females, except in the case of those receiving 2400 rads when all were followed.

Engorged weight (expressed as percentage difference from own control) showed a significant negative correlation with log radiation dose (fig. 4), while the duration of the feed increased. The estimated yields of larvae from the controls varied from 1000 to 4000. Nevertheless, at a dose of 600 rads the live larval production was greatly reduced and it was consistently nil at the two highest doses (fig. 4). Females that laid eggs, whether or not they had been irradiated, usually started to do so 3–6 days after detaching from the host and completed the process in a further 18 days, by which time they had converted 40–50% of their freshly engorged weight into eggs. However, 65% of the females that had received 1200 rads survived for an average of 54 days, and 88% of those with 2400 rads survived on average for 59 days (table 4), generally without laying any eggs at all. These females were weighed twice, and 16 that had received

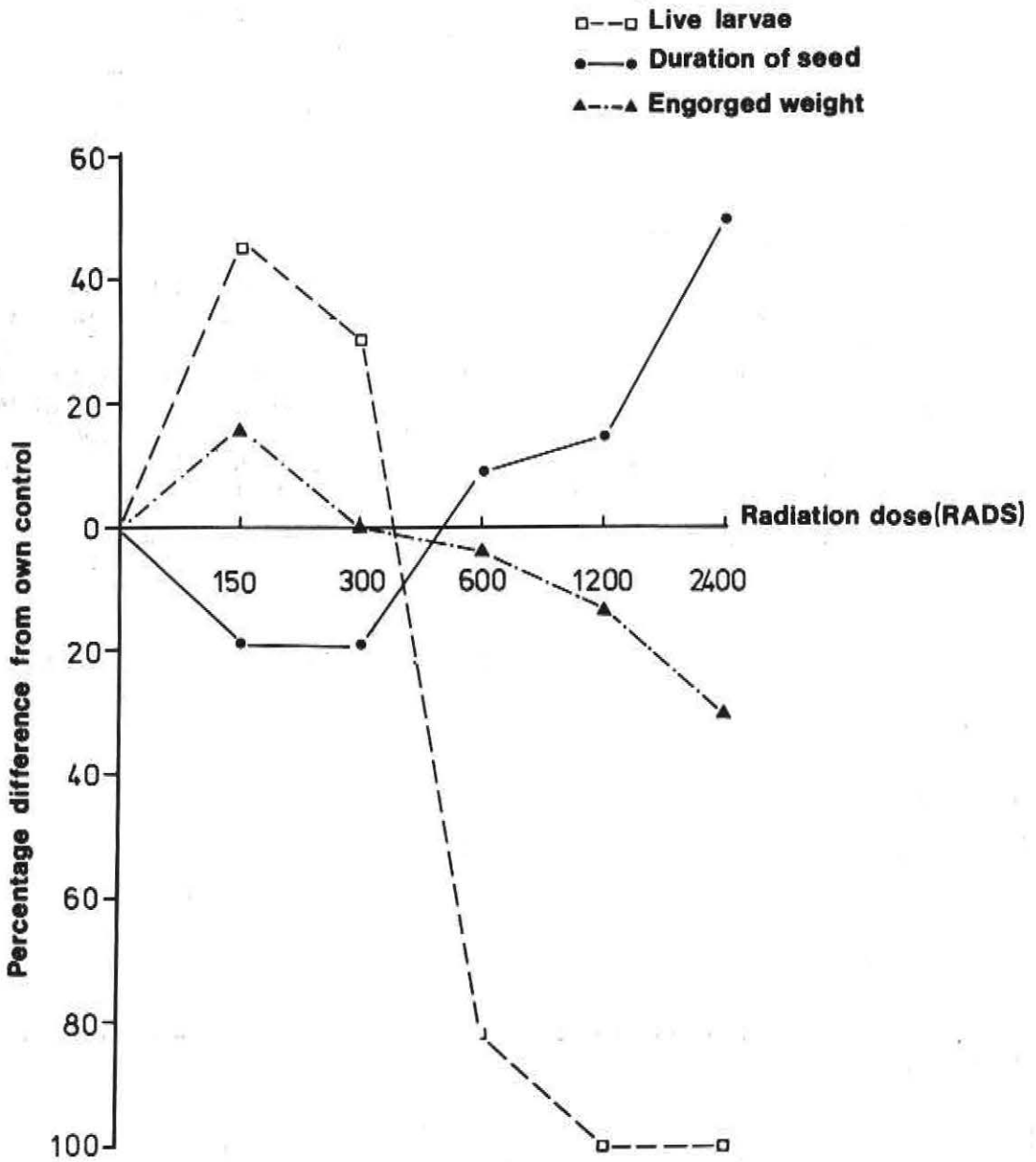


Figure 4. Effects of gamma irradiation, applied to both sexes, on *Rhipicephalus appendiculatus*

Table 4: The effect of radiation dose on egg-laying in *R. appendiculatus* expressed as percentage in each category

Radiation dose (rads)	Died : no lay	Died before 10th day of lay	Full lay	Prolonged survival	Sample size
0	2.3	16.3	81.4	0.0	86
150	0.0	20.0	80.0	0.0	10
300	0.0	10.0	90.0	0.0	10
600	10.0	60.0	30.0	0.0	10
1,200	23.5	11.8	0.0	64.7	17
2,400	9.5	0.0	2.4	88.1	42

2400 rads showed an average loss in weight of 35% after 50 days or more.

It appears that doses of 150—300 rads on males and females may have been mildly stimulating to the ticks, but doses of 600 rads or more progressively lengthened the duration of feeding, reduced the engorged weight and resulted in total sterility above 1200 rads, mainly by preventing the conversion of blood meal into eggs. The affected females were, however, still able to use this source of food to maintain their own metabolism for a long period. Further work was therefore concentrated on the use of radiation doses of 1200 and 2400 rads.

Effect of cross-mating irradiated with untreated ticks.

Twenty male and 20 female irradiated ticks were applied to one ear of a tick-naive rabbit and the same number of untreated ticks on the other ear. Each combination was replicated three times.

The results are shown in table 5. The females were more severely affected than the males where the final production of live larvae was concerned, and in the field their contribution to the larval population would be negligible compared with the likely output of the indigenous ticks. There is normally a two-to-three-fold surplus of males on the host, so the probable effect of any irradiated males is likely to be small, but they would reduce productivity of any wild females with which they mated.

In this case the effect of irradiation on the other parameters was not detectable, and the irradiated ticks are probably similar to untreated ticks in inducing Type I resistance.

Effect on viability of treatment of unfed ticks before application

Batches of 55 males and 55 females (all in the weight range 2.0—2.9 mg) were irradiated at 0, 1200 and 2400 rads and stored for 70 days. On examination the survival was found to be 90%, 90% and 73% respectively, which indicated that treating the unfed ticks a few days before application to the host would have very little effect on their viability.

Table 5: Effect of a radiation dose of 1,200 rads on feeding and breeding in *R. appendiculatus*

	Radiation dose (rads)		Engorged weight (mg)	% fed	Duration of feed (days)	Estimated live larvae
m	0	L	341	95	7.8	2818
f	0	R	334	77	7.5	2528
m	1,200	E	313	88	8.7	7
f	1,200	C	305	95	8.2	2528
m	1,200	E	315	93	7.7	1868*
f	0	C	325	95	8.1	2905
m	0	E	310	87	8.3	5
f	1,200	C	326	97	8.5	2875

Notes
Table shows mean results for 20 males (m) and 20 females (f) fed on groups of 3 rabbits for each treatment
* Larvae of low viability.

L — Left ear
R — Right ear
E — Experimental on one ear
C — Untreated control on the other ear of all remaining hosts.

Degree of resistance

We planned to compare the degree of resistance engendered in 1½-year-old cattle by feeding different numbers of *R. appendiculatus* on them. All the ticks were irradiated at a dose of 1200 rads; they were assumed to be males and females in equal proportion. The ticks were applied at the rate of 0, 2, 4 and 8 per kg body weight of the hosts (which weighed 110-160 kg) with groups of three cattle per treatment. Up to 400 ticks were fed on each animal in ear bags; the remainder were placed under stockinette patches on the neck. Control feeds of 40 irradiated ticks (20 males: 20 females) were done on one ear of each of three tick-naive rabbits, with the same numbers of untreated ticks on the other ear. The cattle had been kept indoors for the previous 8 months when their only known contact with ticks had been in two assessments of resistance to *R. appendiculatus* and two to *Amblyomma variegatum*, which in every case had consisted of feeding 100 nymphs on each host. They were also known to have been exposed earlier to *Boophilus decoloratus* infestation.

It became obvious almost immediately that the cattle had a considerable degree of resistance to ticks. They showed signs of intense irritation at the sites of tick attachment and started to dislodge the neck patches. We therefore removed the ticks on the necks, quantified the tick challenge by the number of ticks applied to the ears (2.5–3.6/kg), counted and weighed the engorged females and compared them with the rabbit-fed ticks (table 6). We then carried out the planned assessment feeds using 20 males and 20 females on one ear of each host and 100 nymphs on the other. The results confirmed that even the three cattle that had not been so far subjected to tick feeding in the present experiment showed a similar effect on female ticks to those that had been. The mean engorged weight of the test nymphs was, however, similar to that from the control rabbits.

We then tried to increase the level of resistance in all the cattle by means of another feed of irradiated adult ticks (treated with 1200 rads). The previously untreated cattle now received 2 adult ticks/kg, making their total known challenge 2.3 adult ticks/kg, including the adults just used in the assessment feed. The other cattle received 2–4 ticks/kg. A second assessment feed using 100 nymphs per host as before was then done 4 weeks later (table 6), when it was found that the cattle that had received the lower challenge now appeared to be similar in response to those that had received the higher one.

The main conclusion to be drawn from these results is that although cattle can easily acquire Type I resistance to tick infestation, their response is very variable and may also be complicated by cross-resistance to other species of ticks. For instance, in the first treatment feed, one animal fed 52% of the females to a mean weight of 172 mg, another fed 100% to 215 mg and the host with the lowest proportion fed (27%) gave the highest mean weight (316 mg) — similar to that from the control rabbits. Seven out of nine cattle receiving three feeds of adult ticks in this experiment showed an average decrease of 20.9% (range 0.5 — 55.5%) in the mean weight of female ticks engorging on them between the first and the last feeds, but two showed small increases over the same period (3.8% and 9.7%).

Table 6: Mean weights of female and nymphal *R. appendiculatus*, and mean percentages of nymphs that fed, when they engorged on test cattle.

Hosts	n	1 mg	2 mg	3 mg	3 %	4 mg	5 mg	5 %	Total ticks/kg
Cattle	3	—	273	7.4	88	175	5.0	76	2.3
Cattle	9	259	251	5.1	82	227	4.8	73	5.7
(a) Rabbits	3	313	333	7.9	89	337	9.0	92	0.0
(b) Rabbits	3	317	—	—	—	327	—	—	—

1. First treatment feed, irradiated adult ticks (2.0 — 3.6 ticks/kg)
2. First assessment feed, untreated adult ticks (20♂♂: 20♀♀)
3. First assessment feed, untreated nymphs (100)
4. Second treatment feed, irradiated adult ticks (2.0 — 4.0 ticks/kg)
5. Second assessment feed, untreated nymphs (100).
6. The accumulated numbers of adult ticks applied per kg of host body weight are shown and (a) corresponding control feeds on tick-naive rabbits with (b) additional feeds of untreated ticks to confirm the performance of the rabbits

Host resistance to *R. appendiculatus* infestation and transmission of *T. parva*

R.M. Newson, J.J. de Castro

A preliminary joint study between ICIPE and the Veterinary Research Department, Kenya Agricultural Research Institute (KARI), showed that resistance to *R. appendiculatus* infestation in cattle appeared to affect the transmission of the protozoan *Theileria parva* which is passed by the tick while it feeds. The following experiments were done to extend these studies.

Transmission of *T. parva* from tick resistant and tick naive cattle

Two groups of five cattle were infected artificially by inoculating with a suspension of *T. parva*. One group was resistant to *R. appendiculatus* as a result of previous prolonged and heavy tick feeding; the other was naive to tick infestation. Initially the cattle were all serologically negative for *T. parva* antibodies.

All the cattle became infected and 9 out of 10 died (table 7). On the 11th and 13th days after inoculation, batches of 2000 *R. appendiculatus* nymphs were put to feed on each animal. The course of the disease was similar in both groups, but the longer survival of the resistant cattle was attributed to their superior physical condition. So few ticks engorged on the susceptible cattle in the second feed that comparisons have been limited to the results of the first feed, also curtailed by the death of the cattle.

After the nymphs had moulted to adults, samples were examined for the presence of *T. parva* sporozoites in the salivary gland acini. The ticks that had fed on the resistant cattle had a lower rate of infection and fewer infected acini per tick (table 7). Thus, 100 nymphs applied to a *Theileria*-infected, tick-resistant host would result, on average, in only 9 infected acini distributed among the resultant adults, whereas there would be 64 infected acini from a susceptible host.

Transmission of *T. parva* to tick resistant and tick naive cattle

Host resistance to ticks reduces the weight at engorgement and also shortens the duration of the feed (confirmed above). We therefore fed 100 *R. appendiculatus* nymphs infected with *T. parva* (at the rate of 12%) on two groups of cattle serologically negative to *T. parva*. One group of five cattle had been rendered resistant to tick infestation by feeding 500 irradiated adult *R. appendiculatus* on them, in addition to long previous exposure to tick feeding. The second group of three cattle had no known previous tick experience.

The results given in table 8 showed that the tick-resistant cattle fed the ticks to a lower weight, but more rapidly, and consequently they developed fatal theilerial infections sooner than the tick-susceptible cattle.

Survival of *R. appendiculatus* ticks under field conditions

D.K. Punyua

A survival experiment, which was carried out at Muguga, showed that adult ticks survived for 20 months, nymphs 14 months and larvae, 8 months in the field. It was then suggested that

Table 7: Mean results for the feeding of *R. appendiculatus* nymphs on 5 tick-resistant cattle (R) and 5 tick-susceptible cattle (S), plus 2 rabbit controls (C). The cattle had been infected with *T. parva* 11 days beforehand

	A	B	C	D	E	F	G	H
R	7.6	10.4	22.8*	21	4.7	3 – 4	21	2.0
S	7.6	10.2	17.8	49	6.7	5 – 7	31	4.2
C	–	–	–	95	7.6	5 – 6	–	–

A Days to detection of theilerial macroschizonts in cattle lymph nodes
B Days to rectal temperature above 39.5^{0}C
C Days to death (* one animal recovered)
D Percentage of nymphs that engorged
E Engorged weight of nymphs (mg)
F Days to peak number of nymphs engorging
G Percentage of adult ticks infected with *T. parva*
H Number of infected salivary gland acini/infected tick.

Table 8: Mean results for the transmission of *T. parva*

	A	B	C	D	E	F
R	9.6	11.6	17.6	59	3.5	4.8
S	12.0	13.7	19.7	59	5.9	8.3
C	—	—	—	80	4.9	4.9

Notes
5 tick resistant cattle (R), 3 tick suceptible cattle (S) plus 3 rabbit controls (C) using infected *R. appendiculatus* nymphs were used.
Column headings A — E as in Table 4; days to engorgement (F)

similar experiments should be carried out in different parts of the country, taking into consideration the ecological differences.

Indigenous strains of the tick *Rhipicephalus appendiculatus* were collected and propagated in the laboratory from the Kiboko area in Machakos District, which is a marginal situation for *R. appendiculatus*, and also from the Lolgorien area in the Trans-Mara division of Narok District, which is typical *R. appendiculatus* country with high rainfall and high altitude. It is also an endemic area for East Coast Fever. The ticks are now ready to be released in the prepared enclosures in these two sites.

Further tick collections will be obtained from the Mbita Point Field Station in the lake region and from Kilifi District in the coast area.

Effect of sulphaquinoxaline an *R. appendiculatus* feeding and reproductive performance

Sulphaquinoxaline has been used in many laboratories as a coccidiostat for the treatment and prevention of coccidiosis in rabbits. This and other sulphonamide compounds have been shown to have a systemic activity against some blood-sucking arthropods when given to the rabbits which are subsequently used to feed these parasites.

The feeding and reproductive performance of *R. appendiculatus* was tested when fed on rabbits that had drunk water treated with 10.32% sulphaquinoxaline sodium. The diet was kept free of coccidiostat.

As shown in table 9, although there was a small reduction in the number of females engorging, their engorgement weights, the weight of their egg batches and the number of egg batches that hatched successfully, this reduction was not significant. Neither was feeding performance of the nymphs significantly affected.

These results suggest that when sulphaquinoxaline is administered via drinking water, at the indicated concentration, it does not affect the feeding and reproductive performance of *R. appendiculatus.*

Survival pattern of *R. appendiculatus* of differing sizes under semi-natural conditions

J. Chiera, D.K. Punyua

The survival of *R. appendiculatus* in relation to host resistance, individual tick size and environmental factors was studied in Stevenson screens between May and December 1981. The unfed nymphs and adults used had been fed as larvae and nymphs on three resistant cattle and rabbits. They were individually caged in small nylon mesh bags and exposed 2 weeks after moulting. Apart from the nymphs and adults

Table 9: The effect of sulphaquinoxaline on the feeding and reproductive performance of *R. appendiculatus* ticks fed on rabbits.

	Sulphaquinoxaline (Embazin)	Controls (Water)
FEMALES		
Engorement (%)	92.5	100.0
Engorgement period (days)	11.8	11.3
Engorgement weight (mg)	340.2	356.1
Weight of eggs (mg)	147.4	174.5
Number hatching (%)	90.0	100.0
Egg hatchability (%)	83.9	79.3
NYMPHS		
Fed (%)	75.3	84.0
Feeding period (days)	6.5	6.0
Engorgement weight (mg)	8.5	7.2
Moult (%)	96.5	99.1

exposed in the Stevenson screens in one of our paddocks, a small proportion of them was maintained in the tick culture room at 85% rh and room temperature. Sampling was done once every 2 weeks and once every 4 weeks for nymphs and adults respectively. Dead ticks were removed and the length of their scuta measured to determine the individual size. Two exposures were carried out. The first one (phase 1) coincided with the wet season in May 1981 while the second one (phase 2) coincided with the dry hot season in January 1982.

The level of host resistance to ticks was determined by the weight of nymphs engorging on each host, the least-resistant host giving the best weights. Environmental conditions were assessed as cumulative rainfall, mean temperature and a mean relative humidity which was calculated from the wet and dry bulb thermometer readings taken at 0900 hrs and 1500 hrs daily.

In phase 1, good survival of the adult ticks in the screens was obtained for the first 36 weeks or so, during which time the environment was wet with high relative humidity and generally low temperatures. But the start of the dry hot season that followed in January through April 1982 (fig. 5, weeks 36 to 48) saw the start of a sharp decline in survival which continued until the end of the experiment, the decline only slowing down slightly in response to improved weather conditions. In contrast, phase 2 adult survival was superb for a good 48 weeks or so, the start of the decline in survival again coinciding with the start of the dry hot season of December through April 1983 (fig. 6, weeks 48 to 64). The dry season that sharply reduced the survival of adults in phase 1 (weeks 36 to 48 in fig. 5 or weeks 0 to 12 in fig. 6) did not affect the survival of the newly exposed phase 2 ticks. Phase 1 nymphs had a mean survival of 31 weeks and a maximum survival of 56 weeks. The corresponding figures for phase 2 were 48 and 72 weeks respectively. On the average then, phase 2 ticks survived much better than phase 1 ticks in the screens. Survival of nymphs and adults in the tick culture room, however, was poor compared to that in the screens. It fell below two-thirds of that in the screens in every case.

Table 10 summarizes the relationships between survival, tick size and host resistance. It indicates that the higher the host resistance the smaller the tick mean size and consequently the lower the survival. Columns 6 and 7 give the regression and correlation coefficients between individual tick size and survival. In general, both parameters increase in direct response to increasing host resistance, which would indicate that survival of individual ticks becomes increasingly more related to tick size with increasing host resistance. It will also be noted that when male and female ticks are fed on the same resistant host, females will, on the average, survive better than males.

Table 10. Relationships between *R. appendiculatus* adult survival, individual tick size and host resistance.

cattle host	mean wt. (mg) of test nymphs	no. of ticks	mean scutal length (mm)	mean survival (weeks)	regression* coefficient	correlation* coefficient
PHASE 1						
			Males			
R64	4.2	67	2.23	56.9	0.36	0.66
R67	4.8	81	2.40	57.6	0.20	0.43
R875	5.7	119	2.73	67.3	0.11	0.25
			Females			
R64	4.2	51	1.11	59.4	0.60	0.48
R67	4.8	58	1.17	63.5	0.68	0.60
R875	5.7	73	1.26	72.9	0.12**	0.09**
PHASE 2						
			Males			
R64	3.7	73	2.21	67.1	0.17	0.69
R67	4.1	74	2.23	65.8	0.16	0.67
R875	7.3	110	2.76	73.7	0.07	0.38
			Females			
R64	3.7	76	1.05	64.6	0.37	0.66
R67	4.1	67	1.12	69.0	0.46	0.76
R875	7.3	88	1.26	78.2	0.31	0.63

*Based on scutal length (mm) and survival (long weeks) of individual ticks.
**all regression coefficients and correlation coefficients significant at $P < 0.05$, except these two.

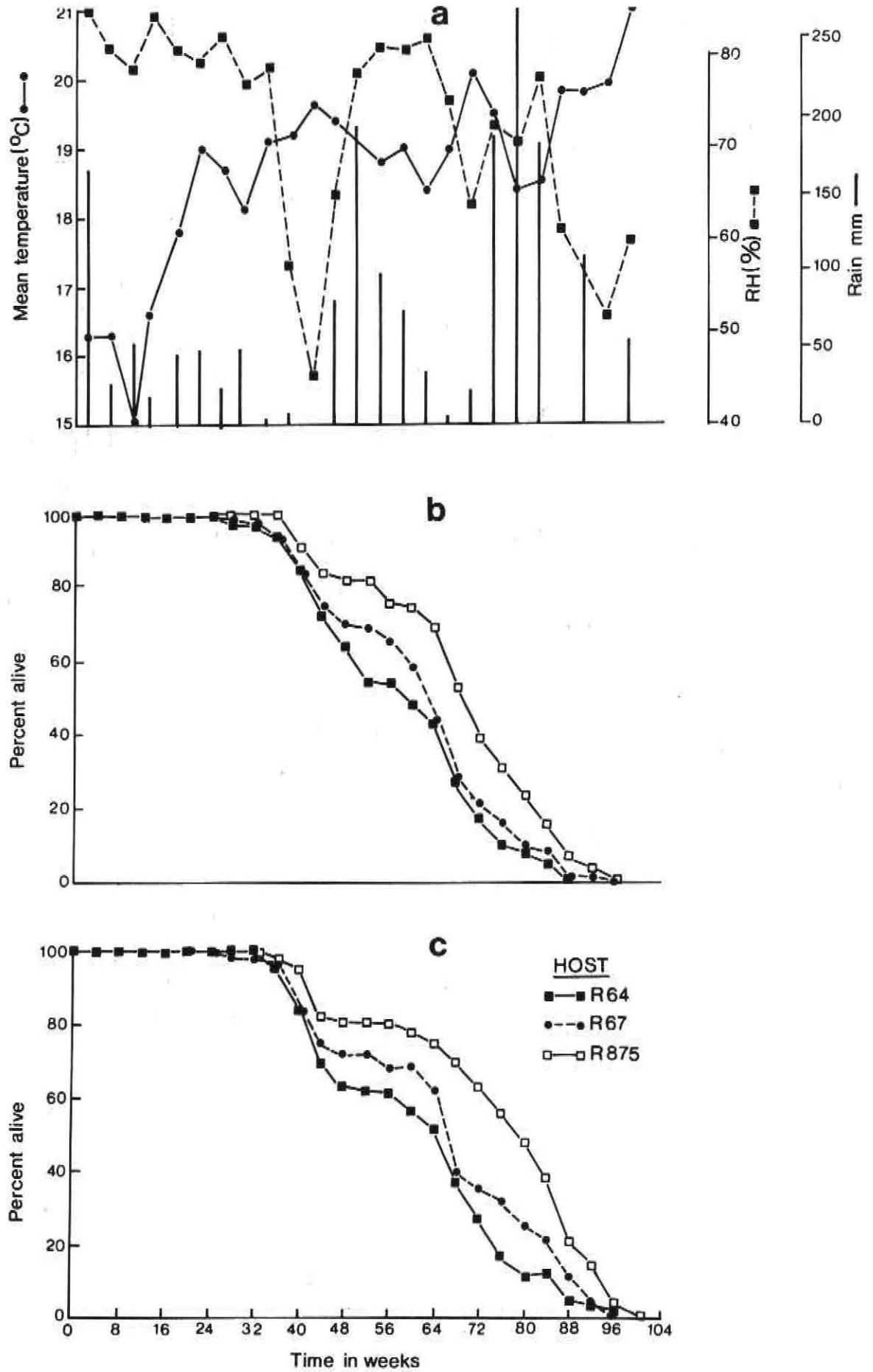


Figure 5. Relationship between temperature, relative humidity (RH) and rainfall (a), male (b), and female (c) *R. appendiculatus* survival as seen in phase 1.

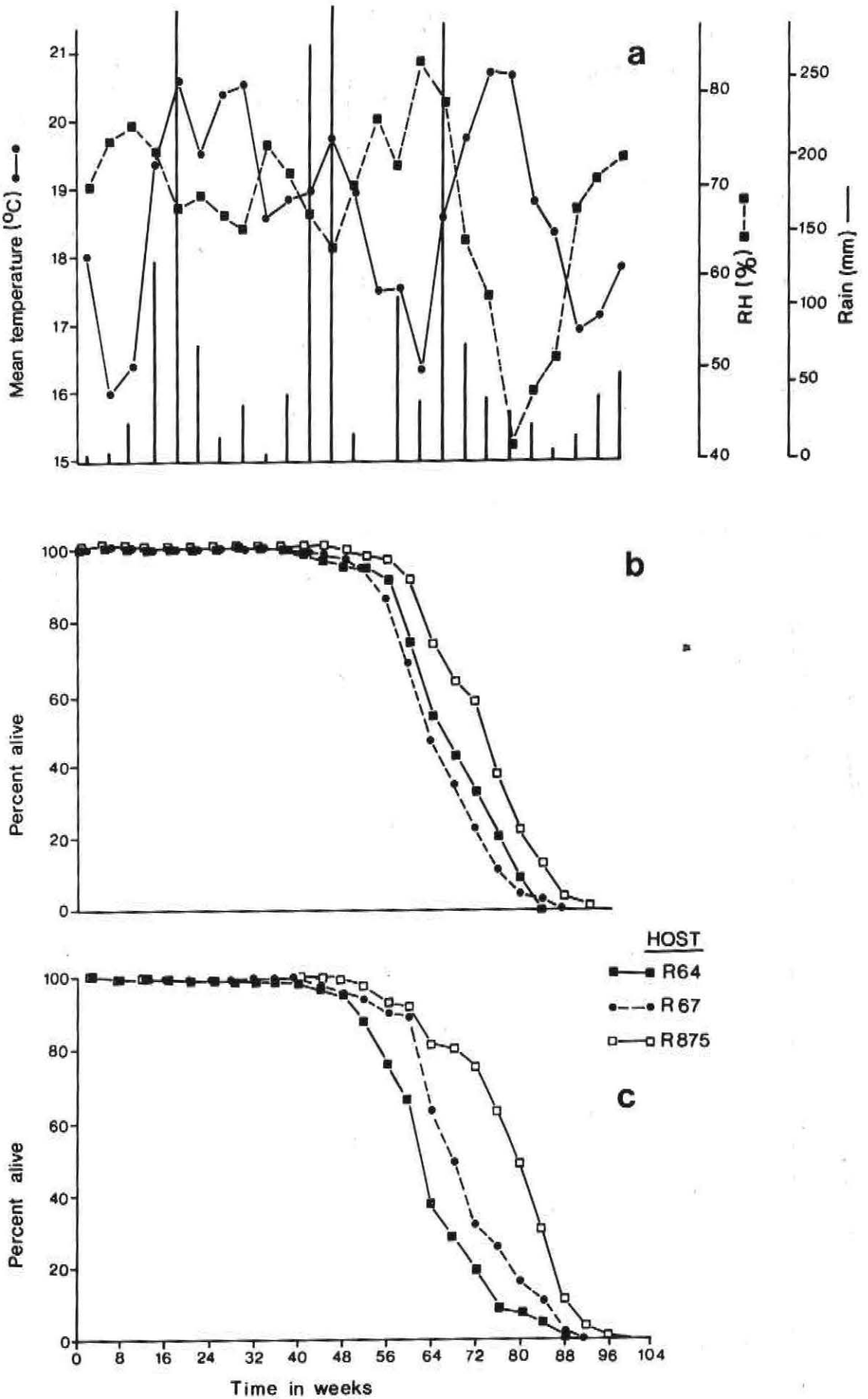


Figure 6. Relationship between temperature, relative humidity (RH) and rainfall, (a), male (b), and female (c) *R. appendiculatus* as seen in phase 2.

Table 11 : Larval and nymphal mortality among ticks fed on immunized and control mice.

	Number Ticks Applied	Per cent Mortality Larvae	Number Ticks Applied	Per Cent Mortality Nymphs
1. A_1 A_u BCD				
Test FCA	100	52	50	52
Control	100	5	50	24
Test FIA	80	52	40	50
Control	100	27.5	30	20
2. MIDGUT HOMOGENATE				
Test FCA	80	72.5	30	40.00
Control	80	11.22	30	3.33
Test FIA	100	33	40	40.00
Control	100	10.0	40	0.00
3. B-PROTEIN FRACTION				
Test FCA	100	23	40	37.5
Control	100	3.22	40	30.0
Test FIA	100	18.67	50	34.0
Control	100	2.00	50	0.0
4. A_1 PROTEIN FRACTION				
Test FCA	80	92.25	40	11.11
Control	80	14.81	20	11.11
Test FIA	60	52.69	20	0
Control	80	0.00	20	0
5. LARVAL HOMOGENATE (UNFED)				
Test FCA	80	6.25	40	17.39
Control	80	6.0	40	9.17
Test FIA	80	0.0	40	4.54
Control	100	0.0	40	11.11
6. NYMPHAL HOMOGENATE (UNFED)				
Test FCA			40	42.81
Control			30	0.00
Test FIA	60	3.77		
Control	60	0.00		
7. A_4 PROTEIN FRACTION				
Test FCA	100	11.38	40	12.50
Control	100	3.49	40	5.26
Test FIA	100	1.11	40	50.00
Control	100	4.16	40	50.00

In summary then, newly moulted ticks appear to be more able to withstand bad weather than aging ticks, and a sharp decline in survival is likely to be triggered by dry hot weather in aging ticks. The survival of ticks fed on highly resistant hosts is much poorer than on susceptible hosts. The relationship between tick size and survival increases directly with host resistance.

Type II resistance in mice

C.K.A. Mango and R. Ojowa

Investigations were initiated in mice to screen both crude and semi-purified tick materials for antigenic activity. The material included crude unfed larval and nymphal homogenate and midgut from partially engorged females; and semi-purified protein fractions from 24 h old *R. appendiculatus* eggs. The protein fractions were A_1, A_4, B and CD

The aim of this work is to identify candidate material for immunizing cattle hosts against ticks in an attempt to control *R. appendiculatus*, the vector of East Coast Fever.

Groups of mice were injected with either crude or semi-purified material in either Freund's complete or incomplete adjuvant. They were boosted fortnightly and were given a total of two boosters and then bled and their sera checked for circulating antibodies by Ouchterlony double immuno-diffusion method. They were then challenged with either larvae, nymphs or adult females. Ticks used in challenge experiments were observed for successful attachment or failure to attach, mortality, successful moulting, egg-laying and hatching.

Preliminary results (table 11) indicate varying degrees of protection based on larval and nymphal mortality in mice immunized with different materials. More experiments in mice are in progress and others are planned to confirm the preliminary results in either rabbits or cattle.

In vitro cultivation of embryonic cells for attempted induction of resistance to tick infestation

M. Nyindo

The *in vitro* cultivation of embryonic cells of *R. appendiculatus* and the use of such cells for the attempted induction of resistance to tick infestation in rabbits and in cattle is in progress. Cells grown for about 8 months were immunogenic in rabbits and high antibody titres were produced as detected by immunodiffusion techniques.

The monoclonal antibody technology is already established and it is anticipated that it will contribute not only to the research in the immunological studies of host resistance to tick infestation but also to other programmes at ICIPE.

Tsetse Research Programme

The programme has endeavoured to strike a balance between basic research and field-oriented ecological work. As is evident from the individual reports below, the field work projects at Nkruman Escarpment in the Rift Valley of Kenya and in Lambwe Valley, western Kenya have placed a lot of emphasis on the ecology of **Glossina pallidipes** *and its role in the transmission of African trypanosomiasis. Laboratory studies on the vector efficiency have indeed complemented the ecological studies.*

One very important aspect of the programme as a whole has been to focus attention on **Glossina pallidipes,** *a notorious vector of human and animal trypanosomiasis in Eastern Africa. The recent success in establishing a breeding colony of this tsetse species at Mbita Point Field Station, is an effort worth all the necessary support. It is hoped that the programme will now be well placed to make an impact in solving crucial problems relevant to tsetse control.*

Nkruman tsetse research project

R.D. Dransfield, M.F. Chaudhury, T.K. Golder, S.R. Tarimo

The Nkruman Tsetse Project commenced in May 1983 with the long-term objective of developing a dynamic epidemiological model of trypanosomiais challenge to cattle. A multi-disciplinary team approach was adopted for simultaneous studies on vector population dynamics and trypanosome infection rates. Sampling is carried out monthly over an 8.5 km transect from the Ewaso Ngiro River to the Nkruman Escarpment where large herds of pastoralists' cattle are usually present. So far work has concentrated on vector/host distribution and abundance and vector infection rate; this will soon be augmented by studies on host infection rates in collaboration with other research institutes.

Vector distribution and abundance

R.D. Dransfield, M.F. Chaudhury, T.K. Golder

Biconical traps have been used for sampling *Glossina pallidipes* and *Glossina longipennis* with pairs of traps 50 m apart, set at 500 m intervals. *G. pallidipes* is the most abundant species at Nkruman and in contrast to other areas in Kenya studied at ICIPE (Coast Province and Lambwe Valley), it has shown a marked seasonal change in distribution and abundance (fig. 1). For several months after the main rains in May it occurred in all vegetation types including the open plains, but by December, numbers had declined and it was restricted to wooded areas. Catches of *G. longipennis* also declined sharply as the dry season progressed.

Research is continuing on the relationship between relative densities given by the biconical traps and absolute densities in the different vegetation types. One aspect of this is to determine whether the site effect, a major source of variability when using visual attraction traps, can be reduced by adding odour to the trap. For this purpose the dose response curves to certain attractive chemicals had first to be determined. So far acetone and methyl ethyl ketone have been tested in replicated 4 x 4 latin squares. With acetone the catch increased significantly ($P < 0.05$) over the range of dose rates used (table 1) but with methyl ethyl

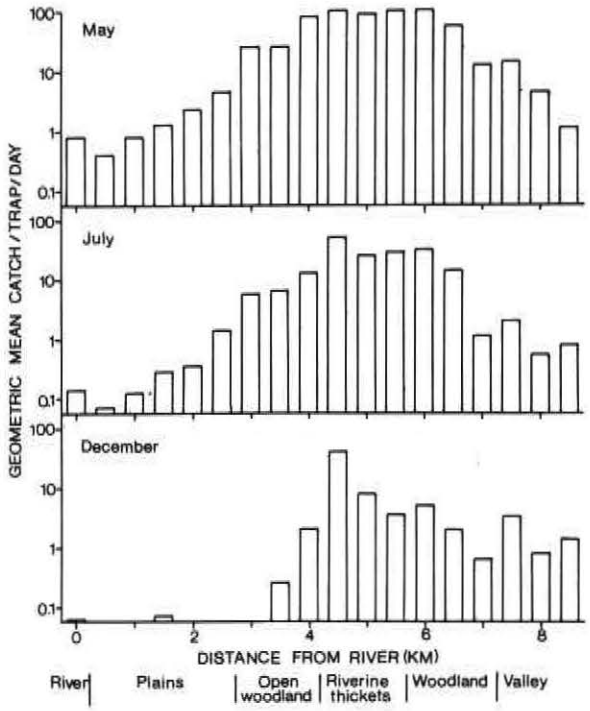


Figure 1. Apparent density of female *Glossina pallidipes* between the Ewaso Ngiro River and the Nkruman Escarpment, 1983.

ketone the catch increased at the lowest dose ($P < 0.05$) and they levelled off or declined at higher doses.

Changes in age structure of the *G. pallidipes* population through the year have been monitored by ovarian dissection for females (fig. 2) and wing fray for males. The percentage of teneral (Oa) females in the catch increased markedly after the rains in May, indicating a variable recruitment rate to the adult population. This will necessitate modifications to current techniques for estimating mortality rates from such data, since they assume a constant recruitment rate to a stationary population.

The rate of natural abortions in the female flies examined was generally low between May and December varying from 0.52% in July to 4.17% in November. Examination of *in utero* larval stages however indicated that at least 10% of the developing larvae had below normal growth rates in November and December; the progeny resulting from such larvae are unlikely to have been viable.

Table 1. Mean number of *Glossina pallidipes* caught per trap per six hour period using different dose rates of acetone.

Approx. dose rate (mg/hour)	Mean number Males	Females
0	17.0	24.5
100	25.5	26.1
500	28.6	35.6
2500	36.4	46.4

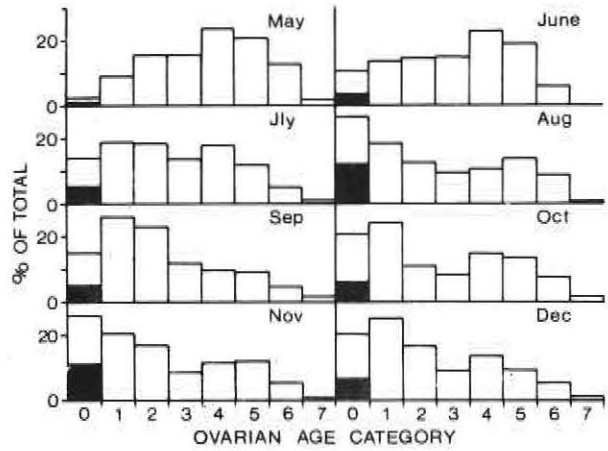


Figure 2. Age distribution of female *Glossina pallidipes* at Nkruman from May to December, 1983 (n = 158, 300, 409, 404, 582, 748, 648, 400 respectively). O category is divided into Oa and Ob.

Highest teneral percentages (7—11%) were found in the riverine thickets and in the valley at the edge of the escarpment. *G. pallidipes* pupae have been found in some sites within these areas usually in isolated clumps of thick vegetation.

Biconical traps and water traps have also been used for sampling other species of biting flies within the area that are likely to be involved in mechanical transmission of trypanosomiasis. These include *Atyolotus agrestis*, *A. fuscipes*, *Tabanus taeniola*, *Stomoxys niger*, *Haematobia minuta*, *Haematobosca uniseriata* and *Musca crassirostris*. Most of these species are commonest in the open plains where tsetse densities are lowest.

Host distribution and abundance

R.D. Dransfield, S.R. Tarimo,

An approximate index of both cattle and wild game distribution over the transect has been obtained by recording sightings made when collecting traps at 06.00, 09.00, 12.00 and 15.00 hours. Several hundred head of cattle were present in the open plains in July and August, together with Grant's gazelle and zebra, but overgrazing resulted in the movement of the Maasai and their cattle to the base of the escarpment and areas further south where better grazing was available. Sheep and goats however were taken into the wooded areas where tsetse densities were highest. Warthog were observed in all vegetation types, but buffalo and bushbuck were restricted to wooded areas with the exception of the valley where the terrain is too rocky. Tsetse blood meal analysis is being carried out in collaboration with ILRAD in order to relate feeding patterns to host availability in the different areas.

Vector infection rate

S.R. Tarimo, T.K. Golder

The infection rate in both *G. pallidipes* and *G. longipennis* has been monitored over the course of the study. The changes over time of the percent infection rate of *G. pallidipes* for the different trypanosome species have been plotted in figure 3. *Trypanosoma congolense* appears to be less common than *T. vivax* and the infection rate was relatively constant throughout the period. The infection rate with *T. vivax* was higher and this species accounted for most of the fluctuation in the total infection rate. Highest infection rates were recorded in May, July and November for males, and May, August and November for females. Infection rates of flies caught in different vegetation types were compared, and the highest incidence was found at the river.

Factors which could be affecting infection rate such as temperature, feeding patterns and age structure of the tsetse population will be related to infection rate as soon as adequate data are obtained. Preliminary results for *T. vivax* however suggest an exponential rather than linear or asymptotic relationship between age and infection rate for both males (using wing fray) and females (using ovarian age). This implies that infected flies are living longer than uninfected flies, or that the probability of picking up an infection increases with age.

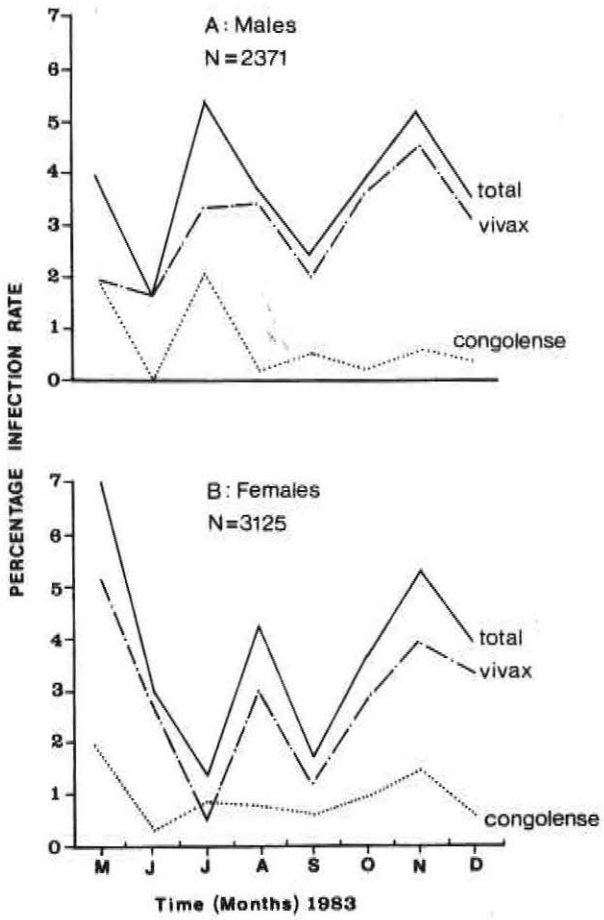


Figure 3. Changes in Trypanosome infection rate in *Glossina pallidipes* through time at Nkruman.

Tsetse trapping studies

M.L.A. Owaga

Trapping studies for *G. pallidipes*, started in 1981 in Nkruman Escarpment, continued. In previous years, revolving attachments to the biconical trap were developed and used to study and improve the quality of trap catches with regard to age structure of females and nutritional condition of the tsetse caught. Various modifications of the biconical trap with stationary components were also developed to enhance the catch size from the biconical trap.

In mid-1983 studies were initiated on olfactory attractants, for tsetse, incorporated in biconical traps. The material chosen was waste products from wild hosts of tsetse, and included faeces, body wash and urine from buffalo. A mixture of the three products was placed in a container inside the traps. The results were promising and the traps baited with this mixture caught significantly more *G. pallidipes* than the non-baited traps. As a result of these preliminary observations, more detailed work was undertaken, each type of waste product being used separately. A 5 x 5 latin square experimental design was used and the three scent baits were compared with the standard biconical trap and a modified trap (4t trap) which had given significantly better results than the standard trap in the previous year. The 4t trap has four black strips hanging from the base of the upper cone of the trap, otherwise it is basically a biconical trap. The results of this work are presented in table 2. The trap baited with buffalo urine performed 10 times better than the standard trap with regard to female tsetse captured, and about 6 times with regard to males. The trap baited with buffalo faeces was equal in effectiveness to the 4t trap and both were 2 times better than the standard trap with regard to female tsetse caught and 1.3 times better with regard to males. The body wash baited trap was not significantly different from the standard trap.

Buffalo urine, therefore, proved to be the most effective olfactory attractant for *G. pallidipes*. Furthermore, it captured large numbers of females, almost twice as many females as males. This is very important if it is to be refined for use in control campaigns.

In collaboration with the Chemistry and Bioassay Research Unit and the Sensory Physiology Research Unit, studies have already been

Table 2. Catches of *G. pallidipes* in scent baited and unbaited biconical traps

Experiment sets	Trap A		Trap B		Trab C		Trap D		Trap E	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
	78	122	22	11	67	38	78	32	1702	625
	123	131	230	107	54	39	284	178	688	334
	120	46	467	251	71	32	57	77	588	484
1	138	62	233	142	98	75	98	63	2328	1557
	53	97	253	73	61	50	315	157	650	425
	4	26	48	35	5	12	52	53	122	82
	19	80	199	140	110	61	86	49	113	101
2	82	52	140	122	27	22	254	199	478	258
	26	15	76	37	14	10	37	20	177	67
	11	32	19	34	13	25	57	55	98	89
	11	11	47	59	51	64	66	71	190	260
	6	24	14	12	6	3	7	11	131	127
3	14	26	3	7	27	60	29	83	126	203
	21	44	12	29	11	27	11	5	53	45
	33	81	27	24	48	59	37	42	226	237
Total	739	849	1790	1084	663	577	1468	1095	7670	4894

Traps
A = Standard biconical trap
B = 4t trap
C = Biconical trap baited with buffalo body wash
D = Biconical trap baited with buffalo faeces
E = Biconical trap baited with buffalo urine

initiated to determine the chemical nature of the attractive elements. Meanwhile, field trials are continuing.

G. pallidipes ecological studies in Lambwe Valley

D. A. Turner

Routine monthly monitoring of *G. pallidipes* using biconical traps continued to the end of 1983 to assess the long-term effects on the population of an endosulfan aerial spraying campaign carried out in 1981. The immediate effects of the spraying were to reduce the population by 99.9% in its classical habitats of thicket and woodland in the floor of the valley and by 99% in the coniferous plantation adjacent to the valley on the eastern escarpment wall.

Nevertheless, on the basis of a crude estimate of total fly numbers in the valley made by extrapolating mark/release/recapture data from a small area, it was estimated that several thousand flies remained after spraying. The population in the thicket subsequently recovered to its original level 12 months afterwards, but in woodland and plantation it only reached 20—30% two years after spraying.

The thicket population maintained a rough equilibrium density after recovery, indicating that density dependent processes had re-established a regulatory effect. There was some evidence that this was brought about in part by massive spontaneous fly movement from a thicket habitat which had attained saturation density into adjacent, virtually unoccupied habitats since density changes in these habitats, at the time the thicket population reached saturation density, were greatly in excess of those which otherwise occurred as a result of the combined effects of population growth, climatically-induced variation and random sampling errors.

A provisional estimate was made of the rate of recovery of the population in thicket, by fitting the post-spray data to a logistic equation of population growth. The value for r, the

instantaneous rate of increase, was 0.015 per day, equivalent to a finite rate of increase of 1.610 per month and a population doubling time of 45 days. The post-spray growth rate was close to the temperature-dependent maximum possible monthly rate of increase for tsetse in the absence of any mortality, and the value for the instantaneous rate of increase was very similar to those obtained for optimally productive laboratory colonies of *G. austeni* and *G. morsitans.*

The rapid growth of the population (in thicket) after spraying and its subsequent stabilization were manifestations of the general weakness of abiotic fores on the population, (bioclimatic conditions of temperature and saturation deficit are in fact optimal for tsetse in the Lambwe Valley) and of strong density-dependent regulation by biotic processes. The actual nature and relative importance of density-dependent processes remain largely undetermined at present. Some evidence of population regulation by fly movement has been mentioned; other studies (see below) have shown that parasites and pathogens of adult *G. pallidipes* play no important role, and that reproductive losses, such as from abortion, are so low that the likelihood of density-dependent processes acting directly on tsetse fecundity was negligible.

G. pallidipes is clearly entrenched in the Lambwe Valley, with environmental conditions entirely in its favour. A rational approach to control or eradication would have to take these factors into account, and it may be that aerial spraying technology has not yet reached the standard of perfection required to surmount the strong environmental constraints which exist there, at least at an acceptable level of cost. In this context it may be mentioned that a further aerial spraying campaign using pyrethrum was carried out in mid-1983. Our monitoring failed to detect any notable impact whatsoever of this on the tsetse population.

A study of the nature, frequency and causes of reproductive loss in *G. pallidipes* populations of the Lambwe Valley was concluded in 1983. Daily collections from biconical traps in which tsetse were entrapped for varying periods up to 9 h were supplemented by a series of hourly captures which gave less biased data. Ovarian abnormalities were rare and abortion was the only major source of loss of fecundity. Total reproductive loss was within the range 1.60-1.97% (0.23% ovarian loss plus 1.37% abortions or 1.74% total empty uteri). The increase of empty uteri was higher in daily collections, due mainly to premature parturitions brought about by the stress of prolonged confinement in traps and handling. Abortions were significantly more frequent in females in their first reproductive cycle (ovarian category 1) than in older parous females, possibly as a result of failure to become inseminated before first ovulation. No relationship between the frequency of abortion and climate, tsetse density or pathogen and parasite infection could be demonstrated.

G. pallidipes population dynamics in relation to the epidemiology of African trypanosomiasis

L.H. Otieno, R. Tarimo, N. Darji

The intention of this study was to examine and compare *G. pallidipes* population dynamics, age structure and the incidence of both human and animal trypanosomiasis in two study sites in the Ruma National Park, Lambwe Valley. Human trypanosomiasis in Lambwe Valley has for a long time been known to be sporadic, involving hunters, poachers and passers-by in transient contact with tsetse flies. The situation is potentially quite dangerous as the surrounding areas may soon be affected. The vegetation around the Ruma National Park has become more dense thus creating favourable habitat for *G. pallidipes* outside the National Park area. Furthermore, the transmission of the disease has recently been reported to be of a peridomestic nature involving all members of a family, and earlier observations have incriminated a high proportion of domestic livestock as hosts of *Trypanosoma rhodesiense.* The implication therefore is that we are dealing with a zoonotic situation in Lambwe Valley.

Recently studies have been initiated in two areas inside the Ruma National Park at two sites situated 15 km apart, quite close to human settlements. One area (Riamakanga) appears particularly bad in terms of human sleeping sickness, as most of the cases reported seem to have been contracted in or near this study site. The other site (Ruma), however, would appear to be free of human trypanosomiasis, but animal trypanosomiasis is highly enzootic in the two study areas.

G. pallidipes population was sampled using biconical traps once every month (for 4 days) beginning from July 1983. The number of flies caught per trap (six traps in each study area) was noted. A sample of flies caught was aged according to the method of Saunders and some of these were dissected and examined for the presence of trypanosome infection. Preliminary results obtained are shown in table 3.

Fly population density appeared to be very high in the Ruma thicket. This was in sharp contrast to observations made in Riamakanga

Table 3. Monthly sampling of *G. pallidipes* in Lambwe Valley (Ruma and Riamakanga thickets)

	RUMA THICKET		RIAMAKANGA THICKET	
Month	No. flies per trap per day	Infection rates %	No. flies per trap per day	Infection rates %
July	302.5	17.1	114.7	18.7
August	334.9	22.7	168.0	18.9
September	371.1	25.0	126.9	14.6
October	382.3	16.2	65.7	16.7
November	469.8	12.7	116.3	15.7
December	251.8	3.5	149.8	4.5

thicket where the density was less than half that obtained for Ruma. In both areas the results obtained indicated no relationship between the fly density and the trypanosome infection rates.

Trypanosome infection rates in flies from Ruma thicket increased steadily during the first three months of study and then declined during the subsequent months. At Riamakanga, trypanosome infection rates appeared stable, except during the last month (December) when there was a very sharp drop in the incidence of trypanosome infection in the flies examined. This decline was observed in both sites.

These observations are being studied in relation to fly age structure. *Trypanosoma brucei*-like trypanosomes observed were isolated and stabilates prepared. These are currently being characterized using blood-incubation infectivity tests (BIIT), isoenzymes and DNA probes.

Low vector strains of tsetse

L.H. Otieno, P. Onyango, M. Chimtawi

Extensive investigations have been carried out to determine factors responsible for the low *T. brucei* infections in the tsetse fly. Recent observations at Langford Laboratories, Bristol, England, suggest that susceptibility of *G. m. morsitans* to *T. congolense* may be under genetic control. In the present investigations attempts are being made to see whether there are any differences between the transmissibility of two different stocks of *T. brucei* by two species of *Glossina*, and if so what factors are responsible.

Two stocks of *T. brucei*, 5 and 11, which were isolated from *G. pallidipes* in Lambwe Valley, western Kenya, were used in the transmission experiments. Three attempts to transmit these stocks by *G. m. morsitans* showed that stock 11 was easily transmissible (5.6—7.1%) by male *G.m. morsitans* whereas transmission of stock 5 failed throughout these attempts.

Since stock 5 was isolated originally from *G. pallidipes*, it was interesting to see if *G. pallidipes* reared in the laboratory would similary fail to transmit it. Newly emerged male and female *G. pallidipes* were fed on rats infected with stock 5 and after 30 days the flies were dissected and examined for trypanosome infection. Results showed that the stock was transmissible by *G. pallidipes*. The following results were obtained: 1.8% salivary glands and 5.3% midgut infections for male *G. pallidipes*, and 2% salivary glands and 8% midgut infections for female flies.

Transmission of *T. brucei* EATRO 1969 by *G. m. morsitans* and *G. pallidipes*

T. brucei EATRO 1969 is a stock easily transmitted by *G. m. morsitans*. It was important to see if laboratory-reared *G. pallidipes* could similarly transmit the stock as readily as *G. m. morsitans*. Newly emerged *G. m. morsitans* and *G. pallidipes* were fed on rats heavily infected with *T. brucei* EATRO 1969. The flies were maintained on rabbits and after 30 days, the flies were killed and examined for trypanosome infection. The results obtained showed that the two species readily transmitted EATRO 1969, however, male *G. m. morsitans* appeared to be a much better transmitter of this stock (37.5% salivary gland infections) compared to male *G. pallidipes* (2.5%) and female *G. m. morsitans* (13.6%) and male *G. pallidipes* (14.7%).

From these observations it would appear that both *G. m. morsitans* and *G. pallidipes* are good transmitters of *T. brucei* EATRO 1969. The failure to transmit stock 5 by *G. m. morsitans*, however, suggests some peculiarity

with this particular *T. brucei* strain. Studies are in progress to determine some of these peculiarities. Two of the female flies with infections in the salivary glands showed also enlarged salivary glands which on electron microscopy (EM) examination revealed virus-like particles. EM micrographs revealed tumor-like protrusions on the surface of enlarged glands. These contained a lot of trypanosomes (see fig. 4).

Survival and fertility of *G. m. morsitans* fed on rabbits immunized with various antigens

G. P. Kaaya

Pathophysiological effects of feeding female *G. m. morsitans* on rabbits immunized with tsetse trypsin, ovaries, thoracic muscles, gut bacteria, brain and thoracic ganglion, or with bovine trypsin or trypsin/chymotrypsin mixture were investigated. A fairly strong cross-reaction between tsetse and bovine trypsin was observed, suggesting the presence of common surface antigenic determinants between the two types of trypsin molecules.

Mortality rate was significantly high only in the flies maintained on rabbits immunized with tsetse and bovine trypsin. The group maintained on the rabbit immunized with a mixture of bovine trypsin and chymotrypsin also showed a high mortality rate but was statistically non-significant. The affected flies showed signs of indigestion for 3 to 4 days following the initial blood meal and then died.

Fecundity, expressed as number of pupae produced per fly per 30 days was significantly decreased in flies maintained on the rabbit immunized with bovine trypsin. Flies maintained on the rabbit immunized with tsetse ovaries also showed decreased fecundity but statistically non-significant. Pupae from the flies maintained on rabbits immunized with tsetse or bovine trypsin had significantly decreased mean pupae weights. Pupae from flies

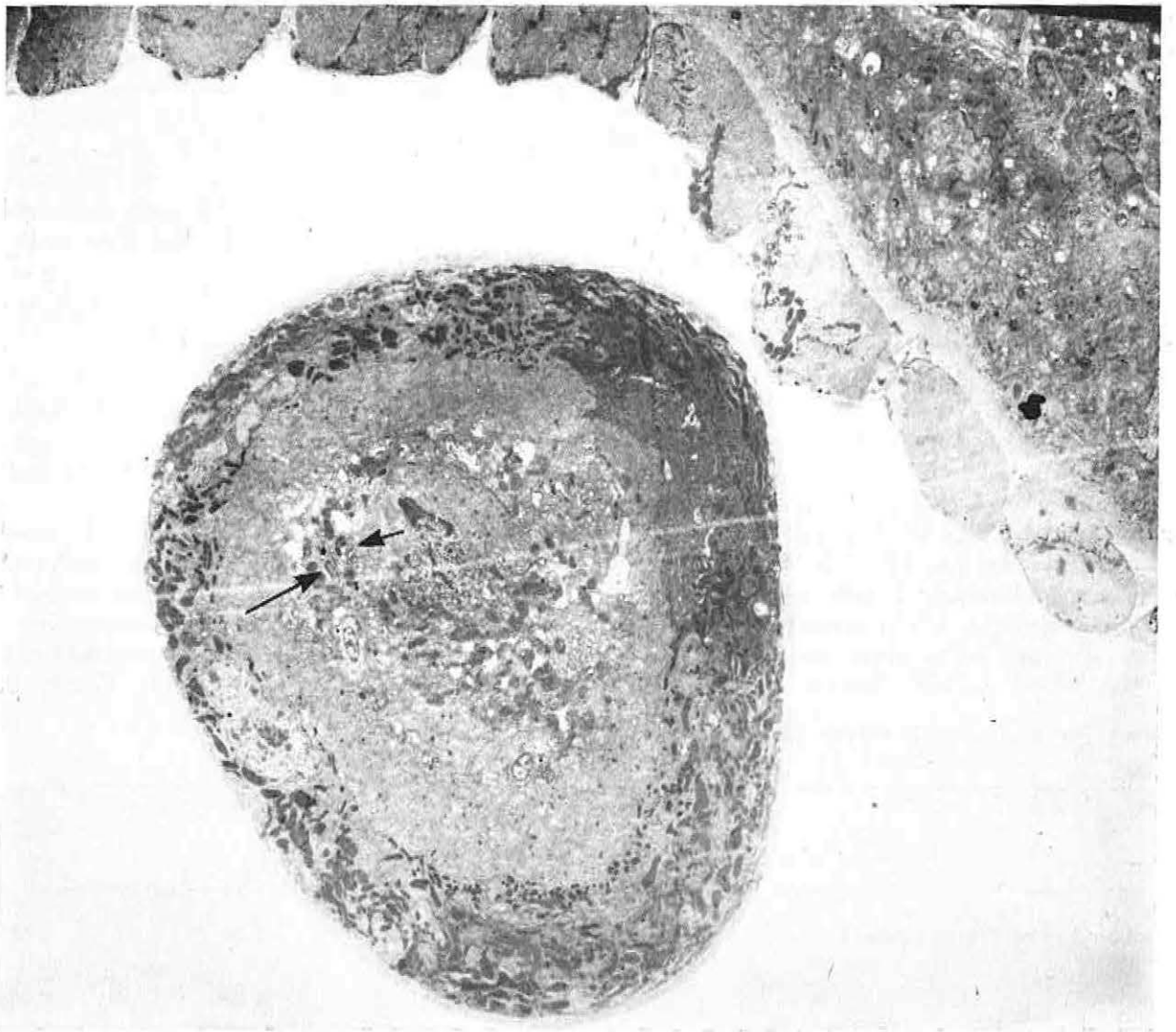


Figure 4. A section of an enlarged *Glossina pallidipes* salivary glands showing a tumor-like protrusion. Trypanosomes can be seen in the tumor (see arrow)

maintained on rabbits immunized with tsetse thoracic nuscles, tsetse trypsin and tsetse gut bacteria had the highest incidence of pupal mortality, although not significant at 5% level. When dissected, puparia were found to be either completely empty, containing decomposed material or containing fully formed but dead flies. A pilot experiment on infection rates with *T. b. brucei* in flies maintained on the immunized rabbits and on saline-injected controls showed no difference.

G. pallidipes rearing at Mbita Point

L. H. Otieno

G. pallidipes collected from Lambwe Valley were introduced to ICIPE's Mbita Point Field Station in March 1983. Wild-caught flies were maintained on rabbit ears in a temporary insectary with neither temperature nor humidity control. There was heavy mortality and abortions when they were first brought to the insectary, and death was thought to have been caused by stress brought about by caging and over-crowding. On reducing the number to 10 flies per cage, abortions were considerably reduced. The first larvipositions in the insectary tended to be under size but subsequent larvipositions were of good weight (33-40 mg). The total number of mated females, as of 15 December 1983, was 1280.

Infectivity of bloodstream form *T. brucei* to *G. morsitans* after cation exchange chromatography separation

T.K. Golder, N. Darji, P. Onyango, E. Mpanga

In the blood stream of their vertebrate hosts, the salivarian trypanosomes are highly heterogeneous. They are highly pheomorphic, variable in surface antigens and there is some evidence that the sexual stages may exist. We have previously reported preliminary results of a chromatographic technique which appeared to separate short stumpy forms from the slender forms in the blood (ICIPE Annual Report 1982). However, after many replicates, we have found that the results are more variable than we suspected. While it is true that our initial results gave us more pure populations of stumpy forms, we now find that the eluting populations can often be heterogeneous with regard to the morphology. Nevertheless, it is of interest that there are two subpopulations of the bloodstream forms which differ in their surface charge when pH and ionic strength of the medium are changed.

ELUTING TECHNIQUE

Rat blood, at peak parasitemia, is passed through DEAE-cellulose column to separate trypanosomes from the formed elements of the blood. Parasites that elute are centrifuged twice (1600 g x 10 min) and resuspended in phosphate-buffered glucose (PSG) pH 7.0 and I = 0.036, then applied to a carboxy-methyl cellulose column equilibrated with the same buffer. Some of the parasites elute rapidly, in a sharp peak, just after the column void volume. The rest of the parasites remain bound to the column. Bound parasites are recovered by the application of PSG pH 8.00, I = 0.22. These parasites also elute in a sharp peak after the void volume. The physiological role of the parasites in the two peaks is unknown, but preliminary results from experiments conducted in our laboratory suggest that peak 2 parasites are more infective to tsetse flies than peak 1 cells.

EXPERIMENTAL PROCEDURE

All glassware, buffers and the membrane feeding apparatus were sterilized previously and extreme care was taken to conduct the experiment under aseptic conditions.

The two peaks of the parasites *T. brucei* EATRO 1969), were eluted by the technique described above, the parasites were washed 3 times with PSG and the approximate number of parasites was estimated in each population by Neubauer haemocytometer counts. Finally the

Table 4: Performance of the colony during the period, August to December 1983

	(Original) flies emerging from pupae larviposited by wild-caught flies	F1	F2
Pupal production (no. of pupae/♀ /month)	1.12	1.23	1.67
Longevity	0.65	0.88	0.74
Fecundity	0.42	0.50	0.51

parasites were suspended in defibrinated clean rat blood and then used for *in vitro* feeding.

Newly emerged male *G. m. morsitans*, were fed on these parasites through silicone membrane. After the first infective blood meal, the flies were maintained on clean rabbits for weeks, and were then dissected and scanned for gut, proboscis and salivary gland infection.

The results of 3 experiments show that the salivary gland infection for peak 1 was 6.2% (n = 113), and for peak two 23.9% (n = 121). X^2 = 14.42 df = 1; P<001. These preliminary data show a significant difference between the infectivity to tsetse flies of the two populations.

Temperature effect on *G. pallidipes* reproduction in the laboratory

M.F.B. Chaudhury, D. Uvyu

The present study was undertaken to investigate reproductive behaviour and performance of female *G. pallidipes* at various temperatures and to find ways to improve the performance of the colony. The temperature generally used for rearing *Glossina* sp. including *G. pallidipes* is about 25°C although *G. pallidipes* can survive and reproduce at higher temperatures in the field. Furthermore, a breeding colony maintained at a higher temperature may be desirable for mass-rearing due to increased rate of development.

The strain of *G. pallidipes* studied originated from the pupae received from a colony maintained in the laboratory of the Uganda Trypanosomiasis Research Organization, Tororo. To study the effect of temperature on behaviour, longevity and reproduction, flies were maintained at 3 different temperatures, 25°C, 27°C ± 0.5°C and 29°C ± 0.5°C with a 12 h photophase and a light intensity of about 100 lux at the level of holding shelves. Relative humidity was 70 ± 10% for all the temperature conditions.

Female *G. pallidipes* reared at 25° ± 0.5°C did not become fully receptive to sexually mature males (12 to 14 days old) until they were 8 to 9 days old. The females mated at this age, 8 to 10 days old, inseminated successfully but most of them (about 70%) did not produce the first larva. However, during most of the subsequent cycles they produced normal larvae. About 12% of the females produced non-viable larvae in two to three consecutive cycles. Total production for the entire life averaged about five larvae per female.

Females emerged from the pupae stored at 25° ± 0.5°C and reared at 27° ± 0.5°C became fully receptive to 12 to 14 day-old males between the age of 5 and 6 days after emergence. Most of these females were successfully inseminated by the males when kept together for 24 h. More than 90% of these females produced their first progeny when they were 18 or 19 days old, although some of these larvae produced pupae of sub-normal size. Total production for the entire life was 8 larvae per female on average.

Rearing females at 29° ± 0.5°C was not successful because most of them did not take large enough blood meal for proper development of reproductive organs.

When pupae were kept at 25° ± 0.5°C, 27° ± 0.5°C and 29° ± 0.5°C for their entire pupal life, the pupal periods (pupariation to emergence of adult) were 29, and 23 days respectively on an average. Less than 50% of the flies emerged from the pupae kept at 29° ± 0.5°C most of which exhibited crippled wings. All flies emerging (95%) from the pupae kept at 27° ± 0.5°C had normal wings. Emergence rate from the pupae kept at 25° ± 5°C was lower (80%) but all flies had normal wings. All flies emerged from the pupae kept at the 2 lower temperatures fed and mated normally.

To study the effect of temperature on oocyte development, adult *G. pallidipes* females emerged from pupae stored at 25° ± 0.5°C were maintained at 3 different temperatures and samples were drawn at intervals to dissect and examine ovarian development. Because of the inability to take normal blood meal, oocyte development was significantly retarded in the females maintained at temperature 29° ± 0.5°C. There was no significant difference between the rate of oocyte growth (at least the first 2 follicles) in females reared at 25° ± 0.5°C and 27° ± 5°C, although the growth in follicles of females reared at the higher temperature was to some extent accelerated. A mature chorionated egg was present in most of the females examined on the 8th or 9th day after emergence when flies were reared at 25° ± 0.5°C, whereas females reared at 27° ± 0.5°C produced the first mature egg on day 8 in most cases and on day 7 in a few cases.

Results from the present study show that slight differences in temperature have some effect on receptivity, longevity, reproduction and to some extent on ovarian development in *G. pallidipes* females. Change in temperature clearly influences puparial development.

There is no doubt that temperature should be considered carefully for successful rearing of *G. pallidipes*. Although temperature of about 25°C has been used to rear species of *Glossina* including *G. pallipipes* by some workers, this temperature would not appear to be the most suitable for rearing this species.

Results of the present study clearly show that by raising the temperature by about 2°C for adult rearing, the receptivity of the females

can be increased, enabling them to mate when they are younger thus not missing the first cycle and perhaps reducing the chances of abortion during later cycles. Although the reason for higher abortion rates at 25°C is yet unknown, the phenomenon of missing the first cycle, when reared at this temperature, appears to be related to late mating, resulting in expulsion of an unfertilized first egg following ovulation which occurs within a few hours of mating.

In some cases, expelled eggs and spermatophores were observed together in the same vials where females were allowed to rest after mating at 8 to 10 days after emergence.

Longevity and fecundity also appeared to be comparatively better when the females were reared at 27° ± 0.5°C.

Results also suggest that the temperature for storing pupae should not exceed 27° ± 0.5°C. and probably should not be less than 25°C.

Related activities

Behaviour and Population Ecology" was held at ICIPE on 23—30 October 1983. The workshop was attended by over 40 scientists from government institutes and international organizations in Africa and Europe.

Dr. D.A. Turner took leave of absence from the centre between November 1983 and February 1984, to act as a short-term consultant for the International Atomic Energy Agency to carry out a tsetse distribution survey on the Island of Zanzibar, Republic of Tanzania.

In the course of the year, the following collaborative research projects have been initiated or are at an advanced stage: Effects of pesticides on trypanosome infected tsetse (with ILRAD); Tsetse blood meal analysis (with ILRAD); Epidemiology of African trypanosomiasis on a ranch; development of trypanosomiasis challenge model [with Tanga Trypanosomiasis Research Institute, (TTRI) Tanzania] Use of insecticide impregnated screens and traps to reduce *G. pallidipes* population and trypanosomiasis risk to man and his livestock in Lambwe Valley, (with KETRI and Ministry of Agriculture and Livestock Development Kenya); Animal faecal matter and urine as tsetse attractants (with the Veterinary Research Laboratories, Kabete and Ministry of Tourism and Wildlife, Kenya).

Advantage was taken of the nutritional status of tsetse flies service at Tsetse Research Laboratory, Langford, Bristol, U.K.

Medical Vectors Research Programme

At the close of 1982 most of the mosquito projects, which were concerned with studies on vectors of malaria and filariasis, were phased out. The "ecological studies on malaria vectors" that appears at the end of this report, is a postgraduate student project. The programme is now concentrating on epidemiological investigations of leishmaniases in the foci of Machakos, Kitui and Baringo districts. Field investigations on the vectors and reservoirs of the disease are complemented by experimental laboratory work. Although there has been no major epidemics of the disease, many cases are still being reported throughout the country. Typing of leishmanial parasites has previously been a drawback to progress in the search for vectors and reservoirs. Consequently, the parasite identification facility was added into the programme at the beginning of the year to provide quick characterization and answers to many questions regarding the identity of the parasites. Collaboration in research on leishmaniasis epidemiology has been very good both with national research bodies i.e. Division of Communicable Diseases Control and Research of the Ministry of Health, the Kenya Medical Research Institute, as well as some international research centres.

Epidemiological investigations

M.J. Mutinga, J.B. Kaddu, D. Omogo, F.M. Kyai, J. Mwandandu, J. Ndambuki, R. Musyoki.

Blood-meal analysis of sandflies from a kala-azar endemic area

Using the sticky sandfly trap, sandflies were trapped from houses, rock crevices, termite hills, animal burrows and latrines. Out of large numbers captured each time it was noted that only a few had fed. The flies that had fed were sorted out, washed in 10% detergent saline, rinsed and placed in gelatin capsules individually. The head of each fly was later severed as the flies were identified into species, while the whole thorax and abdomen were shipped in gelatin capsule to Dr. Killick-Kendrick of Imperial College, England, for blood-meal analysis. During the year, a total of 483 blood-meals were collected from the three foci of the disease, Kalawa, Masinga and Tseikuri.

Many of the *Phlebotomus martini* and *P. duboscqi*, the known vectors of visceral and cutaneous leishmaniasis, were found not fed, but those with digested blood were dissected. *Sergentomia garnhami*, which is now featuring as a potential vector of visceral leishmaniasis revealed some interesting results. As has already been observed in both Kitui and Machakos, this species is both a mammalian and reptilian feeder as confirmed by blood-meal analysis. *S. garnhami* is mainly found in termite hill ventillation shafts. Those shafts are the homes of mongooses, genet cats, snakes, and various rodents. Sandflies have been trapped from these ventilation shafts and several isolates were made during this year. Some experiments were also conducted on infectivity of parasites in laboratory animals. *Sergentomia bedfordi* and *S. antennatus* proved to feed mainly on reptiles. This study is still in progress and more specimens are being collected to get comprehensive data.

Studies on host preference using live bait

Laboratory investigations have been carried out using sandflies caught wild in Machakos District, to determine host preferences. During these studies it was demonstrated that *Sergentomyia* genus with the exception of *S. garnhami*, preferred reptilian to mammalian hosts for blood meal. *S. garnhami* feeds on both mammalian and reptilian hosts. On the other hand the *Phlebotomus* genus, except *P. rhodhaini*, preferred mammalian hosts.

In order to study natural preference, a study offering sandflies various hosts has been initiated at Marigat, an active focus of visceral and cutaneous leishmaniasis. Because of intensity of work, only one host (domestic dog) is being investigated to begin with, as it has been incriminated as a sandfly reservoir in West Pokot, Kitui and Machakos and it was therefore felt that it is the best host to start with in the study of natural host preference.

A dog was placed in a wooden cage measuring 96 x 46 x 29 cm with 2 cm^2 wire mesh to allow flies to come in. Around the cage a castor oil trap was placed (plastic sheeting raised 30 cm from the ground). The animal was placed in the trap at 20.00 hrs and taken back in the morning. The flies from inside the trap were removed and the ones with fresh and undigested blood were removed carefully for dissection. All the female flies were dissected for promastigotes. The results are shown in table 1 and cover the months of March through July 1983.

The highest percentage preference for the dog was shown by *P. martini* (42%), followed by *S. adleri* and then *P. duboscqi*. The above findings are significant in view of the fact that the dog has been incriminated as a reservoir of *L. donovani*, and that dogs play an important role in everyday life in the arid zone of Kenya. In this area, dogs are commonly used as guards and yet are not fed enough and therefore have to go out at night and hunt in the environment to supplement their food. This usually includes rodents and birds.

This study has revealed that *P. martini* rests inside rodent burrows like *P. duboscqi*. If indeed there is a wild animal reservoir for *L. donovani*, as has been established for *L. major* in the area of investigation, then the dog is a very important potential link between man and the wild reservoirs for both *L. donovani* and *L. major*. The role of *S. adleri* and *S. ingrami* in the transmission of *L. major* must be investigated, in view of the fact that they are possible mammalian feeders and have been found inside burrows in close association with rodents from which *L. major* has been isolated. Similarly their role in the transmission of *L. donovani* needs to be investigated by isolation and typing of parasites as they have revealed a strong tendency to feed on mammals and hence their role in the transmission of *L. donovani* in the wild could not be ruled out. Laboratory experiments using some of the *Sergentomyia* species are under way. It is believed that it is during their night hunting sprees that the dogs become infected in nature. Other times when the dogs become infected could be when there is a case of visceral leishmaniasis in a homestead. Infected flies can transfer infection from man to the dog.

Our host preference experiments using live wild rodents met with some technical difficulties. At Marigat the local population interfered with the bait, and often removed them. It is suspected that some of these rodents are eaten in the area and were therefore taken out for a meal. We are planning to construct locable traps.

Studies on resting sites at Marigat

As *P. martini* disappeared from Kalawa, Machakos District, our vector studies were shifted to Marigat, Baringo District, which is a kala-azar endemic focus. Previous investigations had revealed the presence of *P. martini*, *P. orientalis* and *P. duboscqi*, all of which are vectors of leishmaniases, so the first step was to establish their resting sites. Using various trapping methods (i.e. smokers, castor-oil traps and light traps) in animal burrows, houses, termite hills, open and wooded areas, tree poles and rock crevices, information was gained regarding various resting sites of the species in Marigat depending on relative abundance.

Table 1. Dog-bait trap around animal burrows in Marigat, Baringo, Kenya

Species	total caught	total fed on dog	% feeding
P. martini	96	40	42
P. duboscqi	99	26	26
S. antennatus	103	1	1
S. adleri	26	9	35
S. ingrami	13	3	23

Infectivity Tests for *Leishmania* isolated from the field

One way of confirming leishmania species isolates from wild animals and flies is infectivity trials in laboratory-bred animals. Some species cause specific characteristic lesions on certain types of laboratory animals. Balb/c mice were innoculated with a known quantity of promastigotes grown in NNN medium in the tip of the nose and or the tail junction. They were then kept for a period of 4 weeks for observation for development of characteristic lesions.

Various leishmanial isolates in culture, obtained from wild-caught sandflies and wild animals were injected into the nose of Balb/c mice to determine the infectivity to these animals. Seventy percent of the animals injected with *P. duboscqi* isolates developed sores which are characteristic of *L. major* infection (fig. 1). Other isolates which caused sores on Balb/c mice were from *S. ingrami*, trapped from animal burrows in Marigat (16%), and *S. garnhami* from termite hills in Kitui (25%).

Because of the various isolates we have made from *P. duboscqi* in Baringo, from animal burrows where we had earlier isolated *L. Major* from rodents, and the fact that this species has been confirmed as a vector of *L. major* in West Africa, there is strong evidence to suggest that this species is the vector of *L. major* in Kenya.

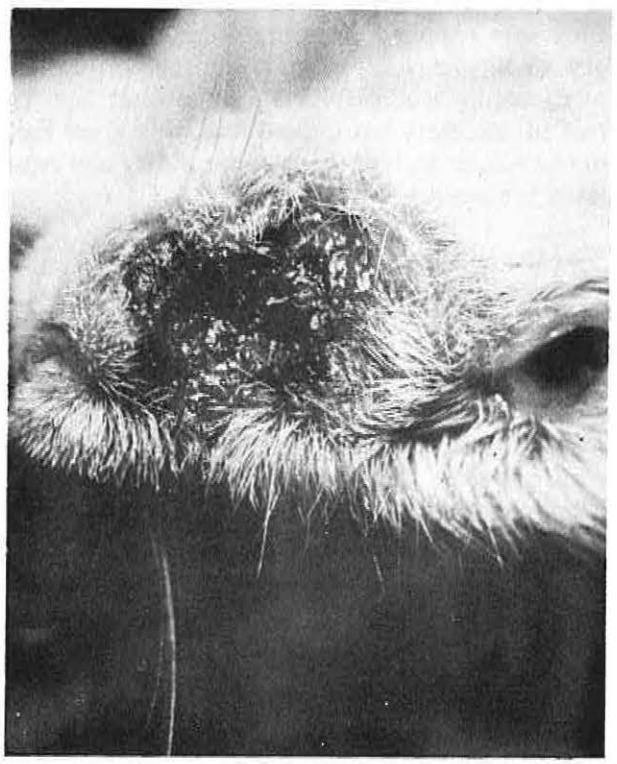


Figure 1. Nose lesions on a Balb/c mouse. The infection was caused by *Leishmania* isolated from *Phlebotomus duboscqi*.

Detection of promastigotes in sandflies from Baringo

SANDFLIES FROM HOUSES

In our previous studies carried out in West Pokot and in Machakos, we established that *P. martini* comes indoors to bite man. In Machakos, we showed that *P. martini* rests indoors, especially during the dry season. So far, the isolation of leishmanial parasites of *L. donovani* type from *P. martini* have only been from outdoors both in Kitui and Machakos districts. The current investigations were aimed at determining whether or not *P. martini*, which has been established to be anthropophilic, does harbour leishmanial parasites and whether it is involved in disease transmission in Baringo.

Homes of active or treated kala-azar cases were selected for intensive trapping using castor oil traps. Traps were set at night along house walls hanging both inside and outside. Flies were collected in the mornings, taken to the field laboratory and dissected. Positive guts were cultured in NNN medium to isolate the parasites for characterization and they were then injected into experimental animals.

Leishmanial isolates were made from *P. martini*, *S. ingrami*, *S. bedfordi* and a new *Sergentomyia* species.

SANDFLIES FROM TERMITE HILLS

The termite hills constitute part of the major natural resting sites of phlebotomine sandflies (fig. 2). Although these natural resting sites have been shown not to be the foci of transmission from man to man, they are capable of serving as reservoirs of phlebotomine sandflies that have infection from wild animals. Consequently, flies were trapped, using the sticky trap set at night, and dissected in the mornings.

The most ubiquitous female species in this area are *S. antennatus*, *S. ingrami*, *S. bedfordi*, *S. africanus* and *P. martini*. Although *S. clydei* and *S. wynnae* were much less prevalent i.e. less than 100 for the whole year of trapping; they had the highest infection rate, i.e. 33.3% and 15.4% respectively. *P. martini*, the main anthropophilic species was not observed to harbour leishmania in these resting sites, *S. antennatus* and *S. bedfordi* had infection rates of 0.93, 0.44 and 0.23 respectively.

SANDFLIES FROM ANIMAL BURROWS

Castor oil traps were placed in and around animal burrows of the type that harbour gerbils from which *L. major* has been isolated in Marigat. All the flies captured were dissected for promastigotes. The highest infection rate encountered in the investigation was in *P. duboscqi*. Other species of sandflies from which promastigotes

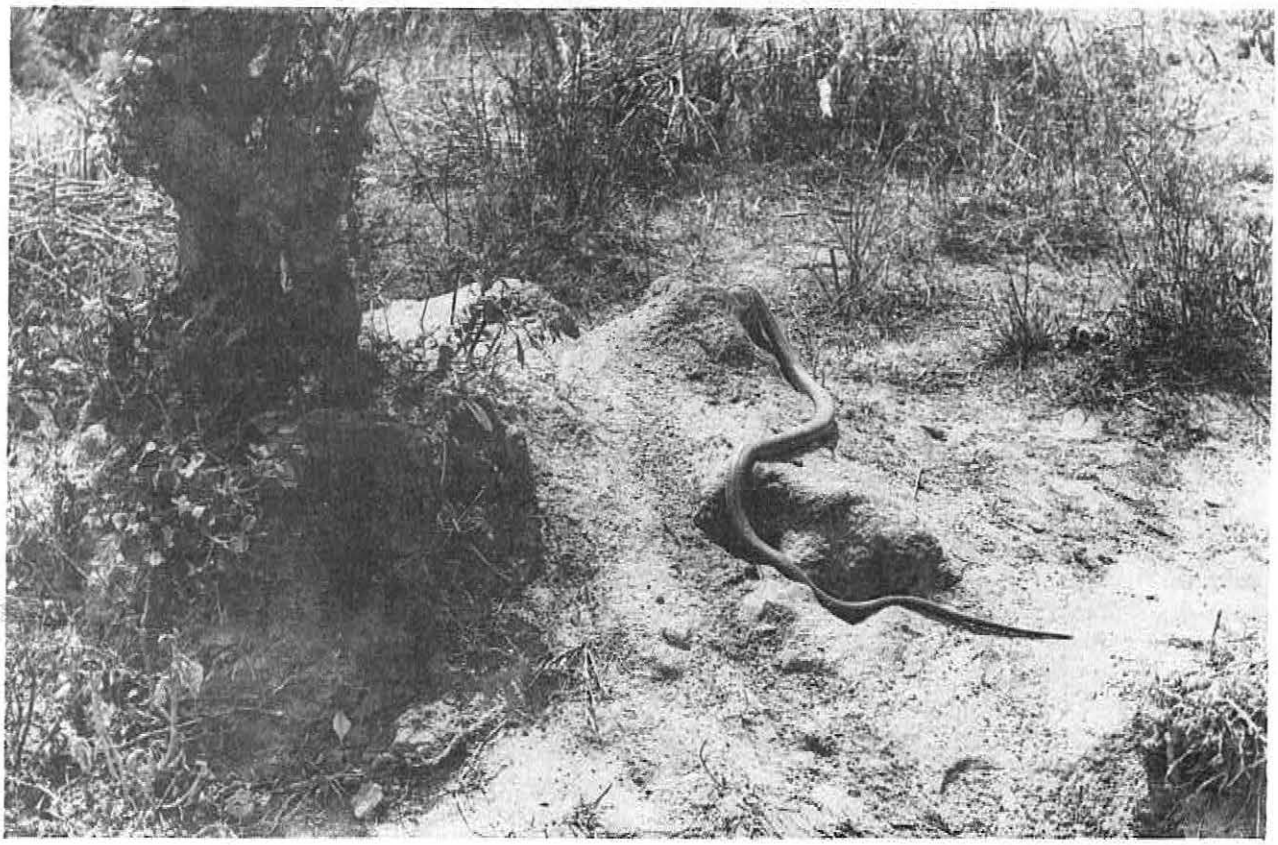


Figure 2. The termite hill is a popular home for sandflies and other fauna.

were encountered included *S. antennatus*, *S. ingrami*, and *S. adleri*. This was further evidence that *P. duboscqi* may be the major vector of *L. major*. The role of the *Sergentomyia* species is still under investigation through parasite characterization and infectivity tests on laboratory insects.

Sandfly colony at ICIPE

M.J. Mutinga, J.B. Kaddu, J. Mwandandu, J. Ndambuki, R. Musyoki

The joint venture between ICIPE and the Walter Reed Army Project to rear a *P. martini* colony have resulted in the establishment of sandfly colonies in the clinical research laboratories. The needs of the Walter Reed Army group for experimental insects made it very difficult to cater for our needs as well. The only solution was for ICIPE to initiate its own colony with the minimal equipment available. Efforts to acquire environmental chambers for *P. martini* at ICIPE are under way, so that the role of *P. martini* can be fully scrutinized. *P. elgonensis* and *P. pedifer* which have already been investigated in the laboratory, (i.e. biology and optimal growth requirements), have recently been brought into the laboratory for transmission experiments and taxonomic investigations.

In the genus *Sergentomyia*, we have established a laboratory colony of *S. antennatus*, *S. schwetzi*; *S. bedfordi*, *S. ingrami*, and *S. adleri*. Other species which are in the process of multiplying for colony establishment are *P. martini*, *P. duboscqi*, *S. africanus*, *S. garnhami* and *S. affinis*.

During the past year, 816 female sandflies were supplied from the programme insectory to investigators carrying out vector-parasite relationship studies. It is hoped that the expected insectary equipment will be a great help in the efforts to rear the high humidity and regulated temperature species.

Vector parasite relationship

J.B. Kaddu, M.J. Mutinga, M.P. Nyamori

Parasites in the malpighian tubules

Previous investigations by Kaddu and Mutinga showed that leishmanial parasites can invade the excretory structures called the malpighian tubules in two species of Kenyan sandflies; *S. garnhami* and *S. antennatus*. Further investigations were undertaken, to find out whether the location of leishmanial parasites in the various parts of the sandfly gut is species-specific.

Sandflies were captured from natural resting sites using the standard suction tube technique. They were processed, dissected and their guts examined under a light microscope

Table 2. Natural infections of leishmanial parasites observed in the guts of sandflies at Tseikuru (Nziitu and Muuna sublocations) Kitui District.

Locality	Species	FLIES DISSECTED		
		Total	Parasitaemic	Parasitaemic in the malpighian tubules
Nziitu	*S. garnhami*	174	30	2
	S. antennatus	12	0	
	S. bedfordi	12	0	
	S. graingeri	16	0	
	S. schwetzi	7	0	
Muuna	*S. garnhami*	5	2	0
	S. antennatus	1	0	
	S. kirki	1	0	

Note. The sandflies were collected between 17—20 January 1983

for the presence of parasites. Electron microscopical observations were carried out on some of the parasitaemic guts.

The species of sandflies examined, and the number of sandflies with leishmanial infection in the malpighian tubules are indicated in table 2.

Out of the six species of sandflies, leishmanial infection was found in the malpighian tubules of *S. garnhami*. These results are similar to our previous findings. The significance of these results, in the epidemiology of leishmaniasis will be elucidated after the parasites isolated from the malpighian tubules have been biochemically characterized.

Artificial infection with *L. donovani*

One of the main drawbacks to the understanding of leishmaniasis epidemiology in eastern Africa is the lack of laboratory evidence about the vectorial capability of various species of eastern African sandflies. Over 30 species of sandflies occur in Kenya but, unfortunately, no information is available about their ability to transmit human Leishmania in the laboratory and which species are vectors.

With the sandfly colony being established at ICIPE, it is becoming possible to carry out experimental laboratory transmission of Leishmania to sandflies in order to pinpoint vector species. In the present study observations were made on the susceptibility of 6 species of Kenyan sandflies to *L. donovani*, namely; *S. schwetzi*, *S. bedfordi*, *S. ingrami*, *S. garnhami*, *S. adleri* and *S. antennatus*.

L. donovani, ICIPE stabilate number 126, previously isolated from a kala-azar patient at Makueni hospital, Kenya, was used. Promastigotes were cultivated in NNN culture medium and transferred to RPMI-1640 culture medium. The promastigotes were harvested at long-phase, concentrated in a sterile test tube by centrifugation and asceptically suspended in about 0.3 ml rat, rabbit or hamster blood in a syringe.

The sandflies were artificially infected using a newly designed feed apparatus (fig. 3). A 70-watts water pump with a capacity of 90 l per minute maintained a steady flow of water through the apparatus at a pre-set temperature which was controlled by a thermostat at temperatures varying from 28 to 31°C. The approximate temperature of the blood was recorded by monitoring that of the water flow.

To prepare the membrane, the skin of a 1– to 2–days-old male chick was carefully removed and fixed on a sterile concave-bottomed end of a test tube using a sterile rubber-band.

About 0.3 ml of the highly parasitaemic blood (at least 50 parasites per microscopic field) was asceptically inoculated into the parasite/blood cavity of the feeding unit covered with a dark cloth for semi-darkness micro-environment. Flies which did not feed within 2 h were offered the infected blood for a longer period ranging from 4 h — 12 h, with an unlimited supply of sugars in form of a piece of fresh apple, and were maintained at 60—80% rh and temperature of 18—26°C. The flies were dissected at various periods ranging from 1 to 9 days post-infection.

In *S. schwetzi*, dissected between days 3 and 9 after the infective feed, parasites were found in the abdominal midgut and the thoracic midgut in 13 of the 69 flies which were dissected on day 4 post-infection. In 20 *S. adleri*, dissected between day 4 and day 7 after the infective feed, parasites were found in the abdominal midgut of 5 flies. Three species, namely *S. bedfordi*, *S. antennatus* and *S. ingrami* failed to feed on the blood.

The number of *S. antennatus* and *S. ingrami* (17 and 7 respectively) which have been tested so far is too small to provide experi-

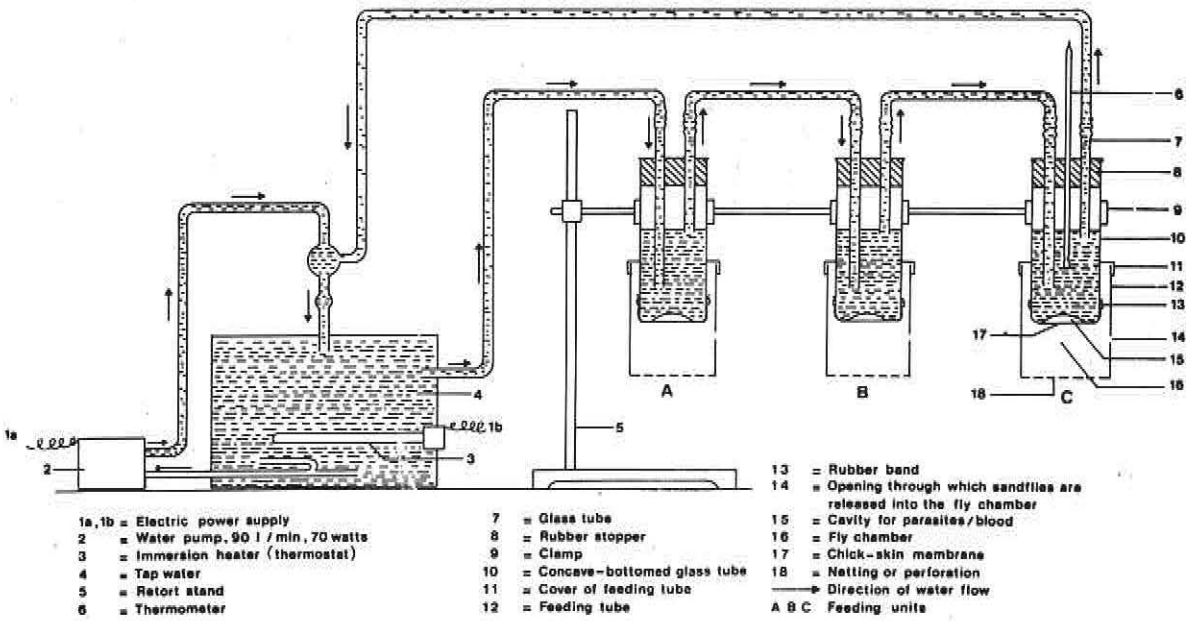


Figure 3. Apparatus for artificially infecting sandflies with Leishmania

mental evidence regarding the importance of these species in the epidemiology of leishmaniasis. However, in the case of *S. bedfordi* the failure of 155 flies to feed in the 19 experiments carried out may reflect *S. bedfordi*'s poor vectorial capacity for human *L. donovani* and that it may be of no significance in epidemiology of visceral leishmaniasis.

Taxonomic studies based on phlebotomine sandflies (Diptera : phlebofomidae)

L.M.N. Rogo, M. Okulo

Two problems exist in the identification of Kenyan sandflies. These are; the occurrence of species complexes within the leishmaniasis vector and the unavailability of an identification key.

Within the vector species i.e. the *Synphlebotomus* complex (vectors of kala-azar), there are three species known in Kenya, namely *P. martini* Parrot, *P. vansomeranae* Heisch, Guggisberg and Taesdale and *P. celiae* Minter. The females of the three species are not easily distinguishable, only the males show small differences in their terminalia. Similarly, the females of *P. pedifer*, Lesis, Mutinga and Ashford (known vectors of cutaneous leishmaniasis) are not easily distinguishable from *P. elgonensis* Ngoka, madel and Mutinga (now known to be a synonym of *P. aculeatus*, Lewis, Minter and Ashford) and *P. longipes* Parrot and Martin (now believed not to occur in Kenya). The only clue to their identity is the male terminalia. A detailed study is therefore being carried out on the immature and mature stages of the vector species in order to find a possible method/methods for distinguishing these vectors.

Immature stages

EGGS

Eggs obtained from females of the above species are processed for chorionic studies using the scanning electron microscope. They are mounted on metal stubs and allowed to rest in chambers with 5% gluteraldehyde fumes, and subsequently double-coated with 100-200A° carbon and Au-pd alloy in a vacuum evaporator.

Examinations and photography were carried out in a Joel scanning electron microscope. Eggs of *P. pedifer* have been examined, but are yet to be compared with those of *P. elgonensis*. *P. martini* eggs from Baringo have also been examined. The chorionic sculpturing of Baringo *P. martini* will be compared with *P. martini* group from Kitui District, the type location for the *Synphlebotomus* complex, in order to find out whether the chorionic sculpture of eggs can be used in the identification of phlebotomine species complexes.

LARVAE AND PUPAE

The chaetotaxy of the fourth instar larvae and the pupae of the various vectors and implicated species is being studied. Fourth instar larvae and pupae were killed in hot water, and mounted in Berlese's fluid. Outlines of larva were drawn with the aid of a camera"lucida". Setal positions were ascertained before a representation of the chaetotaxy by eye. The larva and pupa of *P. martini* and *P. pedifer* have been drawn. The former will be compared with those of *P. celiae* and *P. vansomeranae* and the latter with *P. elgonensis*.

ADULTS

BIOCHEMICAL IDENTIFICATION

Electrophoretic studies are being carried out on the adults of *P. pedifer* and *P. elgonensis*. The terminalia of males are removed and mounted in gum chloral in a microscopic slide. This is used to confirm the biochemical identification of the males. The females are processed whole. Each fly was homogenized and applied to a thin starch gel (10-12%) and electrophoresed at 400V. Staining procedures follow recipes of Harris and Hopkinson (1978). The following enzymes have been investigated: malate dehydrogenase (MDH), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), hexokinase (HK), isocitric dehydrogenase (ICD), superoxide dimutase (SOD), pyruvate kinase (PK), phosphoglycerate kinase (PGK), and L-threonine-3 dehydrogenase (TDH). Six of these have so far yielded results that can be used to comment on the identities of *P. pedifer* and *P. elgonensis*. Similar biochemical studies are about to start on the *Synphlebotomus* complex. Very few members of this complex are found on traps, therefore there may be some delay on their identification.

Identification key for Kenyan sandflies

Most of the sandflies for this purpose have been collected from leishmaniasis endemic areas of Kenya. These are now preserved and awaiting key preparation after controversial species (sysnonyms etc) have been compared with type specimens and identifications confirmed.

Preliminary studies on *Leishmania* identification

B.M. Okot-Kotber, B.N. Odero

Parasite identifications are important in the event of vector and reservoir incrimination, in epidemiological studies of leishmaniasis. Previous isolates from possible vectors and reservoirs have been sent overseas for identification, sometimes resulting in delays and possible loss of isolates. It has therefore proved imperative to establish parasite identification facilities within the ICIPE project. A new facility for identification work has therefore been established and a few equipment and reagents purchased. Culture facilities have been improved and we have been able to mass-culture a number of isolates.

All the isolates from the field were routinely passaged in NNN culture medium and RPMI 1640 medium before banking in liquid nitrogen. As NNN medium contains rabbit blood which may interfere with biochemical studies of the parasites, all the isolates for these investigations have to be reconditioned to grow in a blood-free medium, RPMI 1640, which is commercially available, supplemented with 20% foetal calf serum. Todate, 10 isolates and reference strains have been mass-cultured in this medium and banked in liquid nitrogen awaiting subsequent isoenzyme identification.

To avoid field fungal contamination, a mild technique for elimination of fungal contamination from cultures has recently been developed involving separation of parasites from fungus, based on differences in surface charge. In short, DEAE 52-cellulose column equilibrated with phosphate buffered saline glucose (PBSG), pH-80 and ionic strength of 0.218, allows stabilization of a column system useful for the separation. The optimum elution of the parasites from the column while the fungus binds to the gel is achieved at pH-8, ionic strength = 0.362.

The recovery rate of parasites is good, as estimated from the number of parasites per microscopic field at X 40 magnification objective. In samples 60 parasites/field, 50 parasites/ field were recovered in the same volume. In the diluted samples of 8 parasites/ field and 1 parasite/field gave recovery rates of 5 parasites and 1 parasite/field respectively.

On the average, the recovery rate was esti-

Table 3. Isolates and reference strains

Isolates	Identification number	Source
1.	ICIPE 126	Human spleen from Machakos Hospital, Kenya
2.	ICIPE 168	*Sergentomyia garnhami* malpighian tubule, from Kitui District
3.	ICIPE 188	*Sergentomyia garnhami* mid-gut from Kitui District
4.	ICIPE 217	*Sergentomyia* mid-gut, from Baringo District
5.	ICIPE 218	*Sergentomyia ingrami* mid-gut, from Baringo District
6.	ICIPE 151	Mongoose spleen, from Kitui District
7.	ICIPE 172	Mongoose liver, from Kitui, Kenya
8.	ICIPE 224	Elephant shrew spleen, from Baringo District
9.	ICIPE 180	Genet cat spleen, from Kitui District
10.	ICIPE 140	Lizard *(Mabunya natalensis)* liver from Kacheliba, Kenya

Reference strains

1	WA/KE /LV457	— London, from Professor Peters
2	MAN/ET/83/Belehu 76/83	— London from Professor Peters
3	MAN/IN/80/DD8	— London, from Professor Peters
4	LRC-L52	— Israel, isolated from man in India in 1954, typed to be *L. donovani*
5	LRC-L119	— Israel, isolated in 1954 from *Tatera* in Baringo and typed to be *L. tropica major*
6	LRC-L137	— Israel, isolated from human in Jericho 2, Israel and typed to be *L. t. major.*
7	LRC-L147	— Israel, isolated from man in Ethiopia in 1971 and typed to be *L. eathiopica.*

mated at over 80%. Occasionally, a fungus spore may escape through. In such a case the new subculture should be allowed to settle for 24 h or so to give time for the spore to grow and change its surface charge, then rechromatograph. This system was found to be most effective and studies are underway to improve conditions so as to be able to detect possible mixed infections with other flagellates, at the same time clearing the parasites.

Studies are also underway to characterize the parasites already mass-cultured using isoenzyme techniques using carefully selected commonly used 13 isoenzymes.

Ecological studies on malaria vectors in Kenya

C. M. Mutero

The *Anopheles gambiae* complex which is the general vector of malaria in the Afro-tropical region comprises six sibling species, three of which are found in Kenya. Out of these three, *A. merus* Donitz is only found along the coast while *A. gambiae s. str.* Giles and *A. arabiensis* Patton are of a much wider geographical distribution. Due to lack of external morphological differences, the only practical method of distinguishing the species is by banding their polytene chromosomes.

Few people can make reliable cytogenetic identification of the different species, consequently, relatively little is known about the differences between their biology or ecology.

In 1978, the Medical Vectors Research Programme initiated several studies on the *A. gambiae* sibling species that were considered not only relevant to the epidemiology of malaria, but also to the planning of appropriate vector control strategies. This work was later extended to include *A. funestus*, the only other locally known vector of malaria. The aspects studied included, among others; the seasonal and geographical distribution of genetically defined populations of *A. gambiae*, their feeding and resting behaviour, as well as their relative vectorial capacities. In an effort to quantitatively assess the vectorial capacity of the four species, the present studies were started in 1981 with the immediate objective of determining how the average survival rate and the interval between blood-meals differ between the species and within each species during different seasons.

Study area

Between May and June 1983, *A. funestus* was studied for 27 consecutive days in Jaribuni, a sparsley populated coastal area lying at latitude 3° 40′ south of the equator and about 50 km north of Mombasa town. Daily sampling of *A. gambiae* s.l. was carried out for 26 days in the Mwea rice irrigation and settlement scheme between August and September during the same year.

The Mwea rice irrigation scheme lies about 96.5 km to the North East of Nairobi at an altitude of 1,159 m above sea level and at latitude 0° 40′ south of the equator. There are slightly over 3 000 tenants on the scheme each growing rice on a basic holding of 4 acres. The tenants with their families live in the 36 villages on the settlement which are located within close proximity of the rice paddies. The paddies are flooded with water for about 8 months every year, thus providing breeding sites to a number of mosquito species, the most abundant of which is *A. arabiensis.* Malaria and water related diseases like schistosomiasis are common throughout the scheme.

Vector ecology

The major requirement of the sampling method was a consistent nightly estimate of the relative biting density and an unbiased estimate of the nightly parous rate. In collaboration with the Liverpool School of Tropical Medicine, a new updraft suction trap was developed and used for the sampling of the adult mosquito population in the present studies. The suction trap was

Table 3. Survival rates of field populations of malaria vectors in Kenya

Date	Species	Locality	Season	Survival rate ± S.E.
May 1981	*A. merus*	Jimbo	long-rains	0.45 ± 0.03
Nov. 1982	*A. merus*	Jimbo	short-rains	0.55 ± 0.03
May 1982	*A. gambiae* s. str.	Msihu	long-rains	0.52 ± 0.04
Sept. 1983	*A. arabiensis*	Mwea	rainy	0.41 ± 0.06
May 1983	*A. funestus*	Jaribuni	long-rains	0.42 ± 0.04

driven by a 12-volt sealed lead-acid battery which was charged daily from a solar panel, Ferranti MST 300. A volunteer was made to sleep under an ordinary mosquito net from 21.00 hrs to 06.00 hrs during which period the suction trap was continously operated at about 40 cm outside the net but above his head. Mosquitoes thus collected were removed in the morning and sorted into various species.

Fed *A. gambiae* s.l., resting inside houses in Mwea, were collected between 08.00 and 09.00 hrs and then kept alive for 18 h after which their ovaries were dissected for polytene chromosomes, to be used in the identification of the sibling species and the various chromosomal types. Preliminary examination of 50 chromosome preparations showed *A. arabiensis* to be the only sibling species in Mwea during the study period. The population had a well balanced polymorphism for the 2Rb and the 3Ra inversions, with heterozygotes near to 50% for both inversion systems. Table 3 provides a summary of the various mosquito populations that were studied between 1981 and 1983. The average survival rate for *A. funestus* and *A. arabiensis* studied in 1983 did not show significant differences, being 0.42 ± 0.09 and 0.41 ± 0.06. However, these values were considerably lower than those recorded for *A. gambiae* s. str. during the long rains and for *A. merus* during both the long and short rains season. There was marked seasonal variation in the survival rate of *A. merus*, the value being highest during the short rains season. This high survival corresponded with relatively low densities of the mosquito. The interval between blood meals for *A. merus*, *A. gambiae* s. str. and *A. funestus* ranged from 2–3 days but was 4 days for *A. arabiensis*.

Implications

Preliminary analysis of the findings demonstrates both species and seasonal variation in the average survival rate of the various adult mosquito populations. The relatively high average survival of *A. merus* and *A. gambiae* s. str. is an indication that these two species have a higher potential to transmit disease than either *A. fenestus* or *A. arabiensis*. However, it will not be possible to make firm conclusions about the disease transmission potential until these field studies have been conducted during at least two seasons for each species.

Recent studies on the intraspecific variation within members of the *A. gambiae* complex have shown that certain inversion karyotypes have clinal geographical changes in frequencies, with evident correlations to climatic conditions. In order to determine whether the seasonal variation in survival rate of the *A. gambiae* sibling species is related to the frequency of certain polymorphic inversions, detailed examination and analysis of chromosome material are being carried out especially with regard to the 2 La inversion in the *A. gambiae* s. str.

RESEARCH UNITS

Chemistry and Bioassay Research Unit

This year's report reflects the unit's research activities in a state of transition. A number of projects, e.g. on tsetse and tick pheromones, are being completed and will soon be phased out. On the other hand the reports on the allomones of a resistant cowpea cultivar and the limonoid project make their first appearance, underlining the changing emphasis in the unit's research priorities. Biochemical reasearch is now almost wholly oriented toward proteins with antigenic potential. Chemical research will henceforth stress studies on allelochemicals implicated in host plant resistance in addition to pheromones and kairomones of selected target insects and vectors. Furthermore, in-depth studies on promising groups of insect-active natural products, e.g. limonoids, have been initiated to complement the on-going programme on the screening of tropical flora. Research on the unit's special project with the International Atomic Energy Agency (IAEA) on microbial depolymerization of lignocellulose is beginning to gather momentum and it is hoped that support for the project will continue beyond the current phase which ends in 1985.

The unit's research equipment is being upgraded and modernized to keep up with the demands of new research projects. A new Varian HPLC has been operational throughout the year and we have recently acquired a pharmacia FPLC for high efficiency protein analysis. The unit plans to instal a fully computerized GC-MS-DS to replace the old Finnigan 1015D instrument which is no longer operational. Other smaller items of equipment like high-speed centrifuge and low-temperature freezer required for biochemical research will be acquired in 1984. The International Atomic Energy Agency (IAEA) is helping ICIPE to set up a well-equipped radio-isotope laboratory which will be available for both training and research. The missing link in the network of instruments required to accomplish the unit's research goals is an FT-NMR equipment which is an essential complement to GC-MS for complete identification of small quantities of allelochemicals and other structurally complex natural products.

There have been no major breakthroughs in the year but several interesting and some significant findings have been made.

A synopsis of the Unit's major accomplishments

A. Hassanali

PROTEIN BIOCHEMISTRY

Two protein fractions from the eggs of *Rhipicephalus appendiculatus* obtained by gel permeation chromatography have now been demonstrated to induce immune response in rabbits. The purification and characterization of the antigens are now underway.

The proteolytic enzyme trypsin has been shown to cause lysis of trypanosomes that infect *Glossina morsitans morsitans*. Feeding *G. morsitans* with blood infected with *Trypanosoma brucei* containing a trypsin inhibitor led to higher infection of the salivary glands and proboscis. The digestive enzymes may thus constitute an important component in the infection barrier against trypanosomes in the vector.

ALLELOCHEMICAL RESEARCH

Feeding assays with *Maruca testulalis* on cowpea discs treated with ethyl acetate extracts of a resistant and a susceptible variety of cowpea have shown that a feeding deterrent(s) is (are) present in the former. Fractionation of the extracts is in progress to identify the active compound(s).

PHEROMONE AND HORMONE RESEARCH

The three stereoisomers of *Glossina pallidipes* sex pheromone have been bioassayed. Only the meso form (R,S-13,23-dimethylpentatriacontane) showed significant activity at doses down to ca. 0.25μg per decoy. This finding is different from that reported for stereoisomers of dimethylheptatriacontane, a component of *G. morsitans* pheromone system.

R. appendiculatus tick has now been shown to contain the ubiquitous 2, 6-dichlorophenol, contrary to an earlier report. The compound has been found in both males and females, but predominantly in the unfed females, diminishing as feeding proceeds. The biological implications of this finding, are unclear but it would appear to argue against the function of this phenol as a sex pheromone in this species.

Guanine, the principal component of tick excreta, active as an assembly factor, has been shown, by improved bioassay, to be active at pheromonal concentrations. However, *Argus persicus* ticks respond to guanine only at low humidities. Exposure of the ticks to high humidities appears to lead first to loss of the assembly response and eventually to the induction of a negative response to the pheromone.

Topical application of JHIII on *G. morsitans* females has been shown to partly abolish the abortifacient effect of 20-hydroxyecdysone. The ecdysone is believed to function as an abortifacient by shutting off JH release from the corpus allatum, which in turn leads to decreased supply of "milk" to the larva from the uterine gland of treated females. Direct effect of the abortifacient on circulating JH levels in the pregnant fly now awaits to be demonstrated.

NATURAL PRODUCTS

Preliminary assays of a number of limonoid compounds including limonin (obtained from citrus seeds) and simple synthetic modifications of it, show promising antifeedant activity against *Eldana saccharina*. The activity appears to be related to the presence of α,β-unsaturated lactone units. Assays against other crop pests are underway. The possibility of systemic protection of crop plant foliage by absorption via roots will also be investigated.

The plant *Tephrosia alata* has been screened for insect-active compounds. Two known compounds have been identified as being responsible for antifeedant activity against the African armyworm, one of which (tephrosin) belongs to the rotenoid group of naturally occurring insecticides. Tephrosin shows potent antifeedant activity at dosages almost 10—100 fold lower relative to its insecticidal activity. The antifeedant activity of rotenoids against crop pests are under study. Rotenoids (and *Tephrosia* species which are widely distributed in the tropics) deserve closer investigation as subtle anti-insect agents, at sub-lethal dosage levels, for rural insect pest management.

A new monomeric indole-type alkaloid as well as three other known ones have been isolated from *Strychnos hennigsii*. The alkaloids show moderate antifeedant activities against 6th-instar larvae of *S. trychnos exempta*. Three iridoid glucosides, one new and two known, isolated from *Mussaenda arcuata* were found to be ineffective as feeding deterrents against *S. exempta*.

LIGNOCELLULOSE PROJECT

Bacterial isolates from *Macrotermes michaelseni* and various other termite species have now been identified. The bacteria have been screened for cellulolytic activity. Only one isolate (out of 8), a yellow gram negative bacillus, showed ability to produce cellulase. On the other hand, five species of *Actinomyces* isolated from the same termites showed evidence of cellulolytic ability.

Species of *Trichoderma* have been isolated from the fungus-comb of *M. michaelseni* (in addition to Fusarium *Aspergillus, Termitomyces*

and *Rhizopus* spp. reported last year). Attempts to culture *Termitomyces*, which has demonstrated high cellulase and β-glucosidase activities, are being made.

A procedure for isolating and purifying cellulase from *Termitomyces* condiophores and fruiting bodies has been worked out and standardized. This has allowed a comparison to be made on the kinetic performance of cellulases from different sources. Similarly, xylanase from *Termitomyces* fruiting bodies has been purified and characterized.

A procedure for detecting polyphenoloxidase, one of the key enzymes in lignin degradation, has been worked out. Polyphenoloxidase activity has been demonstrated and quantified in a number of isolated fungi.

BIOASSAYS

Two new feeding bioassays against *(M. testulalis* and *E. saccharina)* have been added to the existing battery of assays. An olfactometer was designed to screen for volatile repellent compounds from plants against grain weevils.

Immunization of rabbits with *R. appendiculatus* egg protein fractions A4 and B

R.M.W. Vundla, M. Brossard

Tick vitellins constitute potential antigens for inducing resistance against tick infestation in livestock. Gel permeation chromatography of egg proteins on sepharose (CL-6B) gave four fractions (ICIPE Annual Report 1980), two of which eluted at MW corresponding to about 2×10^6 (protein fraction A_4) and 1.06×10^5 (protein fraction B) respectively. A4 and an isoelectric point (pI) at about 6.3; B which separated into three major protein bands (as seen after isoelectric focussing), had pI in the range 8.5—9. Immunization experiments were carried out with the objective of determining:

- the immunogenic performance of the two egg protein fractions;
- the relative efficiency of two different immunization procedures in rabbits; and
- if the protein fractions conferred any cross-protection to *Ixodes ricinus* infestation.

Rabbits were immunized with 250 μg of protein/rabbit. In immunization scheme I, the protein was injected intradermally in Freund's complete adjuvant, with boosters on days 14 and 29. Scheme II rabbits were injected subcutaneously with saponin (0.5 mg/kg body weight) as adjuvant. These rabbits were boosted on days 14 and 21. All the rabbits were bled from the ear vein on days 9, 21, 31, 43, and 56. Antibody titres were determined by the micro-ELISA technique. Antisera were also tested by the double diffusion precipitin (Ouchterlony method). On day 58, the rabbits were challenged with adult *I. ricinus* ticks, 15 males and 15 females placed on the right ear of each rabbit.

The antibody titres obtained for the two immunization schemes using fractions A_4 and B are summarized in figure 1. The two fractions were equally good immunogens. Immunization scheme II was faster, peak titres being attained at about day 20 as compared to day 30—40 for scheme I. However, scheme II was characterized by a drop in antibody titres after the peak. This decrease in titre persisted even after challenge. Scheme I antibody titres were stable. The results of the double diffusion test (antigen B only) are shown in figure 2. Two precipitin lines were associated with the homologous reaction. There was a strong cross-reaction between antigens A_4 and B. However, this cross-reaction was associated with only one of the two precipitin lines. When the immunized rabbits were challenged with adult *I. ricinus* ticks, there was no effect on the ticks, indicating that there is no cross-protection.

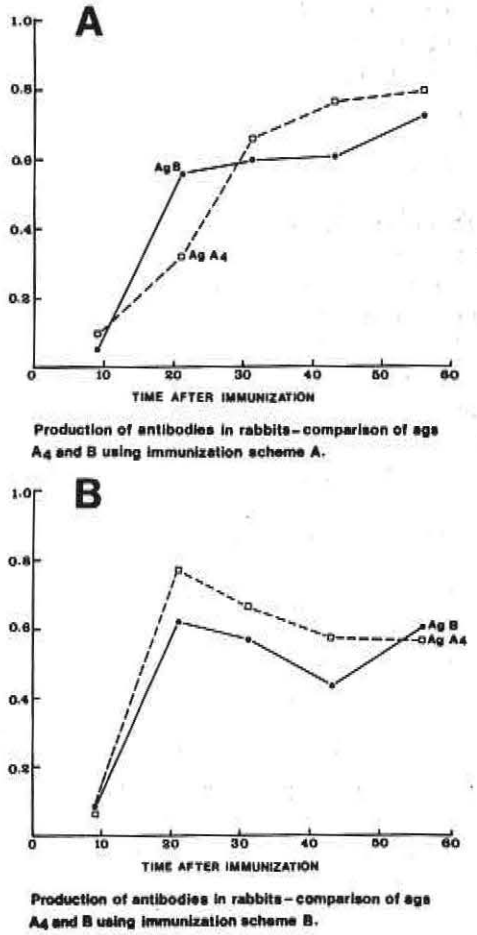


Figure 1. Production of antibodies in rabbits — comparison of anti A_4 and B using immunization scheme A and scheme B.

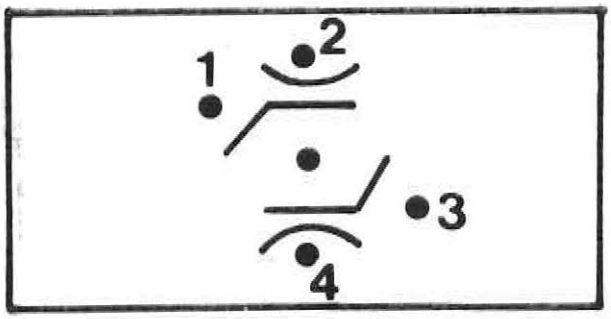


Figure 2. Double diffusion of antigen B vs Rabbit Anti A_4 and Anti B. Antigen B is in the centre well. 1, 3 = Anti A_4. 2, 4 = anti B.

Effect of trypsin inhibition *in vivo* on the establishment of *T. brucei* in *G. m. morsitans*

R.M.W. Vundla, L.H. Otieno, G. Achieng'

The natural frequency of *Trypanosoma (Trypanozoon) brucei* salivary gland infections in tsetse flies is usually very low. These low frequencies may be caused by failure of the trypanosomes in a blood meal to establish cyclical infections in the fly. A variety of factors are thought to be responsible. We feel that digestive enzymes of the tsetse may retard the establishment of *T. brucei* in the fly, and hence form part of the establishment barrier to trypanosomes. The work reported here is a follow-up of our earlier work on the interactions between digestive proteases of *Glossina morsitans morsitans* and the bloodstream form *T. brucei* (ICIPE Annual Report, 1978, 1979 and 1980). The data is preliminary.

Newly emerged female *G. morsitans* were membrane-fed on whole blood infected with *T. brucei* (first peak), containing (a) 10^{-3}M and (b) 2×10^{-3}M N-tosyl-L-lysine chloromethyl ketone (TLCK), a trypsin inhibitor. Control flies were similarly fed on infected whole blood, but containing no TLCK. Thereafter, flies were maintained on uninfected rabbits. Thirty days after the infectious meal, the gut, salivary glands and proboscis were examined for infection. The results are summarized in table 1.

Table 1. Percent infected (as % of survivors)

	Gut	Salivary gland	Proboscis
Control	18	11	11
10^{-3}M TLCK	56	17	17
2×10^{-3}M TLCK	64	24	31
	$P < 0.05$	NS	$P < 0.05$

The presence of trypsin inhibitor in the fly gut had a positive effect on infection. At the higher concentration of TLCK (2×10^{-3}M), the results were significant at the 5% level (χ^2 test) for the gut and proboscis. These results are in agreement with observations that trypanosomes are damaged *in vivo* during the first 8 h after a blood meal. We have observed a similar effect *in vitro*, where the degree of lysis appears to be directly proportional to the proteolytic enzyme activity of the tsetse gut homogenate. Our results therefore indicate that trypsin is probably a component of the establishment barrier to trypanosomes in the tsetse.

Effects of extracts of stems of TVu 946 on the feeding behaviour of *M. testulalis* (Geyer)

D. A. Otieno, P.E.W. Njoroge

TVu 946, a cowpea line, has been identified by the International Institute for Tropical Agriculture (IITA) as having resistant characteristics. The bases of this cultivar's resistance to several pests including *Maruca testulalis* (Geyer) are being studied further at ICIPE. It has been shown that chemical factors are responsible for the resistance of TVu 946 stems to *Maruca* infestation. In the past year, further systematic study of extracts of TVu 946 stems was carried out first to investigate any antifeedant properties, and secondly to identify the deleterious constituents.

Crude EtOAc extracts of the stems used in the study were obtained from 40-day old plants in the usual way. The extract was assayed for antifeedant properties using a variant of the standard Chemistry and Bioassay Research Unit bioassay method. Leaf discs from VITA I, the susceptible cowpea cultivar, were used and 100 μg of the crude extract was applied on each disc. Only 5th-instar *Maruca* larvae were used in the bioassays.

Extracts of VITA I showed significantly less feeding inhibition than extracts of TVu 946 in all the tests (table 2). The feeding response to discs treated with extracts of VITA I was equal to that of acetone treated discs thus showing that acetone had no effect on the feeding behaviour of *Maruca* larvae.

Fractionation of TVu 946 by column chromatography and HPLC and assays of the different fractions are now in progress to establish the identity of the deterrents.

Table 2. Effects of extracts of stems of Tvu 946 and VITA I on the feeding behaviour of *Maruca testulalis*

Extract	Dose µg/disc	*Control disc	*Treated disc	† Percentage feeding deterrency
Tvu 946	100	2.64	0.441	83
VITA I	100	3.23	3.07	4.95

Notes: * The figures show quantities in g of the disc consumed after 6 h of uninterrupted feeding, and represent averages of 3 experiments, each involving 10 replicates.

† Calculated using the formula:
$$\% \text{ feeding deterrency} = 100 \times \left(\frac{\text{quantity control disc consumed} - \text{quantity treated disc consumed}}{\text{quantity control disc consumed}} \right)$$

G. pallidipes sex pheromone bioassays: synthetic analogues and stereoisomers

P.G. McDowell, A. Hassanali

We have previously indicated that a number of synthetic analogues of the *G. pallidipes* sex pheromone have been synthesized and were undergoing bioassay (ICIPE Annual Report 1982). Figure 3 shows compounds I to VII. Of these, only I to V have been synthesized and tested. We also reported that the diastereoisomers of compounds I and II were being synthesized by Professor K. Mori in Japan. We have now received and tested four stereoisomers; VIII (R, R) - 13, 17 - dimethylpentatriacontane, I X (S, S) - 13, 23 - dimethylpentatriacontane, X (R, R) - 13, 23 - dimethylpentatriacontane and XI (R, S) - 13, 23 - dimethylpentatriacontane (fig. 4).

The standard bioassay for these tsetse sex pheromones has already been described elsewhere. Bioassays carried out on compounds I to V have confirmed earlier reports that II, although not the natural pheromone, is active at a similar dosage to I, the naturally occurring isomer. Compounds III to V show little or no activity in the bioassay at dose levels found to be active for compounds I and II (in the 1 µg to 30µg per decoy range-using cork decoys). Compounds III and V show some activity in the 50 to 100 µg per decoy range but the full copulatory behaviour is still rare.

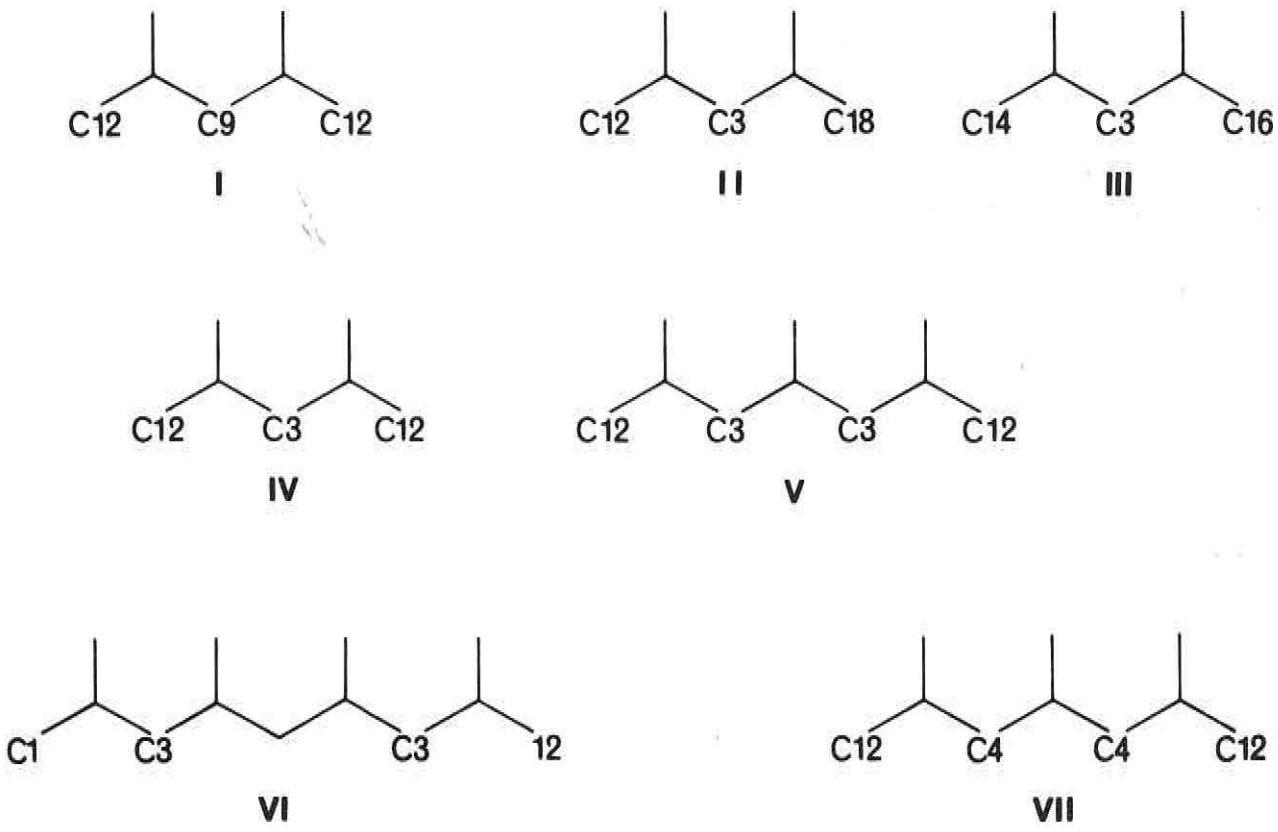


Figure 3

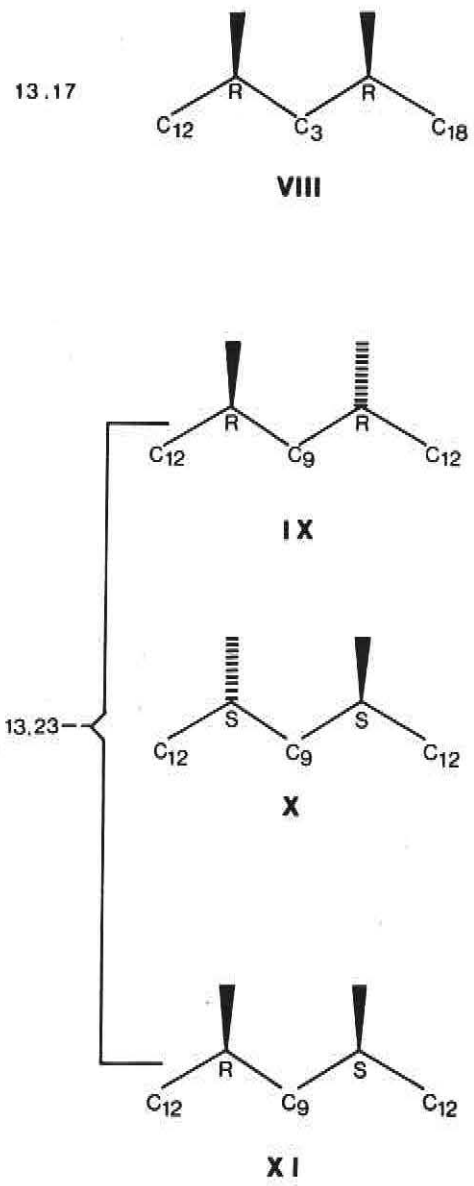


Figure 4. Stereoisomers of *G. pallidipes* pheromones

The most interesting results obtained were from the bioassay of the diastereoisomers synthesized by Professor Mori, Japan. Of the three isomers of the natural pheromone, 13, 23-dimethylpentatriacontane, compounds IX to XI, only the R,S diastereomer, XI showed significant activity at doses down to ca. 0.25 μg per decoy. Both the R, R- and S, S-diastereomers showed little activity, even at high doses (ca. 30 μg per decoy), other than simple arrestment of the test male. On rare occasions at the higher doses full copulation was observed. The one diasteriomer of 13, 17-dimethylpentatriacontane available, compound VIII, the R, R-isomer, proved to be inactive.

The bioassays run so far indicate that XI is more active than the recemic mixture I which would be expected since the achiral synthesis of I would lead to a mixture containing ca 50% R, S-, 25% R, R- and 25% S, S-diastereomers.

Several questions remain to be answered. Firstly, which of the other 13, 17-diastereomers is active? One might expect that the S, S-isomer will prove to be inactive and 13R, 17S-isomer prove to be active, but will the 13S, 17R-isomer prove to be active also? These questions must await the completion of the chiral syntheses of the 13,17-diastereomers. Secondly, is the natural pheromone a racemic mixture, I, or is it specifically the R,S-diastereomer, XI? The latter question is being addressed by carrying out a series of comparative bioassays on the isolated pheromone, the racemic mixture I, and the active diastereomer, XI.

It is noteworthy that in the case of *G. morsitans* pheromone components, diastereomers of the less active dimethylheptatriacontanes do not show significant differences in their activity. The separate stereoisomers of the more active trimethylheptatriacontane have not yet been reported and it will be interesting to see if there is some stereochemical requirement for activity in these molecules.

2,6-dichlorophenol in the tick *R. appendiculatus* — A reappraisal

P.G. McDowell

The presence of 2,6-dichlorophenol (I) has been reported in nine tick species since its first discovery in the lone star tick *Amblyomma americanum* where its principal role was that of a sex pheromone. Other phenols such as phenol itself, p-cresol and salicylaldehyde have also been proposed as sex pheromones in ticks.

Rhipicephalus appendiculatus together with *R. pulchellus*, *R. compositus* and *R. simus* were first investigated here at ICIPE by Wood and colleagues. They reported the presence of phenol (II), p-cresol (III) and salicylaldehyde (IV) in these ticks but did not find evidence for the presence of 2,6-dichlorophenol. On the other hand *R. appendiculatus* males were found to be highly attracted to this chemical in their choice bioassay system. The electrophysiological responses to these compounds were subsequently investigated in the Sensory Physiology Unit (Dr. S.M. Waladde). Of the compounds noted by Wood et al., only (I) gave responses at low dosages, the others required much higher amounts before eliciting responses. For this reason the chemistry of this tick has been reinvestigated with a view to confirming or denying the presence of (I) in *R. appendiculatus*.

Extracts of both 6-day fed and unfed male and female adult *R. appendiculatus* were made using hexane and phenols, and extracted using IM sodium hydroxide solution after strong acids had been extracted using sodium

bicarbonate solution. These extracts were investigated by electrophysiological bioassay, capillary gas chromatography with electron capture detection (ECD) and by mass spectrometry and ultraviolet spectroscopy.

Bioassay of the extract fractions obtained by acid-base extraction of female *R. appendiculatus* extracts confirmed that the activity occurs in the phenolic fraction. Gas chromatography (GC) analysis of the extracts revealed the presence of a component with the retention time of (I) in females, fed and unfed, and in unfed males. Figure 5 shows a typical capillary chromatogram of the unfed female extract. No 2,6-DCP was detected in the fed males. The largest amounts appeared in unfed females, while fed females and unfed males contained similar amounts as follows: unfed females ca. 12 ng/tick; fed females ca. 2 ng/tick; unfed males ca. 2 ng/tick; fed males ca. 0.ng.

The component suspected of being (I) was GC coinjected with an authentic sample and coincidence occurred on all columns, 5% OV-17 (packed column), and CP Sil 5 and CP Wax 51 capillary columns.

To confirm the identity of the suspected 2,6-dichlorophenol peak a large number of unfed female ticks (2880) were extracted. The suspected (I) (component 1, fig. 5) and the following major peak (component 3, fig. 5) were separated by preparative GC on an OV-17 column. Preparative fractions were collected in hexane. The remainder of the extract was collected as one fraction. Re-analysis of the fractions 1 and 2 by capillary column GC showed fraction 1 to be a clean single component while fraction 2 contained a minute amount of the first fraction.

Ultraviolet spectra were obtained on these two fractions. Fraction 1 displayed a spectrum similar to that of an authentic sample of (I) with maxima occurring at 268, 276 and 284 nm. The slight difference in relative intensities of the peaks were shown to be due to a background absorption by stationary phase bleed from the preparative OV-17 column. The mass spectrum in the region of the molecular ion cluster showed isotope ions at m/z 162, 164 and 166 in the ratio of ca. 8.9:6·6:1.0 which approximates to the theoretical value of 9:6:1 for a compound containing two chlorine atoms.

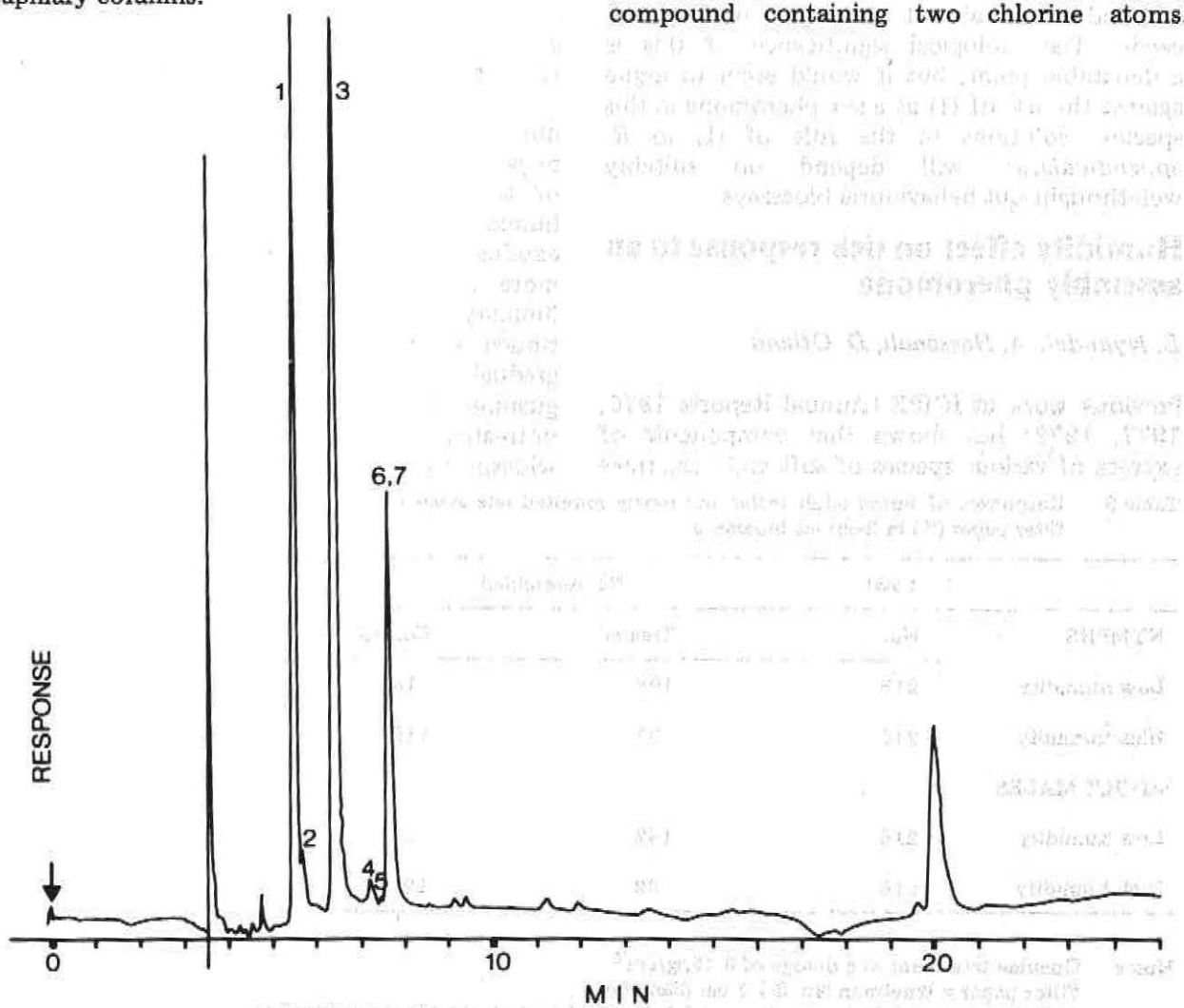


Figure 5. Gas chromatogram of extract of unfed female *R. appendiculatus*. GC — Capillary CPS115, 50 m column, i.d. 0.22 mm; Isothermal operation at 170°C.

From these data it was concluded that 2,6-dichlorophenol is indeed present in *R. appendiculatus* males and females, predominantly in unfed females.

The structure of component 3 in preparative fraction 2 (see figure 5) as yet remains unknown. The UV spectrum revealed little of its nature. Based on its GC retention time it does not appear to be any of the other common di-and tri-chlorophenols available for comparison viz. 2,3-, 2,4-, 2,5- and 3,5-DCP and 2, 4, 6-, 2,4,5- 2,3,4- and 2,3,6-TCP.

Electrophysiological assays carried out in the Sensory Physiology Unit indicated that both fraction 1 and 2 produced strong electrophysiological responses, however, fraction 3, containing all other components, was not active

It is apparent then, that *R. appendiculatus* does indeed contain the ubiquitous 2,6-DCP (I) contrary to the original report from this laboratory. 2,6-dichlorophenol has been found in both males and females of the species, predominantly in the unfed females. This appears to reduce as feeding proceeds. Unfed males start with about the same level as fed females and reduces to almost nothing as feeding proceeds. The biological significance of this is a debatable point, but it would seem to argue against the use of (I) as a sex pheromone in this species. Solutions to the role of (I) in *R. appendiculatus* will depend on suitably well-thought-out behavioural bioassays.

Humidity effect on tick response to an assembly pheromone

E. Nyandat, A. Hassanali, D. Otieno

Previous work at ICIPE (Annual Reports 1975, 1977, 1979) has shown that components of excreta of various species of soft and hard ticks were responsible for the assembly behaviour of nymphal and adult ticks. HPLC analysis of saline and water extracts and subsequent bioassays on filter paper discs showed that guanine was the principal active component of excreta. By application of successive dilutions of aqueous solution of purified commercial guanine we have now demonstrated that the pheromone can induce assembly behaviour at concentrations as low as 1 ng/cm^2 (10-11 mole/cm^2) of the filter paper.

Of particular interest is the observation that the response of *Argus persicus* nymphs to guanine is weather-dependent, the assembly behaviour being lost during the rainy days. Similar observations had earlier been made by the late Dr. T. Hefnawy on the assembly behaviour of *R. appendiculatus* adults on filter papers treated with water extracts of nymphal and adult excreta (ICIPE Annual Report 1975). We investigated the assembly behaviour of nymphal and adult male *A. persicus* at low (30—35%) and high (85-90%) humidities and table 3 shows typical results we obtained during exposure at these humidities lasting for about two weeks. The response to guanine is clearly lost at high relative humidities. However, the behaviour is reversible.

The transfer of ticks from high to low relative humidities restores their positive assembly response to guanine. The biological implication of these observations are not clear but the humidity effect may help to prevent premature exodus of ticks from their resting sites until more favourable conditions prevail. Our preliminary observations have shown that continued exposure to high humidity results in gradual induction of a negative response to guanine, with ticks showing preference for untreated control discs. This effect may be quite widespread in ticks and may be an important

Table 3. Responses of unfed adult males and newly moulted late stage *Argus persicus* nymphs to guanine treated filter paper (T) in 2-choice bioassays

	Total	No. assembled		Significance
NYMPHS	No.	Treated	Control	
Low humidity	216	198	18	<0.01
High humidity	216	97	119	N.S.
ADULT MALES				
Low humidity	216	182	34	<0.01
High humidity	216	92	121	N.S.

Notes Guanine treatment at a dosage of 0.15μg/cm^2
Filter paper = Whatman No. 3 1.7 cm diameter
2-choice bioassays: in 6—cm glass petri-dishes kept in dark chambers incubated at 32°C
Low humidity 30—35% rh
High humidity 85—90% rh

factor in the sequence of behavioural changes leading to the abandonment of the resting sites and the search for hosts after the onset of more humid conditions.

Mode of action of an abortifacient active in the tsetse

D.L. Whitehead, L.S. Thomas, E.N. Ole-Sitayo

Feeding 20-hydroxyecdysone (20-OHE) to *Glossina morsitans morsitans* just after larviposition (L1) causes abortions in L2 and L3 pregnancy cycles (ED_{50}=ca 5.2 and 6.9 nmol respectively). The causes appear to be related to subsequent smaller meal sizes and decreased supply of "milk" to the larva from the uterine gland of treated females. In conjunction with Dr. M.F.B. Chaudhury (Tsetse Research Programme) we established that topical application of JHIII (5μg) on day 2, 4 and 6 of the second cycle, abolished most of the effect of feeding 5 μg of 20-OHE on the diameter of the gland distal tubule (table 4) and on development of the larva *in utero*. One possibility this result suggested was that 20-OHE had an allatostatic effect i.e. shutting off juvenile hormone (JH) release from the CA. Since we were able to demonstrate the presence of ecdysteroids by RIA in the abdomen (1.116 ± 0.044 pmol fly^{-1}) as well as in the thorax (0.433 pmol fly^{-1} on day 7 when the uterine gland normally ceased synthesis of "milk", the hypothesis gained support. However, due to limitations in the chemical method of JH assay, we have so far been unable to show that feeding 20-OHE depresses circulating JH levels in the pregnant fly. Incorporation of label from ^{14}C-methionine into JH would, we hope, give us a sensitive assay and facilitate identity of the tsetse hormone to be established.

The ovaries are the source of ecdysteroids (0.0473 ± 0.011 pmol) found in the thorax on day 7 and 8. Most of the hormone from the ovaries is carried into the egg to be used by the embryo and stage I larva during development (fig. 6).

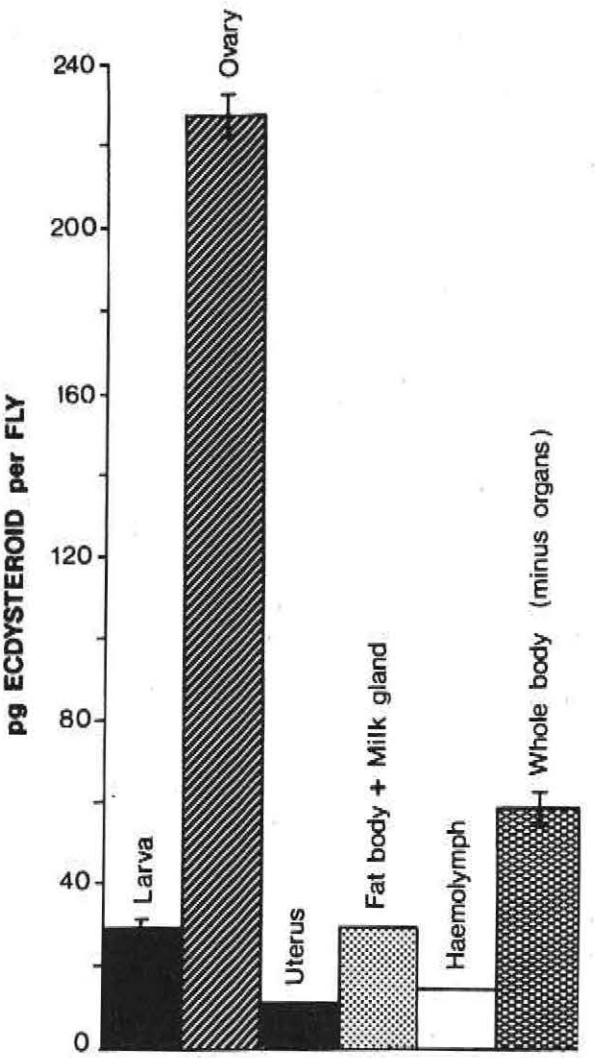


Figure 6. Mean value ± SEM for ecdysteroids present in female abdominal tissues on day 7 of the L2 pregnancy cycle.

Evaluation of limonoids for anti-insect activities

A. Hassanali, M. Bentley, P.E. Njoroge, N. Ole-Sitayo, A. Chapya

Among the various classes of natural products previously studied as anti-insect compounds, the tetranortriterpenes known as limonoids appear to be most promising as models for potential insect-control agents. The most prominent example, azadirachtin, is a very potent

Table 4. Effect of treatment with 20-hydroxyecdysone on tsetse larval development and uterine gland diameter on day 7 of the pregnancy cycle

Fed	Treatment Topical	Diameter of distal tubules (minus lumen) μm ± S.E.M. (n)	Percent larvae retarded
20—OHE	Nil	53.0 ± 1.4 (25)	80
20—OHE	Acetone	41.5 ± 2.1 (10)	90
20—OHE	JH III	49.6 ± 5.2 (16)	56
NIL	JH III	37.4 ± 4.6 (10)	10
NIL	Nil	68.0 ± 2.2 (25)	0

systemic feeding deterrent against a wide range of insect pests and ingestion of the compound by insects causes disruption of normal growth, particularly ecdysis. The high structural complexity of limonoids precludes application of synthetic compounds comprising the entire limonoid structure, and utilization will necessarily be limited to the natural products as such, simple synthetic variants of these compounds, or simple analogues comprising only the active moieties of the natural compounds. The longer-term goals of the limonoid project at ICIPE are: to carry out detailed structure activity studies on limonoids from *Meliacea* and *Rutaeae* on a number of ICIPE target insects; to identify potentially useful naturally occurring limonoids or readily accessible derivatives; and to synthesize and evaluate simple analogues of active limonoids.

In the present report preliminary results obtained for anti-feeding activities of limonin(I), deoxylimonin(II), acetylcitrolin(III), obacunone (IV) and harrisonin (V) against *Spodoptera exempta*, *Maruca testulalis* and *Eldana saccharina* are given.

Limonin(I) was isolated in pure form from grapefruit seeds by acetone extraction, hexane precipitation, gradient chromatography and repeated recrystallizations. Deoxylimonin(II) was prepared by deoxygenation of limonin with HI. 23-acetylcitrolin(III) was formed by reaction of deoxylimonin with HBr in acetic anhydride/acetic acid. Obacunone (IV) and harrisonin (V) were isolated from *Harrisonia abyssinica* by methanol extraction, $CHCl_3/H_20$ partitioning, gradient silica gel chromatography and rectrystallizations. The purities of the compounds were verified by HPLC on reverse and normal phase columns.

Feeding deterrent activity against *S. exempta* (6th-instar larvae) was measured by determining the relative extent of feeding for 2 h on 2.0 cm diameter treated and untreated discs. Dosages of 100 µg and 10 µg for discs of the test compounds were used.

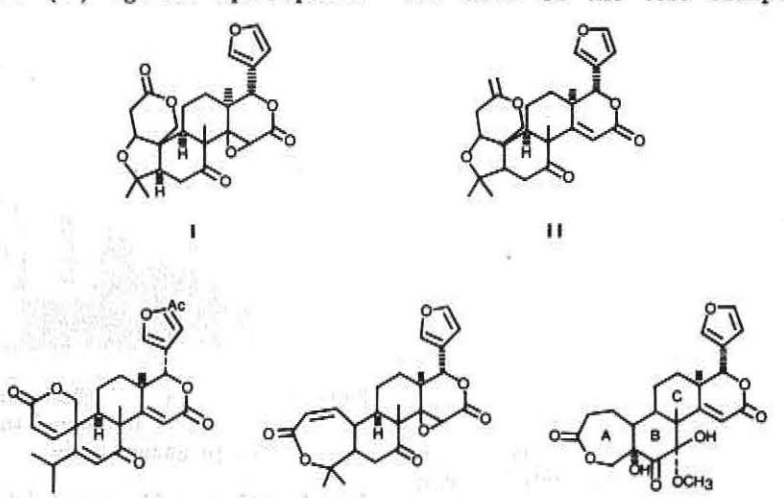


Figure 7

Table 5. Feeding deterrent activities of some limonoids against the larvae of *Spodoptera exempta*, *Eldana saccharina* and *Maruca testulalis*.

Test compound	Dose (µg/disc)	Feeding inhibitory activity (+ 10%)		
		S. exempta	*E. saccharina*	*M. testulalis*
I	100	b	54	69
	10	b	42	68
II	100	b	93	69d
	10	b	90	65
III	100	b	91	e
	10	b	76	e
IV	100	10	91	e
	10	b	87	e
V	100	33	83d	e
	10	b	79d	e

Notes:

1 — Feeding inhibitory activity calculated as $1 - \left(\frac{\text{Wt of test disc eaten}}{\text{Wt of control disc eaten}}\right) \times 100$

b — No significant activity
c — Toxic effect at this dosage evidenced by regurgitation and lethargic behaviour of the worms
d — Based so far on single observations on sets of 10 larvae
e — Results not yet available

Activity against *E. saccharina* (5th-instar larvae) were similarly determined using 1.8 cm diameter maize leaf discs over a feeding duration of 24 h. Activity against *M. testulalis* (5th-instar larvae) were determined using 1.8 cm diameter cowpea discs over a feeding duration of 6 h.

The preliminary results so far obtained are summarized in table 5. Particularly noteworthy is that limonoids containing α, β-unsaturated lactone units appear to be potent antifeedants against *E. saccharina* and it will be interesting to assay other readily available simple analogues of the limonoids. Initial results with *M. testulalis* are promising and assays with other crop borers will be undertaken in the coming year. The effect of longer term feeding of the active limonoids at lower dosages on growth and development of the insect pests will also be studied. Finally, the possibility of systematic action of the compounds by absorption via the root into the foliage of crop plants will be investigated.

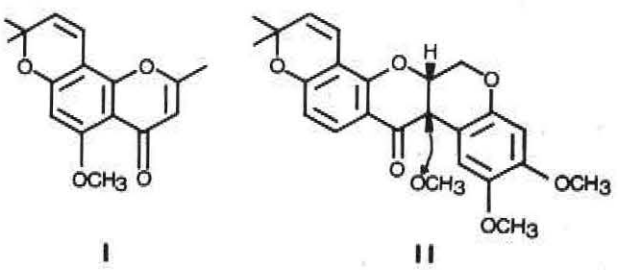
Insect antifeedants from *Tephrosia alata*

M. Bentley, W. Lwande, A. Hassanali, N. Ole Sitayo

Screening studies demonstrated significant activity for *Tephrosia alata* seed extract in *Spodoptera exempta* feeding assay. The product obtained from methanol extraction was extracted with chloroform and chromatographed on a silica gel column. Bioassay of fractions followed by further purification on preparative TLC silica plates yielded isopongaflavone(I) and tephrosin (II) (a well-known natural insecticide) as the compounds responsible for the observed activity.

Quantitative bioassay against *S. exempta* was carried out and results are summarized in table 6. It is interesting that tephrosin is such a powerful antifeedant against the African armyworm at dosages well below its toxic effects. Rotenone, another well-known insecticide containing the chromanochromanone ring system of tephrosin, has also been found to be equally active. Assays against other insects are underway.

We were extremely interested to find a complex of insects associated with *T. alata* seed pods. In collaboration with the National Museum of Kenya and specialists in the United Kingdom, identifications are complete on most of the species. The way these insects have adapted themselves to tephrosin, remains an intriguing chemical ecological question.



I II

Figure 8

Feeding deterrents from plants

A. Chapya

Strychnos henningsii (Gilg) Loganaceae

From the root bark of this typical African *Strychnos*, four main alkaloids, monomeric indole-type (80% of the basic extract) were isolated by counter-current distribution (CCD) at discontinously decreasing pH. Three of them were identified by direct comparison with authentic samples as diaboline (I), hostiine (II), already isolated from *Stychnos henningsii* and a third one as N (a)-acetylstrychnosplendine (III) previously isolated only from *Strychnos fendleri.* The fourth alkaloid was a new one, mp. 200—2°, $C_{22}H_{28}N_2O_4$, $[\alpha]\ ^{20}D$ =+101.1 c=1 CHI_3) which was identified as N(a)-acetyl-11-methoxystrychnosplendine (IV) on the basis of its spectroscopic data. Using the conventional leaf disk "choice" bioassay, each of the isolated alkaloid was tested for feeding deterrency against the 6th-instar larvae of *S. exempta* (armyworms). All compounds showed moderate degree of activity, diaboline at 100 ppm, holstiine (acetyl derivative) at 250ppm, N(a)-acetylstry-

Table 6. Effect of isopongaflavone and tephrosin on African armyworm feeding

Test compound	Dose (μg/disc)	Mortality	Antifeedant activity
Isopongaflavone	100	—	10%
Tephrosin	10	16%	93
	1	—	91

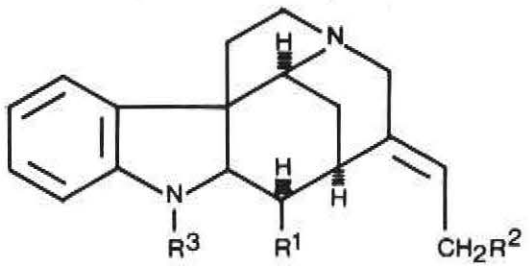
Note: The definition of antifeedant activity has been given in

chnosplendine at 250 ppm and N(a)-acetyl-11-methoxystrychnosplendine at 250 ppm.

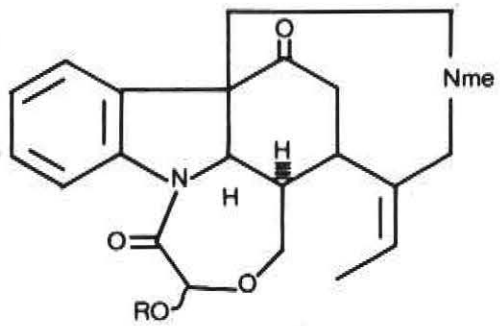
Mussaenda arcuata (Lam) and *Tarrena graveolus* (S. Moore) Rubiaceae

By the CCD technique again, using organic solvents, two iridoid glucosides, shanzhiside methyl ester (V) and a new one, 8-acetyl shanzhiside methyl ester (VI) m.p. 106–8°, $C_{19}H_{28}0_{12}$, $[\alpha]\ ^{20}D$ = .61.2 (c =0.8 MeOH) were isolated in pure form, from the root barks of the plant.

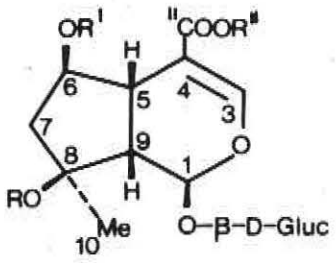
Moreover, ixoside (VII) and two new, 6-feruloylschanzhiside (VIII) named tarennine, m.p. 158–60°, $C_{26}\ H_{32}\ 0_4$, $[\alpha]\ D^{20}$ = 117 (c = 0.6 MeoH), ixoside 11-methyl ester (IX) m.p. 180-2°, $C_{17}H_{22}0_{11}$, $[\alpha]\ ^{20}D$ = 33.6 (c = 1 MeoH) were identified on the basis of spectroscopic data and derivatization. Laboratory assay on these iridoids showed that they are inactive even at the concentration of 5,000 ppm.



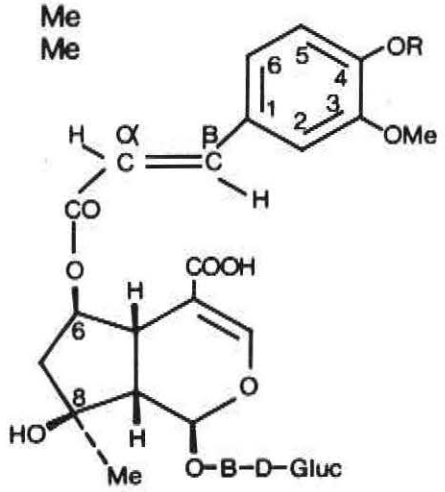
	R^1	R^2	R^3
I	CHO	OH	AC
III	H	AC	
IV	OMe	AC	

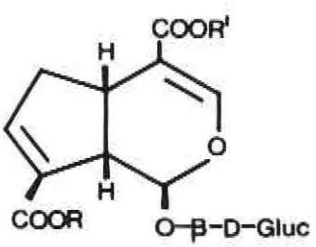


	R
II	H



	R	R'	R"
VI	Ac	H	Me
V	H	H	Me





	R	R'	R"
IX	H	Me	H
VII	H	H	H

Figure 9

IAEA special project on the biology and biochemistry of microorganisms for improved biomass degradation

H. Osore, M.A. Okech

The cooperative programme between the IAEA and ICIPE has now been operating for nearly two years. Since our last report (ICIPE Annual Report, 1982), we have made some progress towards isolating pure cultures of microorganisms (aerobic bacteria and fungi). We have also screened for various cellulolytic and ligninolytic enzyme activities and carried out detailed purification and characterization studies.

Microbiology

We have previously, reported the isolation of various species of bacteria from *Macrotermes michaelseni*, that were awaiting identification using the API 20E identification kit. The isolates have now been identified and the isolation work extended to other termite species such as *Odontotermes*, *Cubitermes* and *Pseudocarthotermes*. Species of bacteria isolated from these species have been similar to those from *Macrotermes*.

The ability of the isolates to secrete cellulase has been tested using ball-milled amorphous cellulose. Of the bacterial isolates only the yellow Gram positive bacillus induced clearance on plates with amorphous cellulose.

Five *Actinomyces* species have been isolated and have been shown to induce diffusion of dye from cellulose-azure in the rapid tube-test for the detection of cellulases.

In addition to the fungi which we isolated from the fungus-combs last year, a *Trichoderma* spp. has been isolated. Three fungi *(Fusarium, Aspergillus,* and *Trichoderma)* have been selected for further detailed studies. Attempts to grow *Termitomyces* have been unsuccessful. It is now well-documented that the fungus is difficult to grow in pure culture once the symbiotic relationship between the termites and the fungus has been disrupted. We believe that it is a fungus of great potential, and are continuing our efforts to grow it. Much biochemical work has been done with conidia and fruiting bodies of *Termitomyces*, clearly demonstrating very high cellulase and β glucosidase activities.

Biochemistry

Much effort has been expended in determining and purifying the components of the cellulase enzyme system of fungi from the fungus-garden and bacteria from the termite gut. The cellulase complex of *Termitomyces* conidiophores and fruiting bodies has been purified by ion-exchange chromatography on DEAE-Sepharose and CM-Sepharose, and by gel filtration on Biogel-P100. The purification stages are shown in table 7.

Kinetic studies have been undertaken to determine the Km values of cellulases from different organisms. The rationale is that, by studying the kinetic parameters of enzymes from different microorganisms, it may be possible to design bioconversion systems for lignocellulose that yield optimum results and avoid inhibition of key hydrolytic enzymes.

We have failed to detect reasonable cellulase activities in all our isolates of bacteria. The isolation of cellulose-degrading bacteria from termites has been the object of many other investigations, most of which have met with mixed success.

Since the hemicellulose content of biomass is usually in the region of 20—24%, the need to study the bioconversion of this biopolymer clearly arises. Xylan forms a large proportion of this hemicellulose. In this project, we have, therefore, attempted to purify and study the properties of xylanase from *Termitomyces* fruiting bodies. A 100-fold purification was achieved by ion-exchange on DEAE-Sephadex-A 25 followed by gel filtration on Biogel P-100 (fig. 10).

We have continued to study the problem of lignin breakdown. Lignin still remains the major technical impediment to the development

Table 7. Purification of cellulase from buffer extracts of *Termitomyces conidia*

Enzyme sample Purification steps	Total protein	Recovery %	Specific activity Protein^{-1}h-r)	Total activity (—fold)
Crude extract (100 ml)	350	100.0	0.99	1.0
DEAE-Sepharose (100 ml)	6.0	1.7	22.75	23.0
Precipitation with saturated $(NH_4)_2SO_4$ then chromatographed on CM-Sepharose	4.0	1.14	29.75	30.0

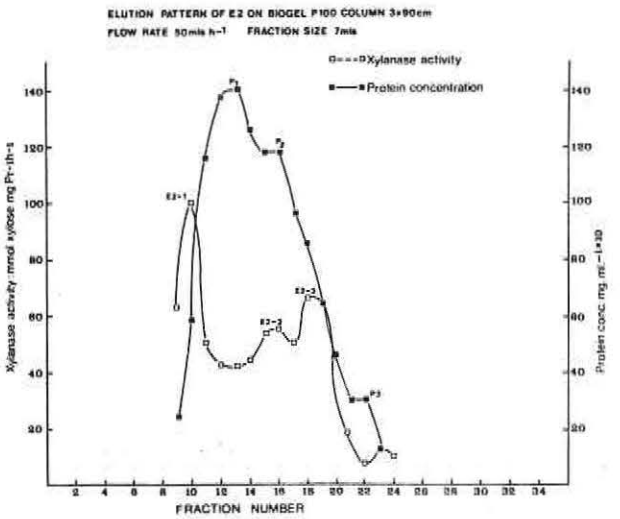


Figure 10. Elution pattern of E 2 on Biogel P100 column 3 x 90 cm flow rate 50 ml h-1 — Fraction size ml

of biological conversion processes for lignocellulosics. We have succeeded in preparing a standardized lignocellulose substrate from cereal straws using solvent extraction. Spectroscopic analysis has confirmed batch to batch reproducibility. This is now being used as a substrate for detection of ligninolytic activity.

Results on the use of HPLC techniques to separate degradation products of lignin dilignols (model compounds), although preliminary, have been encouraging. Using acetonitrile: water: acetic acid (49:49:2), solvent system, mixtures of different dilignols can be separated on a reverse phase column. We are vigorously persuing the synthesis of these compounds for use as lignin models for enzymatic breakdown studies.

In our studies of ligninolytic activity, one of the enzymes we have studied in some detail

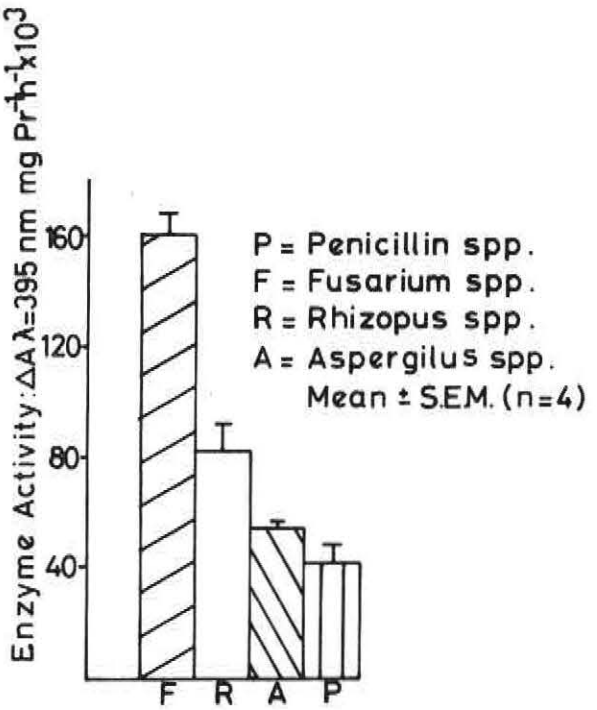
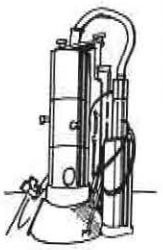


Figure 11. Polyphenoloxidase activity of microorganisms associated with the fungus comb of *Macrotermes* spp.

is polyphenoloxidase. Using a variety of substrates such as protocatechuic acid, catechol, and dopamine, we have demonstrated and quantified polyphenoloxidase activity in a number of isolated fungi (fig. 7). Polyphenoloxidase may be a key enzyme, in the degradation of lignocellulose, as it oxidizes the phenolic rings of lignin.

Future studies will hinge on elucidating the optimum breakdown of lignin leading to the release of cellulose for subsequent bioconversion into reducing sugars and hence ethanol.



Histology and Fine Structure Research Unit

The work of the unit during 1983 was directed towards investigating structural aspects of tissues in close collaboration with a number of ICIPE core programmes. The research topics represented three main areas of immediate interest, involving correlation between fine structure and function, notably; reproduction (in Glossina*), neuroendocrinology (*Glossina*), sensory physiology (in crop borers) and taxonomy (in sandflies). A summary of results obtained during the year are given in the reports below.*

Reproduction

Sperm development in *G. morsitans*

E. D. Kokwaro

During mating in *Glossina*, sperm and associated male accessory reproductive gland secretions are packed into a spermatophore and transferred to the female uterus. Soon after deposition, the sperms migrate into the spermathecae where they are stored until required for fertilization. The question is how those sperms found in the spermatophore are formed.

All pupal stages, from larviposition to the end of pupal instar which lasted approximately 30 days, were used. Specimens were obtained from a colony reared in an insectary at temperatures of 23—26°C, rh 65—70% and light conditions of 12 h. These materials were then processed for light and electron microscopy following standard procedures.

Observations made show that the paired testes of *Glossina morsitans* larvae are almost kidney-shaped organs. They are broadest distally and narrow proximally to the vasa deferentia, (fig. 1). As growth and development ensue, the testes become increasingly elongated and coil up as a result of the rapid growth of the spermatozoa (fig. 2).

As shown in figure 3, the testicular wall consists of flattened cells enclosing numerous cyst cells. The cysts, which are spherical at the time of larviposition, soon acquire an elongated form by the time pupae are 4 days old. The cyst cells contain large, round nuclei with prominent nucleoli. In pupae aged 4 days, the cyst compartments are closely apposed and the entire testis increases in size. Space formation between adjacent cells and cyst compartments reaches its peak in 5-to-10 days old pupae and numerous vocuole-like structures appear in the cytoplasm. The largest number of cells in meiotic stages are observed in the cysts situated in the middle part of the testes obtained from 8-to-9 days old pupae (fig. 4). Thereafer, the developing spermatozoa contained within pockets are a prominent feature.

When examined by electron microscope, the cell nuclei in pupae 0-to-4 days are electron-dense and contain one or two nucleoli. The cytoplasm contains many small mitochondria, numerous free ribosomes frequently arranged as polysomes. Although Golgi complexes are evident, rough endoplasmic reticulum is scarce while microtubules are resolved throughout the cytoplasm. Adjacent cells are linked together by intercellular channels which contain organelles similar to those found in the cytoplasm.

Maturation begins from the proximal cysts between 8 to 9 days. Some of the cysts contain cells with round or oblong-shaped mitochondria in the cytoplasm. Spermatocyte and early spermatid mitochondria contain occasional small, electron-dense granules within their matrix.

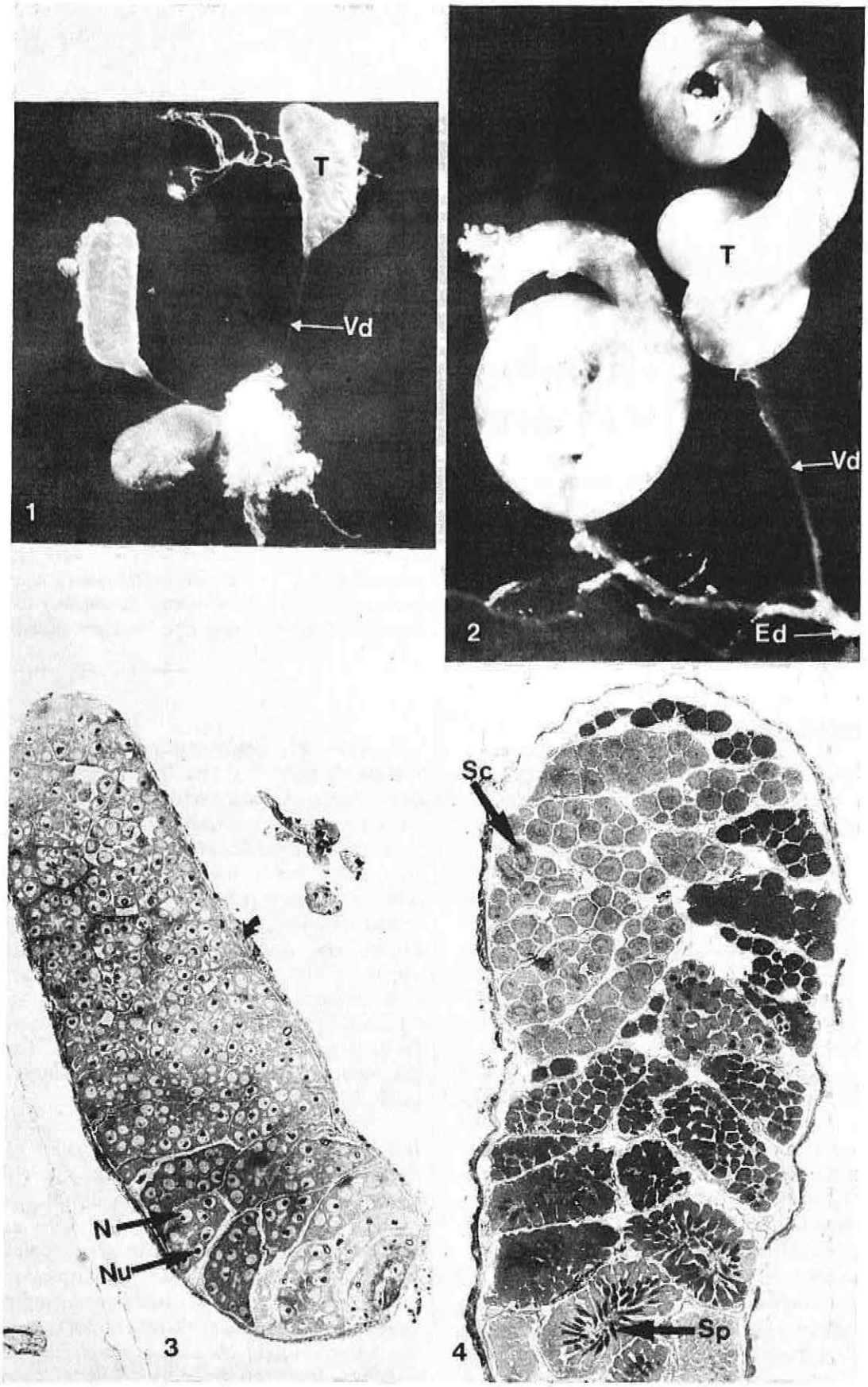


Figure 1. *G. morsitans* male pupal testes, 3 days old. Testes (T), vas deferens (Vd) x 36.

Figure 2. Pupal testes, 23 days old. Testes (T), ejaculatory duct (Ed), vas deferens (Vd) x 19.

Figure 3. Photomicrograph of a thick longitudinal section through a 0-day pupal testis. An epithelium of flattened cells (arrow) surrounds cyst cells containing round nuclei (N) each with a prominent nucleolus (Nu) x 226.

Figure 4. Pupal testis, 8 days old. Spermatocytes (Sc) in the process of meiotic division and early spermatids (Sp) are present x 226.

The transformation of a young spermatid to a spermatozoa involves aggregation and fusion of mitochondria at the proximal end of the nucleus between 8 to 10 days and transformation and fusion of mitochondria into large nebenkern between 10 to 14 days giving the immature spermatids tadpole-like shapes. During this period, filamentous elements show attachments to the centriole which is located at the posterior end of the nucleus. The nucleus chromatin forms a loose system of fibrillar appearance. The axoneme beginning at the centriolar complex is of the 9+2 pattern and is surrounded by mitochondria. A narrow margin of cytoplasm surrounds the spermatid and contains free ribosomes and numerous microtubules.

The spermatozoa are grouped in long, more or less entangled bundles and fill the testis towards the 15th day. During mating, these are transported with the secretions produced by the male accessory reproductive glands to the female uterus as a spermatophore. The spermatophore is thereafter rapidly emptied of sperm which actively enter the spermathecae for storage to be released when required for fertilization.

The present studies have resolved the cellular organization of the pupal testis of the tsetse *G. morsitans* and recorded ultrastructural changes which characterize the transformation of cells during the process of spermatogenesis.

Fine structure of nurse cell nuclear pores in *G. morsitans*

M. Chimtawi

During oogenesis, nurse cells function as the primary synthetic structures and pour their cytoplasmic components into the egg. Generally, it is known that in a developing egg chamber there are three main cell types: the oocyte, the nurse cells, and the follicle cells which encases the other two cells (fig. 5). In a developing chamber it is known that there is communication between those cells and that nuclear pores are involved. The present study was undertaken to follow, in thin sections, the specific pattern of nuclear pore distribution and size in the nurse cell nuclei and their relationship to the cell's function.

At the electron microscopic level, the nuclear envelopes of nurse cell nuclei have many pores (fig. 6) and abundant chromatin-like material associated with both the outer and inner membranes (fig. 7). The commonest nuclear pore arrangement is of a regular pattern. In some instances, there was evidence of small groupings of pores separated by areas of similar size without pores.

Immediately behind the nuclear membrane margin, small aggregations of membrane particles are occasionally observed. Mitochondria, free ribosomes, and microtubules are some of the organelles found in the cytoplasm adjacent to the pores.

These preliminary results suggest that nurse cell nuclear pores facilitate movement of material between the nucleoplasm and the cytoplasm. The relationship between nuclear pores and ovarian development is being investigated.

Cuticular spines in the uterus of *G. m. morsitans*

J. A. Kongoro

The tsetse-fly, *G. m. morsitans* is a viviparous insect. Following ovulation, the egg is fertilized in the uterus, which is a uniquely modified bursa characteristic of glossinids. The embryo hatches into a first instar larva which in turn is transformed into a second and later into a third instar larva which is deposited by the female. The chorion from the egg and the first larval exuvium are deposited in a depression just posterior to the choriothete while the exuvium of the second instar larva remains as a loose pellicle round the third instar larva. The choriothete is a glandular structure on the ventral part of the uterus which has been implicated in the removal of the chorion and the first larval exuvium but the mechanism by which it accomplishes this is not fully understood.

The aim of the present work is to elucidate further the morphology of the uterus in order to understand its role in the development of the larva.

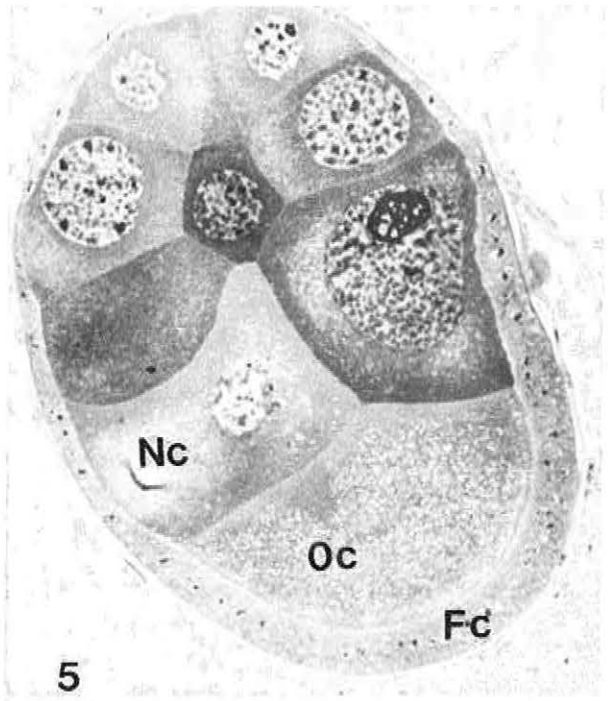
Our light microscopic and ultrastructural studies have shown the presence in the uterus of cuticular spines which can be classified as major and minor. The major spines, which are about 6 μm long, are found at the anterior ventral part of the uterus and are probably concerned with the orientation of the egg at fertilization. The minor spines are about 0.5-2.0 μm long and like the major spines are thicker at the base and taper towards the tip. These spines, which occur in rows of 5-10, and are clustered in groups of 10 or more, (fig. 8) have neither lumina nor sensory structures (figs. 9 and 10). It is suggested that they are mechanically involved in the removal of the chorion from the embryo and the exuvium from the first instar larva.

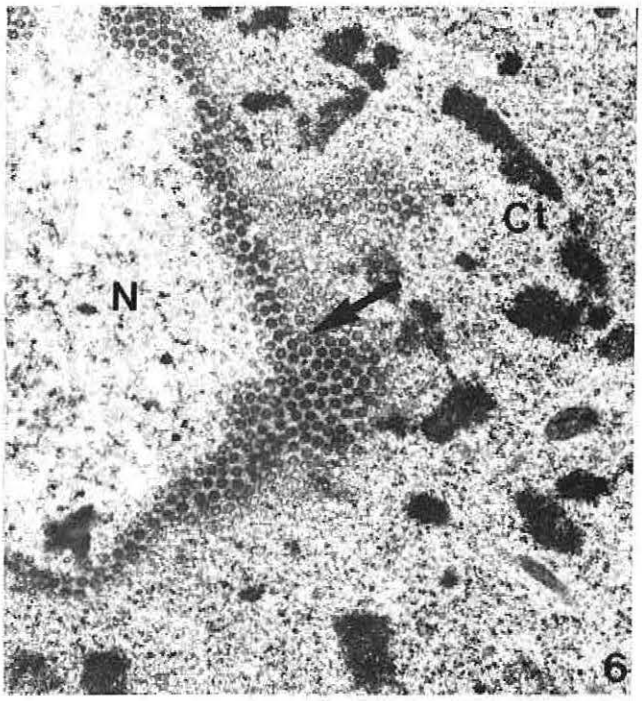
Neuroendocrinology

Structure and function of corpus cardiacum in *G. morsitans*

L. R. S. Awiti

The neurohaemal function of the corpus cardiacum (CC) in insects is well known. In addition





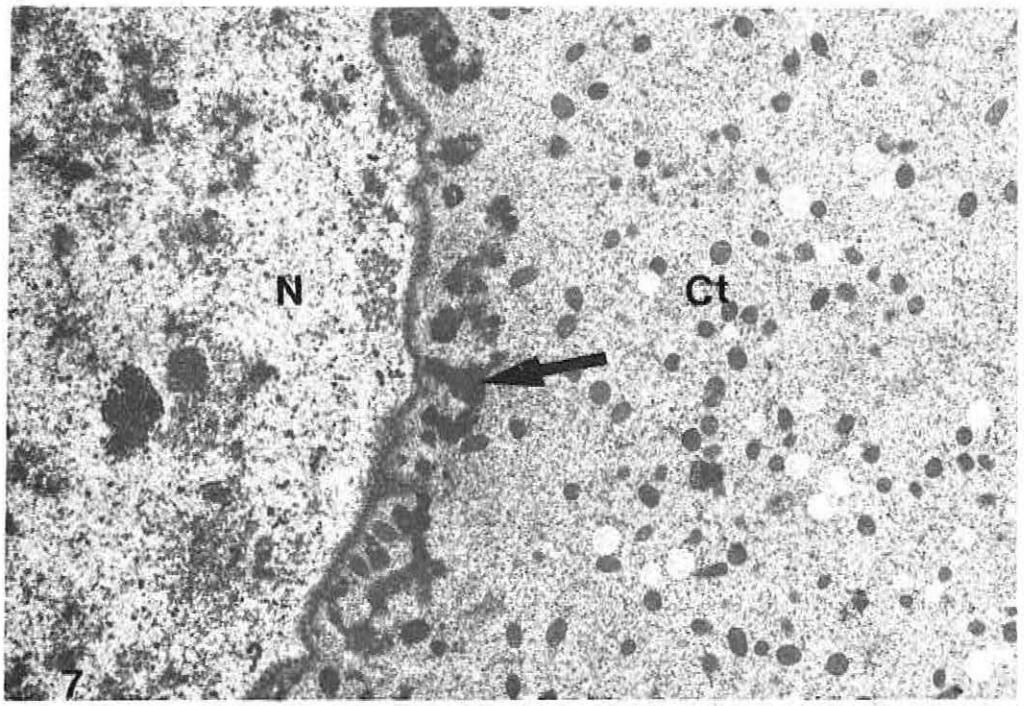


Figure 5. Photomicrograph of a 2 h-old adult egg. Columnar follicle cells (Fc) surround the oocyte (Oc) and nurse cells (Nc) x 400.

Figure 6. A characteristic feature of the nurse cell nuclear envelope is the presence of numerous large nuclear pores (arrow). These pores connect the nucleoplasm (N) to the nurse cell cytoplasm (Ct) x 15,000

Figure 7. The outer surface of the nurse cell nuclear envelope is studded with electron-dense material (arrow) x 6000

to its neurohaemal function the CC is known to produce its own intrinsic secretion which may be hormonal in nature. More recently the wall of the aorta has been found to act as an important neurohaemal site in some insects. This study was undertaken to examine the fine structure of CC cells in order to elucidate their secretory activity and to compare the neurohaemal activities of the CC and the wall of the anterior aorta in tsetse-flies.

The corpus cardiacum is closely associated with and surrounds the ventral and lateral walls of the aorta, just beneath the mid-posterior tip of the corpus allatum. The ventral wall of the aorta in direct contact with the CC is thin and has only a few axons. In contrast the dorsal wall, which is usually in contact with corpus allatum, is thick and has many axons and axonal endings (fig. 11).

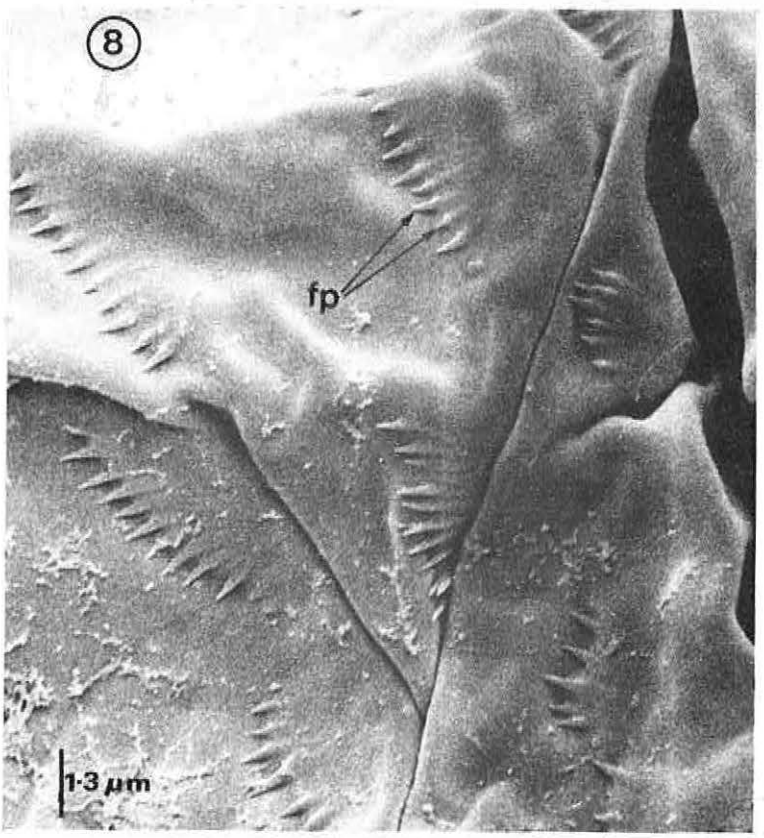


Figure 8. Scanning electron micrograph showing finger-like projections or spines (fp) on the luminal surface of the uterus.

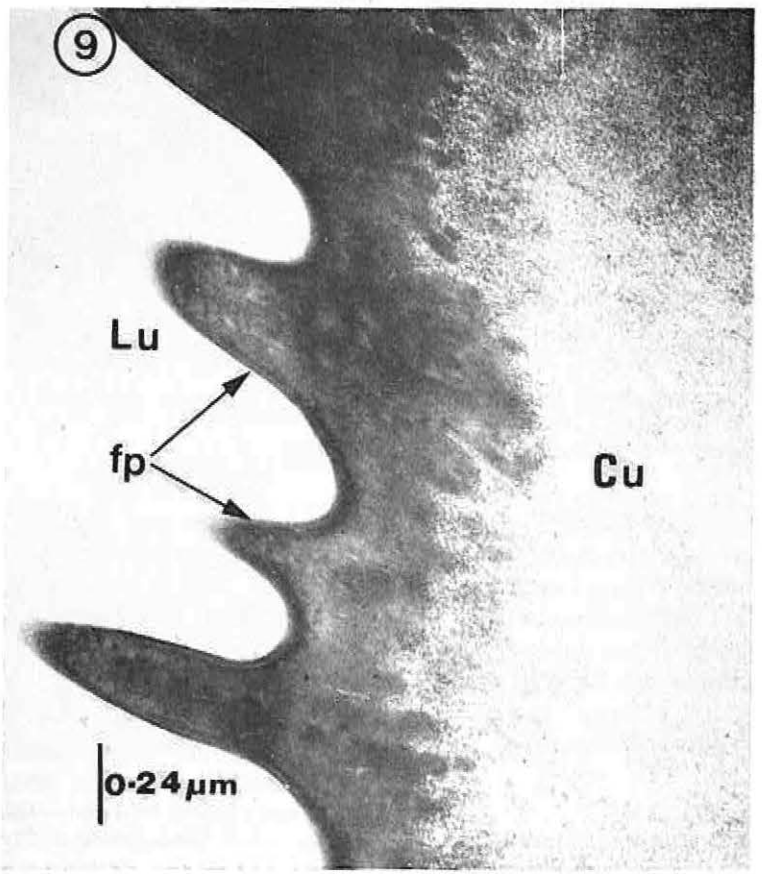


Figure 9. Section of finger-like projection (fp) on the luminal surface of the choriothete, (Lu) lumen of uterus

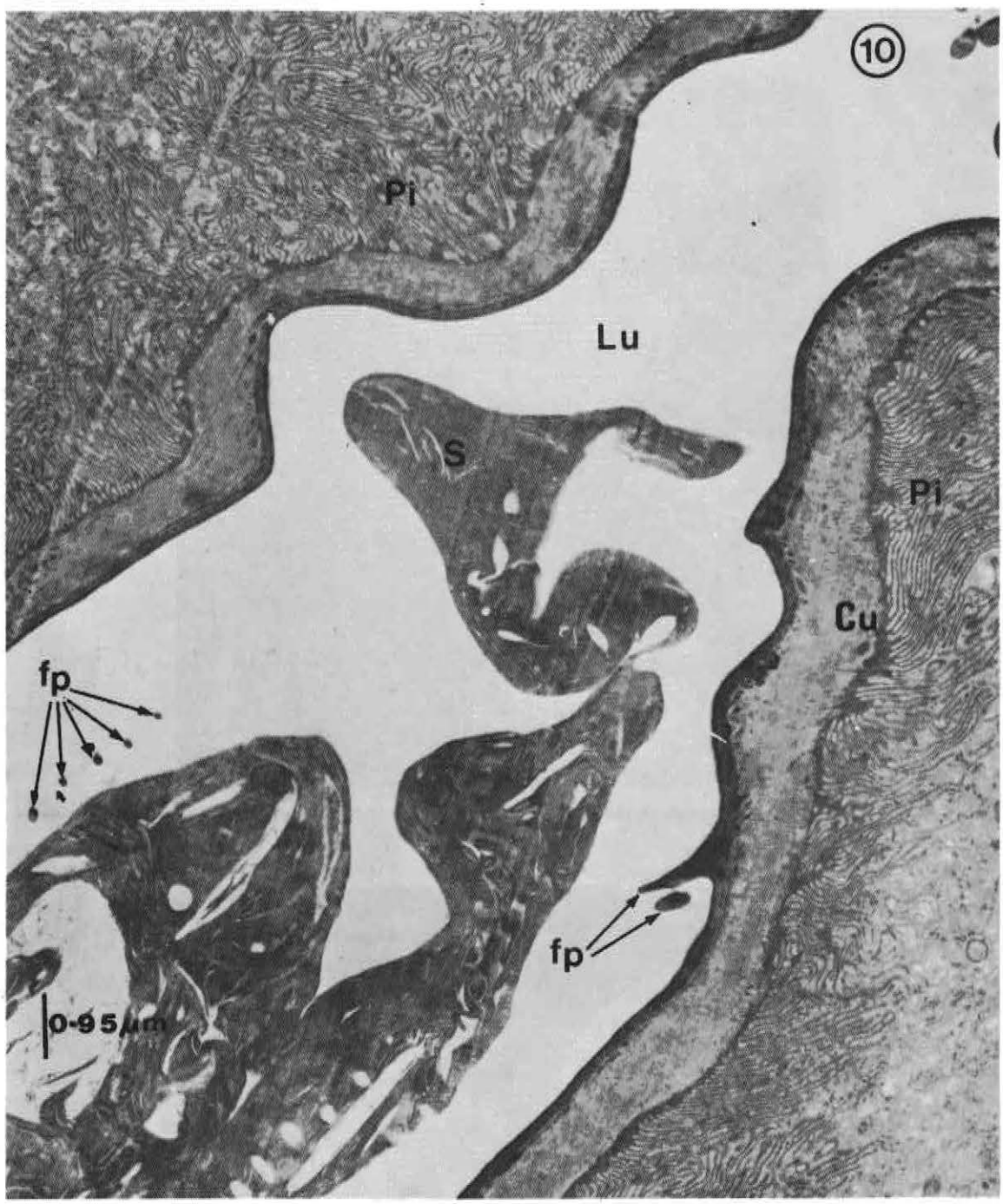
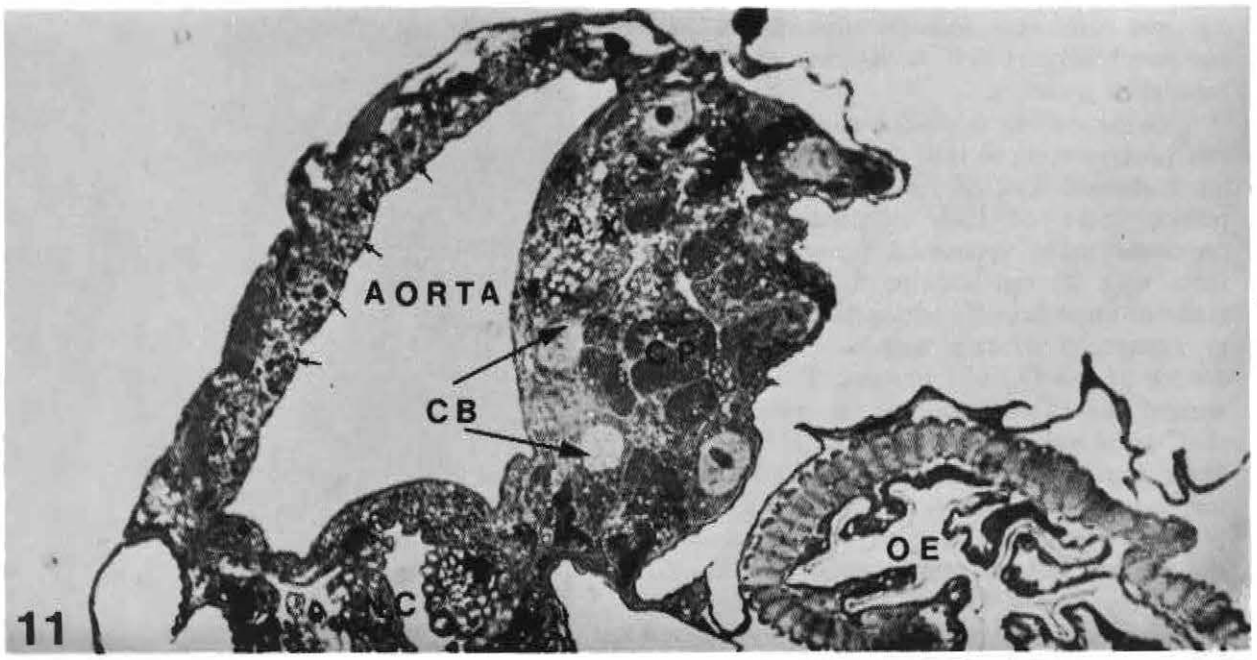


Figure 10. Low power electron micrograph of the posterior part of the uterus showing a secretion (s) in the lumen (Lu), finger-like projections (fp) on the luminal side of the choriothete and an extensively infolded apical plasmalemmal membrane (Pi), (Cu), cuticle

The CC is composed of large loose cells, interspersed by axons branching off from the nervi corpori cardiacii (NCC), and axon-like cell processes. Each CC cell comprises a large irregular cell body with an oval or elongated nucleus. The cytoplasm is made up largely of free ribosomes, mitochondria, dense bodies, and microfilament-like structures resembling neurotubules of nerve axons. The rest of the cytoplasm is taken up by large electron-dense secretory granules. The granules are usually concentrated in the Golgi apparatus whose outer vesicles contain electron dense material (fig 12).

Arising from each CC cell body is a cell process similar to the axons which arise from neurons. The processes are of variable length (figs 11 and 12). Each process proceeds towards the lateral and dorsal walls of the aorta where they become embedded among the axons of the NCC which invade the aortic wall further anteriorly. Before terminating in the aortic wall, each process probably becomes forked into two or more axon-like endings. The latter are distinguished from the true axons by the often large and irregular meandering of their profiles, and their cytoplasmic remnants of the CC cell-body



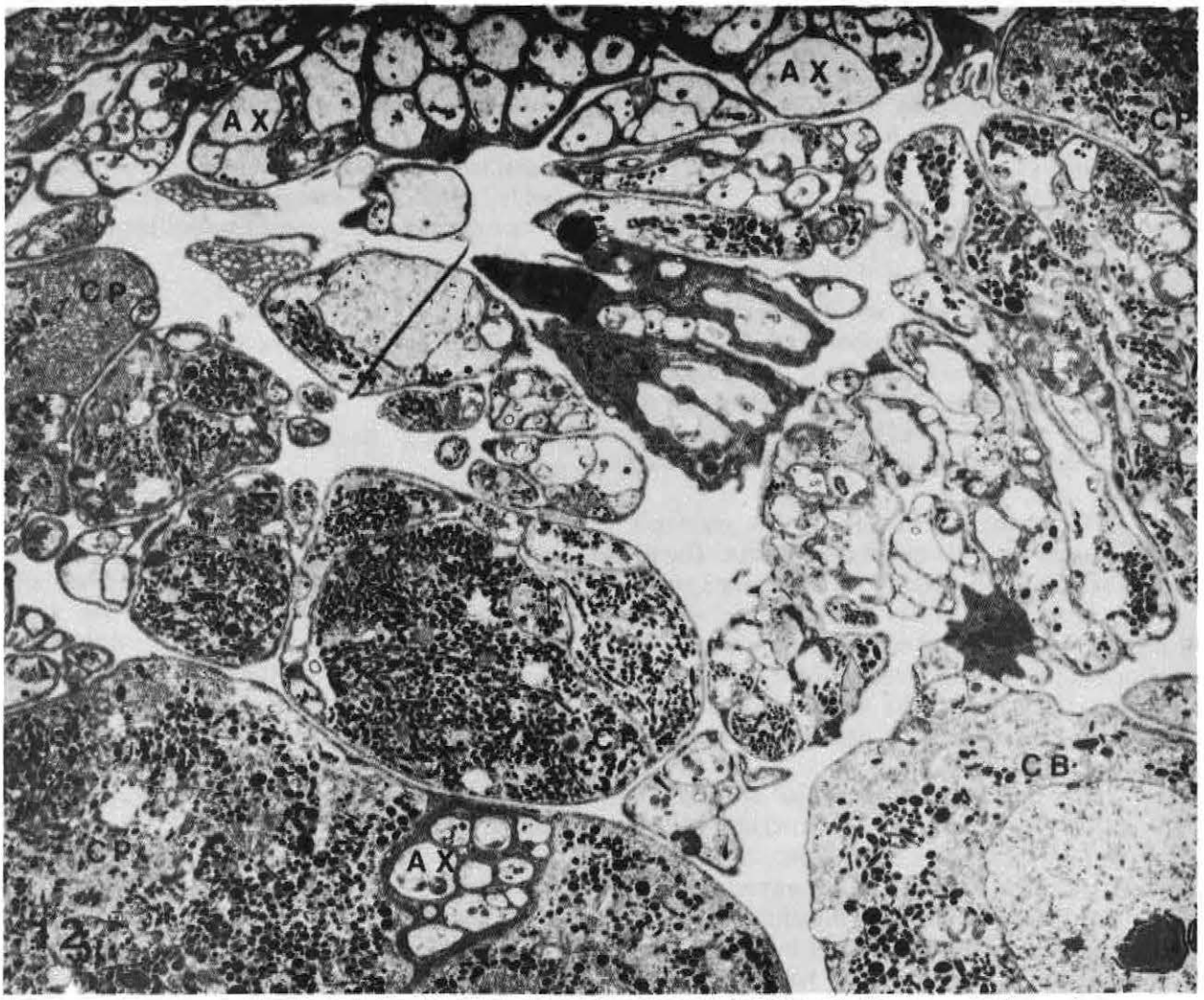


Figure 11. A photomicrograph showing the relative positions of oesophagus (OE), the nerve (NCC), and the corpus cardiacum which consists of large peripheral cell bodies (CB) and a neuropile of axons (AX) and cell processes (CP). The dorsal aortic wall contains axonal endings (small arrows) of NCC axons and CC cell processes.

Figure 12. An electron micrograph showing a CC cell (CB) with secretory granules and a neuropile consisting of CC cell processes (CP) and axons (AX) from the CC. Note the scarcity of axonal endings in the neuropile.

e.g. free ribosomes, mitochondria, dense bodies and neurotubular-like structures which occur among the granules.

Some of the axon-like endings of the CC cell processes come into close contact with the inner stromal wall of the aorta and most likely release some of their contents here. Other processes make synaptoid (synaptic-like) contacts with axonal endings from the NCC. As many as three axonal endings have been observed in synaptoid contact with a single axon-like ending of the CC cell process. The NCC axons around the CC cell bodies are surrounded by glial tissue and lamina external. It is most unlikely that a substantial amount of neurosecretory granules is released in the CC itself.

It is suggested that in *Glossina morsitans* the CC has lost its neurohaemal function. The CC cells are neuron-like and synthesize a granular secretion which is carried by axon-like cell processes to the aortic wall to be released. It is further suggested that the main neurohaemal site for neurosecretory granules, leaving the brain by way of the NCC, is the aortic wall. The aortic wall, in addition, acts as the control point for the CC cells, via synaptoid contacts, to either synthesize or release their own intrinsic secretion.

Sensory physiology

S. Waladde, E.D. Kokwaro, M. Chimtawi

In collaboration with the Sensory Physiology Research Unit, the morphology and ultrastructure of the antennal sensilla of larvae and adults of *Maruca testulalis*, *Eldana saccharina* and *Chilo partellus* have been examined to form a basis for electrophysiological and behavioural experiments.

The antenna consists of 75 segments in *Maruca*, 54 in *Chilo* and 44 in *Eldana*. The scales are distributed profusely on the dorsal surfaces. The population density of sensilla is maximum on the ventral/lateral surfaces. Scanning electron microscopy (SEM) revealed four morphological types of sensilla on the antenna of *Chilo*. They are sensillae trichoidae, basiconica, ceoloconica and styloconic (fig. 13).

The importance of these sensillae with reference to their internal structure and their physiology has been determined by ultrastructural and electrophysiological experiments. Preliminary transmission electron microscopy (TEM) findings show clear differences in these 4 sensilla. Briefly, sensilla styloconica have no pores but have thick dendritic sheaths around the dendrites and are probably innervated by two sensory cells. TEM studies are being extended to other sensillae. The sensillae involved in detecting a wide range of environmental stimuli, such as plant odours, are under investigation.

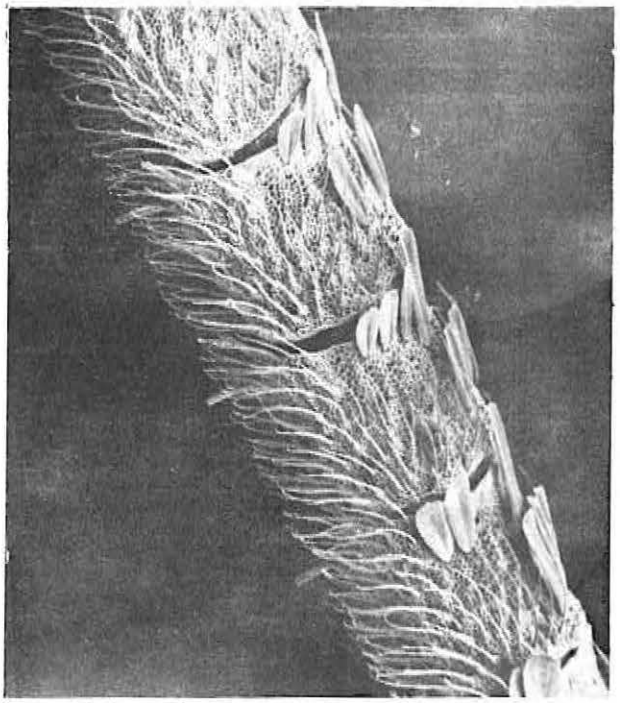


Figure 13. Scanning electron micrograph showing various sensilla on the antenna of *M. testulalis*.

Taxonomy

Morphology and ultrastructure of sandfly eggs

L. Rogo. E.D. Kokwaro

Phlebotomine sandflies, vectors of leishmaniasis, are a serious problem in the tropics. In Kenya, more than one vector species are known to exist but workers have been unable to morphologically separate adult female species caught.

In collaboration with the staff of the Leishmaniasis Project the unit is examining the morphology and ultrastructure of the eggs and larvae of closely related species obtained from different localities in Kenya. The aim of this work is to find out whether sandfly eggs can be a reliable systematic tool to supplement keys based on adult morphology in the identification of sandflies.

So far, preliminary SEM results show that the outer chorionic surface forms irregular ridges. It is hoped that further studies of these structures will facilitate species identification.

Sensory Physiology Research Unit

Research activities within the Unit support the core programmes by investigating the role which sensory organs play in insect behaviour. It is also intended to define the effect of certain environmental factors on insect behaviour. The resultant information is essential in the development of pest management methods by manipulation of insect behaviour.

Most of the work reported here has been done in collaboration with the Crop Pests and the Tsetse Research Programmes. Attention has been focussed on the stimuli which induce mating, host-plant selection, oviposition, and feeding among the crop-borers. Acoustic communication and the chemical stimuli involved in host and mate selection among the tsetse flies were also investigated. The functional morphology of relevant receptors were investigated in collaboration with the Histology and Fine Structure Research Unit and the biologically active substances were investigated in collaboration with the Chemistry and Bioassay Research Unit.

Gustation in stem borer and African armyworm larvae

C. J. Den Otter, H. M. Kahoro

It is known from previous studies that sorghum plants release phenolic acids when the plant tissues are damaged during feeding by an insect. Naturally, the phenolic compounds are present in the plant as esters which are converted to acids as soon as the plant tissue is bruised by the biting action of the insect. It has been established that the phenolic acids have a deterring effect on *Locusta migratoria* and contribute to the resistance of young sorghum plants to attack by locusts. On the other hand, sugars appeared to be strong phagostimulants for these insects.

We followed up these findings by doing electro-physiological experiments to find out whether sugars and phenolic acids may be important in inducing feeding behaviour in the stem-borer and African armyworm larvae.

We have investigated the responses of taste cells in the palpal sensilla and the medial and lateral sensilla styloconica of *Chilo partellus*, *Eldana saccharina*, *Maruca testulalis* and *Spodoptera exempta* larvae. Thirteen carbohydrates (ribose, arabinose, glucose, mannose, galactose, maltose, cellobiose, sucrose, melibiose, trehalose, raffinose, inositol) and eleven phenolic compounds (p-hydroxybenzoic acid, gentisic acid, gallic acid, vanillic acid, quinic acid, trans-*O*-coumaric acid, trans-*p*-coumaric acid, caffeic acid, ferulic acid, chlorogenic acid) have been used as taste stimuli.

These studies have revealed that, as far as the carbohydrates are concerned, the stem borer larvae respond to sucrose only, whereas *Spodoptera exempta* responded to sucrose ribose and mesoinositol. Apart from *Chilo* which responded to chlorogenic acid no response to phenolic compounds was recorded from the other stem-borers. However, *Spodoptera exempta* responded to at least four phenolic compounds (vanillic, gallic, p-hydroxybenzoic acid and chlorogenic acids).

Antennal sensilla of the pyralidae

C. partellus, E. saccharina and *M. testulalis*

S. M. Waladde

The pyralidae species *Chilo partellus, Eldana saccharina* and *Maruca testulalis* are representatives of the crop boring pests. Their antennal sensilla are capable of detecting a wide range of environmental stimuli including plant odours, pheromones, temperature, humidity and others. These stimuli play a crucial role in host-plant selection, mating, oviposition and feeding behaviour patterns. The objective of the current work is to obtain information about the antennal sensilla types on the adult and larval stages of the crop borers. It is also necessary to identify the general and specific stimuli acting on the sensilla. So far, most of the work reported here has been done on the *Chilo* moth antennal sensilla; examples of single cell responses from the larval antennal sensilla are also given.

MOTH ANTENNAL SENSILLA

Of the three pyralidae moths, *Maruca* has the longest antenna, consisting of seventy-five segments, whereas *Chilo* and *Eldana* have fifty-four and forty-four segments respectively. The dorsal antennal surface is covered by scales whereas the ventral/lateral surfaces are equipped with various sensilla. Scanning electron microscopy has revealed that most of the antennal segments of *Chilo* have four types of sensilla, namely sensilla trichoidae, sensilla basiconica, sensilla coeloconica and sensilla styloconica. There is now sufficient evidence to show that sensilla styloconica function as thermoreceptors.

SENSILLA STYLOCONICA

The distal segment of the antenna usually bears a pair of sensilla styloconica at its tip. On the other segments this sensillum is found at the distal ventral end of the segment. On all the three moth species examined so far, ten to thirteen segments at the proximal end of the antenna lack the styloconica sensilla. As reported by other workers, this sensillum is usually situated at the end of a tylus where a cuticular collar surrounds the base of a conical sensilla (fig. 1). Observations in our laboratory have confirmed that one of the sensory cells in sensillum styloconicum is a cold receptor responding with increasing frequency as the temperature decreases and is inhibited by increasing temperature. The same cell is also inhibited by incident light (fig. 2). The styloconica sensilla on male moths respond in a curvilinear manner and are much more sensitive to temperature change than their counterparts on the female moths which respond in a linear fashion (fig.3). The behavioural significance of these differences still remain to be identified.

Transmission electron microscopy has confirmed that sensillum styloconicum is a typical no pore (np) sensillum similar to those described on other lepidoptera species. Towards its distal end, the sensillum shaft contains a dendrite

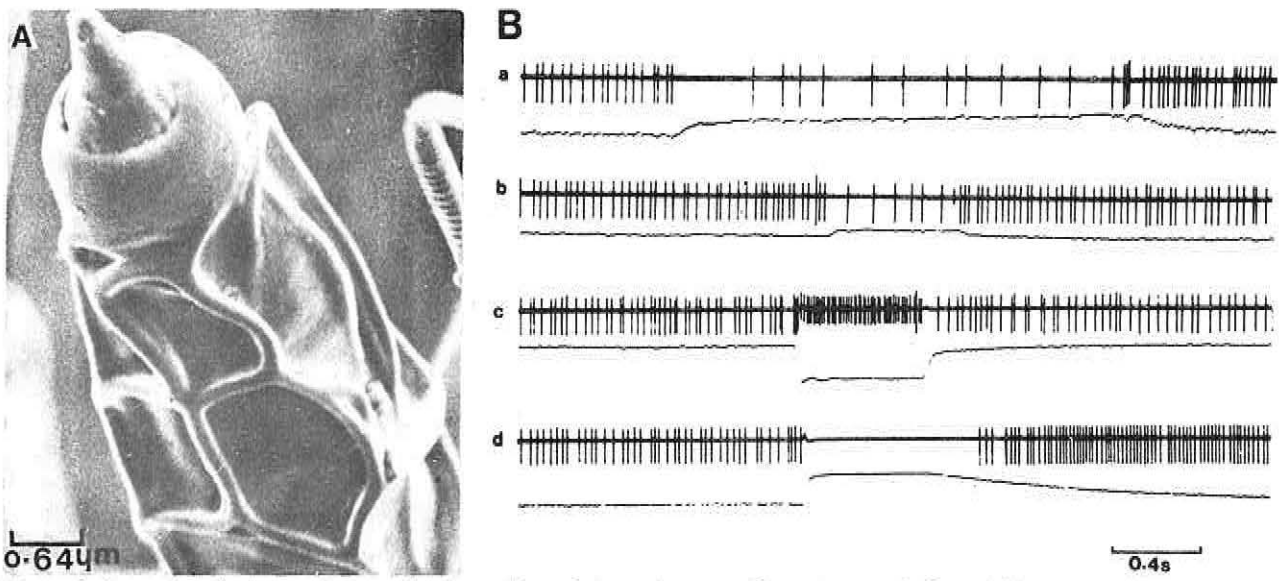


Figure 1: Scanning electron micrograph of sensillum styloconicum on the antenna of *C. partellus*

Figure 2. Single cell responses from a cold receptor unit among one of the cells innervating sensillum styloconicum of *C. partellus*. Below each AC trace there is a corresponding DC trace; up and downward arrows indicate onset and end of stimulus application.

a. Inhibitory effect of incident light from the fiberoptic lamp.
b. Slight inhibition caused by a puff of air at room temperature.
c. Strong inhibitory effect caused by heated air.

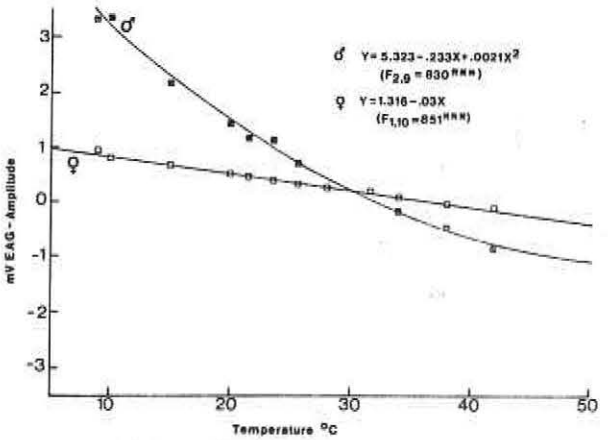


Figure 3: EAG-amplitudes (temperature response curves) from the antenna of *C. partellus* males and females evoked by air puffs at different temperatures.

sheath (Scolopale) enclosing two tightly packed unbranched dendrites and the dendrite sheath is physically connected to the sensillum cuticle. Proximally, the dendrite sheath loses its connection with the cuticle, it is completely separated from the cuticle by an extracellular space and a third dendrite appears. The latter dendrite differs from the other two dendrites in that it does not extend to the sensillum tip and its distal portion is transformed into a stack of lamella (fig. 4 (a-d); these structures are similar to those described on other lepidopteran species. Electrophysiological studies on other insects have revealed that sensilla with the above features have two antagonistic humidity receptors (moist air unit, dry air unit) and a single cold receptor.

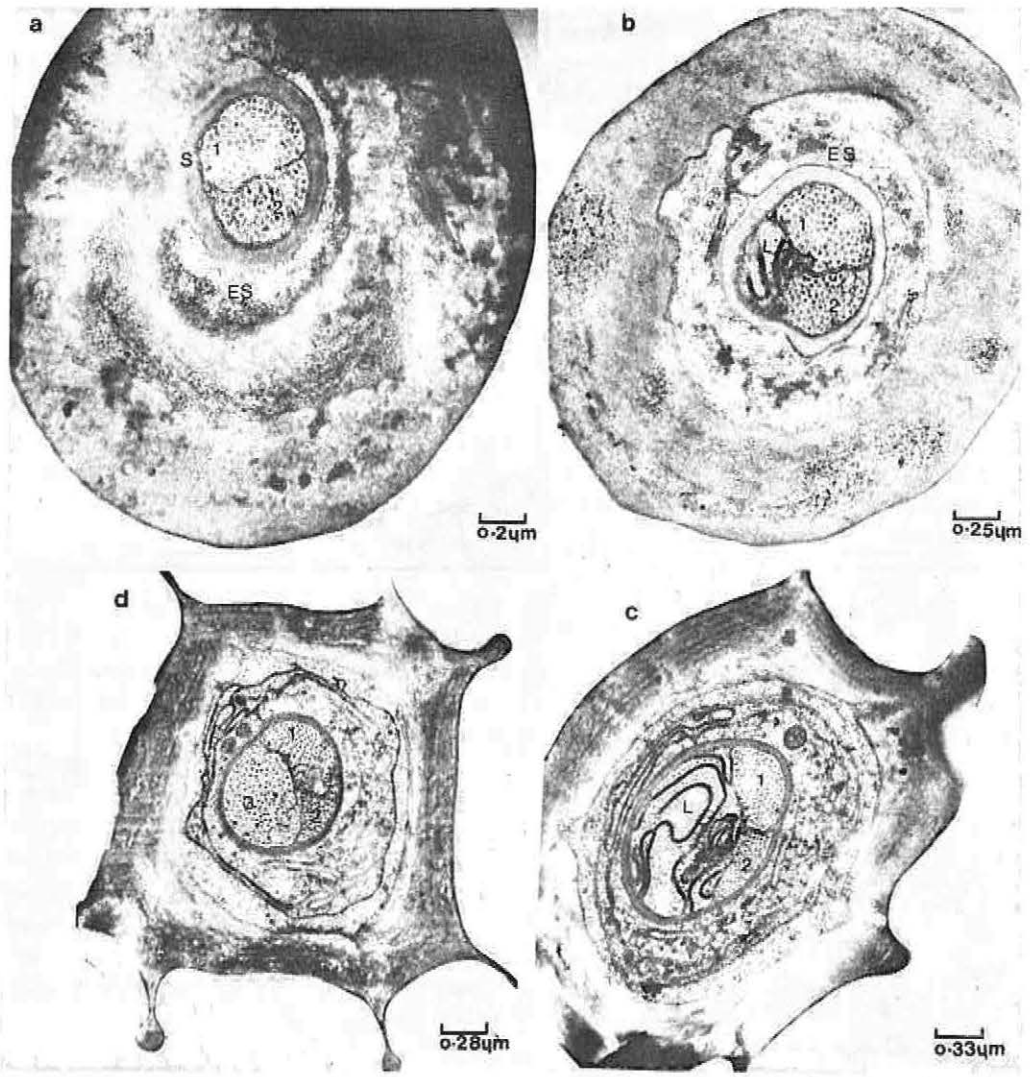


Figure 4: Transmission electron micrographs obtained from different levels of sensillum styloconicum of *C. partellus*.

Notes:
a. Distal region showing the no pore cuticle enclosing two dendrites (1 & 2) tightly surrounded by thick cuticular sheath(s); note the extracellular space (ES) almost surrounding the cuticular sheath.
b. The cuticular sheath completely surrounded by the extracellular space and appearance of a third dendrite signified by the lamellae (L).
c. Third dendrite showing stacks of lamellae adjacent to dendrites 1 & 2.
d. Proximal section showing that the stacks of lamellae are a transient feature which gives way to a large dendrite (3) with features common to those of the other two dendrites.

PHEROMONE RECEPTORS

Attention has been focussed on those sensilla responsible for the detection of *Chilo* female sex pheromone. Dr. B.F. Nesbitt and her colleagues at the Tropical Development and Research Institute (TDRI) examined the *Chilo partellus* species from India and indentified just the aldehyde component as lure; the exact function of the alcohol component was uncertain. In Malawi the same pheromonal components failed to attract either singly or combined. Catches obtained using the same components at Mbita Point Field Station, (MPFS) have been much lower than expected. These observations call for further studies on the *Chilo* pheromone.

Single cell as well as electroantenogram (EAG) methods are being used to test various pheromone analogues supplied to us from TDRI. On the basis of the results obtained from these tests and further field tests it may be possible to identify the most active formulation. Results from these observations may warrant further investigations to find out whether the pheromonal components of the East African *Chilo* are significantly different from those of the Indian species. EAG tests on *Chilo* from the MPFS colony have confirmed that the aldehyde pheromone component is more active than the alcohol component; it evokes a linear response. Preliminary results indicate that the response curve has no clear-cut plateau or levelling-off as one would expect when the pheromone concentration is increased (fig. 5). If further experiments continue showing

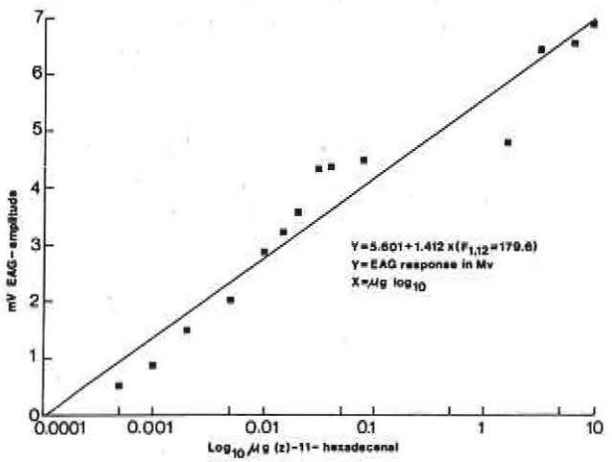


Figure 5. **EAG amplitude from the antenna of *C. partellus* males evoked by increasing concentrations of (Z) — 11 — hexadecenal, a component of the female sex pheromone.**

linear response curves this may indicate that we may be dealing with something less than a true pheromone. In normal circumstances a response curve to a pheromone should have a plateau which signifies saturation of the pheromone receptor sites in the sensitive sensilla.

The majority of hairs on the antenna of male *Chilo* are the long grooved sensilla basiconia (fig. 6). Single cell recordings from those sensilla have shown that the aldehyde component inhibits the spontaneous activity from the sensilla. However, the DC component responsible for the recorded EAG is not inhibited (Fig. 7). This indicates that another sensilla type on the antenna is positively sensitive to the aldehyde pheromone component. It will be interesting to identify the stimuli evoking positive responses from the long grooved sensilla.

LARVAL ANTENNAL SENSILLA

Recent reports on larval movement from oviposition to feeding sites suggested that olfaction may be a significant factor which enables *Chilo* larvae to distinguish between susceptible and resistant maize cultivars. It was reported that after hatching there was a notable larval migration from the resistant CMT 324 plot to the suceptible Inbred A plot. It is therefore essential to find out whether there are any specific olfactory stimuli likely to bring about that kind of selective behaviour.

Plant odours will be collected from susceptible as well as resistant cultivars and antennal responses to the odours will be analyzed using the electrophysiological data obtained from single cell and receptor potential recordings. There is almost no data in the literature about larval antennal responses to odour. This is because larval antennae are retractable and when they are retracted their sensilla are inaccessible. Recently we succeeded in overcoming antennal retraction and we are able to use the cut-tip recording method to obtain resproducible single cell and receptor potential responses from the antennal sensilla (fig. 8).

The available data indicates that the sensilla have multiple innervation and it is possible to identify the response pattern of certain sensory cells. This potential will be fully exploited when investigating the effect of plant odours on the sensory responses from the antennal sensilla. Appropriate behavioural studies are to be developed in order to complement the electrophysiological tests.

Chemocommunication in tsetse flies

C.J. Den Otter, R.K. Saini

Studies are in progress to investigate the perception of the female sex pheromone in *Glossina* by recording the activities of the tsetse taste and olfactory organs during stimulation with the pheromone. Electrophysiology, however, has

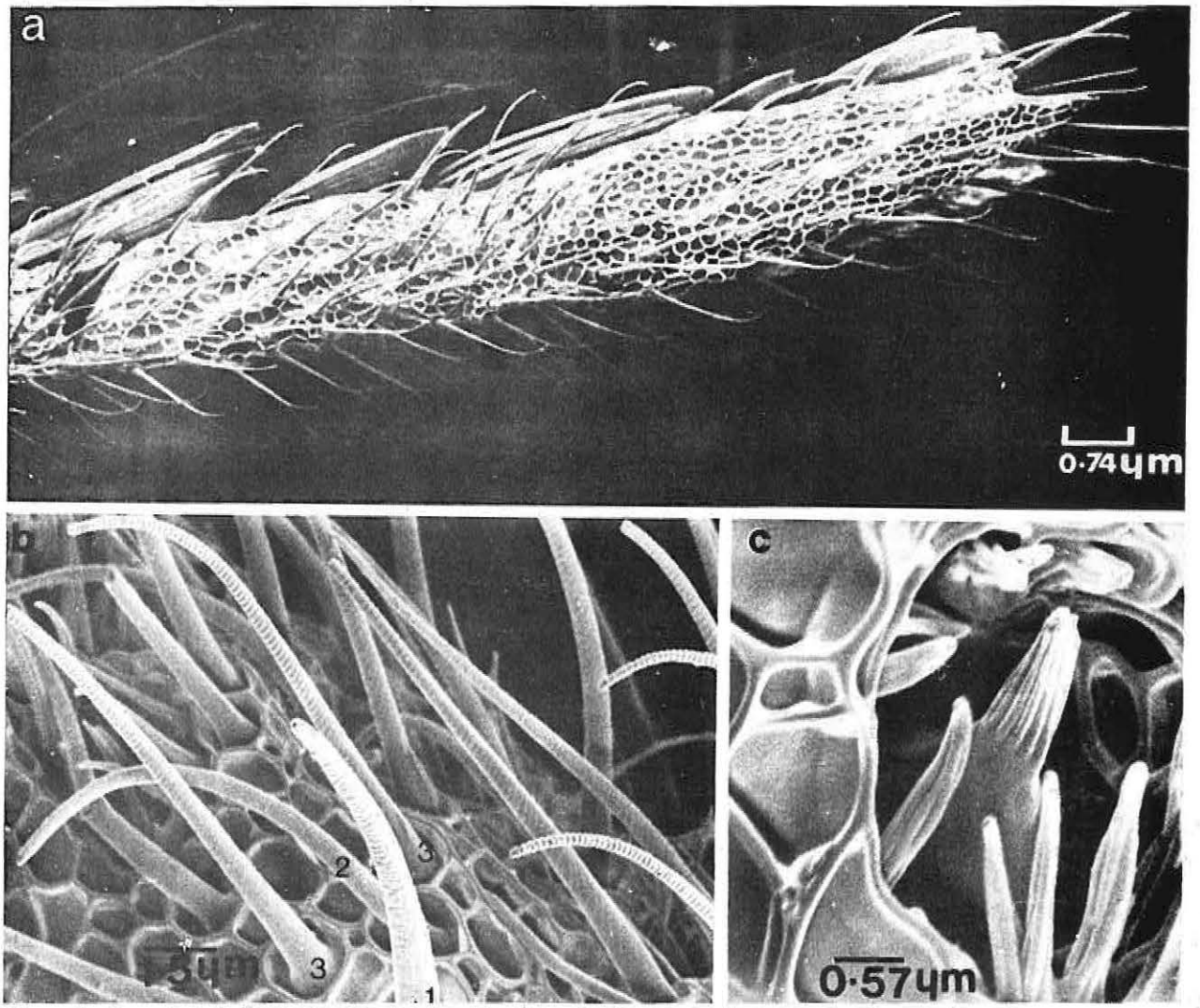


Figure 6: Scanning electron micrographs showing various antennal sensilla of *C. partellus*.

a. Distal antennal segments with sensilla on the ventral/lateral surfaces and scales on the dorsal surface.
b. Details of the antennal sensilla types 1-3 where type 3 constitutes the majority.
c. Details of sensillum coeloconicum; this sensillum type is widely distributed on the antenna.

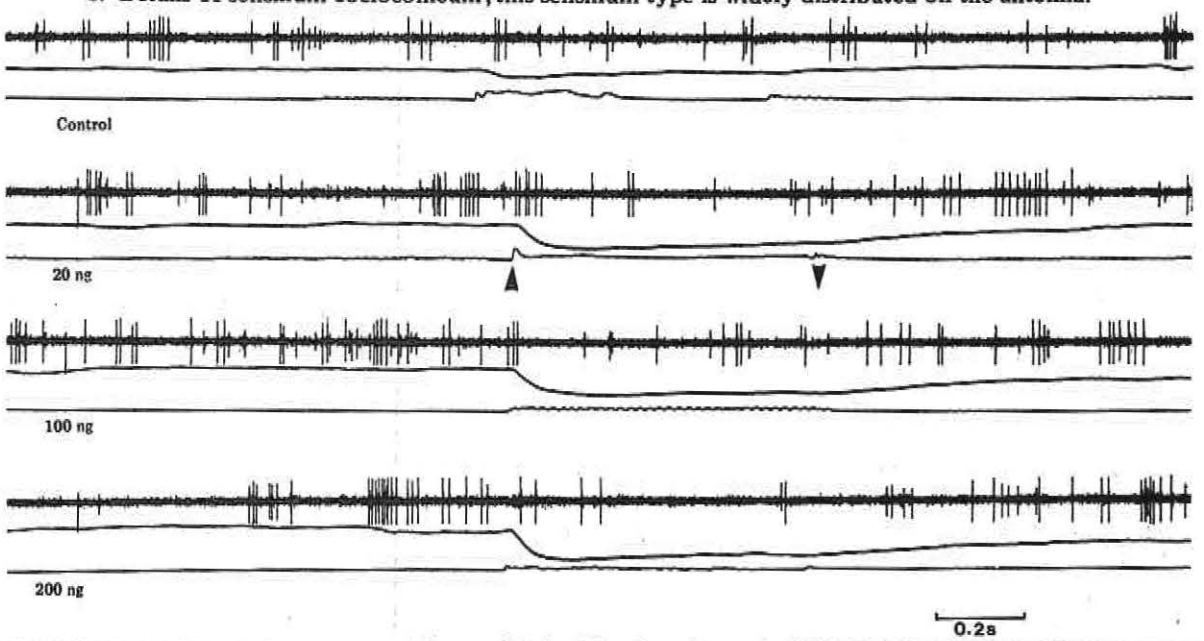


Figure 7: Responses to various concentrations of (z) - 11 - hexadecenal obtained from the long grooved antennal sensilla of *C. partellus*. The AC trace indicates that the aldehyde has an inhibitory effect but the DC trace shows the opposite effect. This suggests that the long grooved sensilla is not responsible for recorded EAG responses. In each recording the first trace is AC, second trace DC and third trace is the stimulus marker where up and downward arrows respectively indicate onset and end of stimulus application.

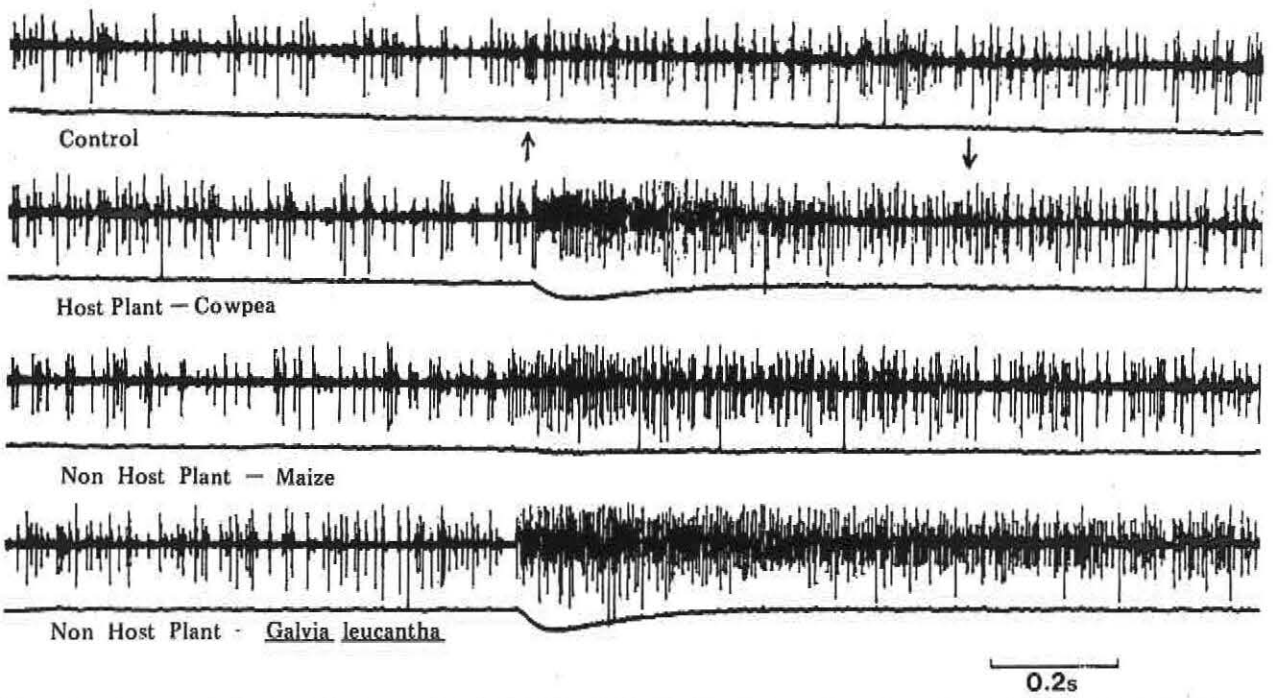


Figure 8: Effect of the indicated stimuli on the single cell responses from the antennal sensilla of *M. testulalis* larva.

failed to show any stimulatory effect of the pheromone on taste hairs on the legs or in the region of the tibial organs. In addition, no response to the common stimuli NaCl and sucrose were found.

Whereas no taste activity of the pheromone could be established, it was observed that injecting the odour of morsilure into a constant air stream blown over the antenna of male *Glossina morsitans morsitans* evoked EAGs, which clearly indicated that the pheromone was smelled by the insect via its antennal receptors.

Behavioural experiments done in our laboratory also unequivocally show that the male is able to smell the pheromone. It was found that flies raised their antennae as soon as a filter paper loaded with morsilure (20 μl) was brought at a distance of about 2 cm from their heads. The number of responses to such a filter paper was significantly higher than that to a clean filter paper ($P < 0.001$). These antennal responses also occurred when a female fly was waved before the head of a male. The responsiveness of males to an unwashed female was significantly higher than to a female from which the pheromone had been removed by washing with hexane (X^2 (1) = 16.3). Responsiveness to morsilure does not show any correlation with the age of the flies, but tends to increase with increasing starvation.

In order to investigate whether the male's antennal responses simply reflect a response to paraffins in general, we compared the number of responses to clean filter paper and to filter paper loaded with common paraffin oil (20 μl). There were no significant differences in the number of male responses to these two types of filter papers. The ability of females to perceive morsilure was also investigated, and the results suggest a low sensitivity of the females to their own pheromone. Thus females may be able to smell their female conspecifics. Our conclusion is that the pheromone is not perceived by contact chemoreceptors on the legs but by olfactory receptors on the antennae. In the mean time, electrophysiological and behavioural experiments to screen a large number of synthetic chemicals and natural products or extracts have been initiated. Screening of chemicals for their effectiveness at the receptor level may help to identify substances which may play a role in tsetse fly chemocommunication and which may be eventually applicable to tsetse control.

Sound production associated with *G. m. morsitans* sexual behaviour

R.K. Saini

Acoustic sexual behaviour of *G. m. morsitans* was investigated. Under laboratory conditions, no distinct precopulatory sexual behaviour was observed in either sexes. In most cases the male paired immediately upon introduction into the vial containing the female. In other cases the vials had to be shaken slightly in order to bring the partners near each other for mating to ensue. Successful mating from the time of mounting to the time of separation, could be divided into three phases: the embrace phase, the middle or copulatory phase and the jerking or ejaculatory phase, respectively.

The embrace phase was characterized by the male mounting the receptive female and the

insertion of his aedeagus into the female genital opening. As the pair was being formed and up to 4—5 min after the genitalia had been engaged, the male rapidly drummed the female thorax and sometimes even the abdomen with the tarsi of his meso and metathoracic legs. Drumming was accompanied by male singing which resulted in his wings and scutellar hairs being set into vibration. In between the male songs, the mean duration of which was 5.3 $\pm$ 1.5 sec, the male opened his wings and sometimes moved them up and down. The female remained passive during the whole of the embrace phase.

The middle phase occupied the greater part of the mating period, i.e. about 125 min. During this phase, the female groomed the dorsal side of the male's abdomen and sometimes even the dorsal side of the male's wings with the tarsi of either one or both metathoracic legs. In addition, she frequently rubbed these legs together. The male hypopygium rapidly moved up and down and from time to time the aedeagus was thrust deep into the female genital opening, whereby the male accessory gland secretion was seen to be transferred to the female. During the middle phase, sounds were produced only when the female became restless or when the pair was disturbed. Whenever this happened, the male sung and at the same time often drummed the female's thorax with the tarsi of his second and third legs till the female was quiet. The mean duration of these songs was a few seconds.

The last phase of mating, i.e. the jerking or ejaculatory phase lasts about 3 min and was characterized by the male's rapid drumming activity on the female's thorax and abdomen. The drumming was initially done with the tarsi of metathoracic legs and, as the frequency of the drumming increased the mesothoracic legs were also used. During drumming activity vertical wing vibrations were observed and both partners swayed from side to side. The male discontinued drumming a few seconds before withdrawing the aedeagus, after which the flies separated. No sound production occurred during the whole jerking phase.

COPULATION WITH DEAD FEMALES

Since many studies have been done using decoys, sound production associated with such mating was also investigated. Males readily mated with freshly killed females and exhibited complete mating behaviour. Sound production was, however, less intense as compared to males mating with live females. This could perhaps be due to the males receiving only limited stimuli from the females.

SPECTRAL ANALYSES

The sounds associated wth mating behaviour consist of trains of pulses which are repeated at regular intervals. Spectral analyses of the male sounds while mating with live females revealed that they are composed of frequencies up to 50kHz. The dominant frequency in the mating songs was centered between 1.5 and 2.5 kHz, with an intensity of 44.8 $\pm$ 3 dB. Peaks may also be observed between 0.5 and 0.8 kHz (having an intensity of 36.4 $\pm$ 2 dB), around 5 kHz (having an intensity of 28.4 $\pm$ 3 dB) and at 8-9 kHz (having an intensity of 20.8 $\pm$ 3 dB). In addition, the male songs during the embrace phase had distinct peaks at 20-40 kHz with an intensity of 17.6 $\pm$ 3 dB in contrast to those produced during the middle phase in which the ultrasonic components (30-50 kHz) are usually less than 15 dB. The male songs produced while mating with freshly killed females, however, lacked ultrasonic components and were of low intensity. In these songs, the dominant frequency around 2 kHz had an inensity of 27 $\pm$ 3 dB, while all remaining frequencies had intensities of about 20 dB or less.

It is evident from these studies that during mating, sounds are not produced at random, but conform to clearly recognizable patterns. The male's singing and drumming during the embrace phase most probably also contribute to the necessary mating stimuli and ensure that the union between the sexes is maintained throughout the long period of copulation. This is further suggested by the fact that the male starts drumming and singing again as soon as the female becomes restless. The male songs may also provide species and sex recognition signals for the female. This is suggested by the fact that preliminary studies indicate that the acoustic sexual behaviour seems to differ between *G. m. morsitans*, *G. f. fuscipes* and *G. pallidipes*.

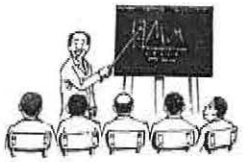
POST-PARTUM SOUND PRODUCTION

Females consistently produce a sound immediately after parturition (97.2% incidence, N=36). Recordings from 22 females indicate the duration of these songs to be 5.8 $\pm$ 0.5 min. Hence these songs are much longer than the brief sounds of only a few seconds duration that have been recorded in the same sepecies in connection with feeding or mating. While the song is being produced, the abdomen which is strongly arched and convulated at parturition gradually straightens.

These post-partum songs contain frequencies ranging from 0.5 to 10 kHz. The dominant frequency was centered around 2 kHz with an

intensity of 35 ± 5 dB. Peaks may also be observed between 0.5 — 0.8 kHz (intensity of 20 ± 2 dB) and around 5kHz (intensity of 22 ± 4 dB). These songs do not contain any ultrasonic components. The behavioural significance of these songs is being investigated.

TRAINING



Training

The launching of the African Regional Postgraduate Programme in Insect Science, ARPPIS, on 1 March 1983, was undoubtedly the most significant landmark in the history of training at ICIPE. ARPPIS is a collaborative project between ICIPE, which serves as the executing agency, and a number of African institutions, particularly universities. It was established to meet the needs of the region in high-level research training in insect pest management.

Research on pests at ICIPE and elsewhere on the continent provides the scientific and technological base, while the training is intended to augment the capabilities of these institutions to meet their own research manpower needs.

ICIPE's strategies to meet these goals have varied over the years but it is now hoped that future efforts will be devoted to the rationalization and consolidation of the selected programmes in postgraduate training (of which ARPPIS is the major thrust), postdoctoral fellowship, training for practitioners of pest management and staff development. These programmes received full endorsement by the first Triennial Review of ICIPE which reviewed content, quality and potential impact of the centre's research and training activities.

The African Regional Postgraduate Programme in Insect Science (ARPPIS)

The ARPPIS programme was launched on 1 March 1983, with an intake of eight students from four countries in Africa: Mr. Richard Bagine, Kenyan, registered with Makerere University; Miss W. Suliman Forawi, and Mr. Latif A. Ibrahim, both Sudanese, registered with University of Khartoum; Mr. Barnabas Njau, Tanzanian, registered with University of Dar-es-Salaam; Mr. J.H.P. Nyeko, Ugandan, registered with Makerere University; Mr. S.H. Okech, Kenyan, registered with Makerere University; and Mr. J.B. Okeyo-Owuor, Kenyan, registered with University of Dar-es-Salaam.

ARPPIS activities started with a one-month orientation course and visits to a number of research institutions in Kenya, such as the Kenya Agricultural Research Institute, the Kenya Veterinary Research Laboratories, the International Laboratory for Research on Animal Diseases (ILRAD), the Division of Vector-Borne Diseases (of the Ministry of Health, Kenya), and the Entomology Section of the Kenya National Museums. The orientation was followed by six-months advanced refresher courses in insect ecology, biostatistics, physiology, biochemistry and pathology. These courses were delivered by visiting academics and senior scientists at the ICIPE. Each course lasted for two to three weeks. Notable academics like Professor S. Friedman and Professor H. Lipke, both of the University of Massachusetts, USA, and Professor R. Galun from the Hebrew University, Israel, one-time ICIPE Visiting Director of Research and currently member of ICIPE Governing Board, offered the course on insect biochemistry. Dr. D. Bignell from Queen Mary College, London offered the course on insect ecology. The students have now embarked on their respective research projects.

— **ARPPIS students (1983) in a laboratory session with Professor Lipke and Dr. Saini of ICIPE**

The ARPPIS Academic Board met twice in 1983. The membership currently includes the following representatives of participating universities: Professor Thomas R. Odhiambo, ICIPE, Professor W.Z. Cocker, University of Ghana, Legon, who is also Deputy Academic Coordinator for the programme, Professor El Iman El-Khidir, University of Khartoum; Professor R. Khumar, Rivers State University of Science and Technology, Port Harcourt; Professor Lutalo-Bosa, Makerere University; Dr C. Magadza, University of Zimbabwe; Professor John Okedi, Academic Coordinator; and Professor A. Youdeowei, University of Ibadan. It is expected that the University of Malawi will also join ARPPIS soon.

At its fourth meeting in December 1983, the Board selected the following nine students for the 1984 intake: Miss Delphina A. Adabie, Ghana; Mr. I.G. Aniedu, Nigeria; Mr. J. Bahana, Uganda; Miss U.M. Elneima, Sudan; Mr; M.L. Kantiki, Malawi; Mr. C. Maranga, Kenya; Mr. M. Ogenga-Latigo, Uganda; Mr. J.P. Omollo, Kenya and Mr. Getachew Tikubet, Ethiopia. The next academic year starts on 1 March 1984.

The ARPPIS programme has generated tremendous interest both on the continent and outside. Although financial support for the project has not been exuberant, it has nevertheless gained support from donors such as the German Academic Exchange Service, DAAD, the Australian Development Assistance Bureau (ADAB) and further support is being negotiated with the Ford Foundation and other agencies.

Postdoctoral Research Fellowships

The ICIPE postdoctoral research scheme has continued to attract young scientists who wish to gain research experience in a tropical environment. A total of eight postdoctoral scientists from Uganda, Sierra Leone, Vietnam, Tanzania, India, Cameroon, Ghana and Kenya were in residence at ICIPE during 1983.

International Group Training Course on Insect Growth, Development and Behaviour

The second course in this series was held from 8 to 24 August 1983 and its main theme was insect chemoreception. This course was co-sponsored by ECRO (the European Chemoreception Research Organization), ICRO (the International Cell Research Organization) UNESCO and ICIPE, and was attended by 14 young scientists from Barbados, Cuba, Nigeria, Sierra Leone, Sudan, Uganda and Tanzania. Lectures and practicals were delivered by visiting academics from Germany and Switzerland, and senior scientists from the Sensory Physiology Research Unit (SPRU) of ICIPE. This course is held once every two years, therefore the next course is scheduled for 1985.

Staff Development Training

In 1983 more attention was focussed on the staff development needs of the centre. It is hoped that an internal programme will be

— At the opening of the "Insect Chemoreception" course, Prof. Thomas R. Odhiambo is seen in conversation with guests including Mr. Hartmut Glimm, (DAAD) and Dr. C.J. Den Otter (ICIPE).

evolved to enable staff to acquire and deepen skills towards realising their full potential at ICIPE, and this is expected to be implemented from early 1984.

Nevertheless a modicum achievement has been attained in the area of technical staff training at Kenya Polytechnic and elsewhere in specialized laboratories. Two scientists from ICIPE core research programmes joined the ARPPIS programme for Ph.D. training. In-house training continues for technical and administrative staff.

Financial and Administrative Management of Research Projects in Eastern and Southern Africa-(FAMESA)

The special project on training for Research and Development (R&D) management hosted two workshops. The workshop on 18-22 January 1983 was held for policy-makers and research directors within the FAMESA constituency as a means of sensitizing the former to R&D policy and management needs. It was well attended and a plan of action was formulated. Throughout the year, the project was engaged in putting together a portfolio on R&D institute needs by conducting institutional survey and profile analyses. The second workshop was for planning curriculum for a course on "Planning and Budgeting for R&D Institutes." On recommendation from the Triennial Review of ICIPE activities and the ICIPE Governing Board, FAMESA is finalizing this exploratory stage and the project is to be phased out from end of December 1983. Proceedings of the second seminar were published in IDRC manuscript report (IDRC — MR87e).

SUPPORT SERVICES

Biostatistics and Computer Services

The Biostatistics and Computer Services Unit became operational in 1983 with the primary goal of advising and assisting ICIPE scientists on experimental design, data analysis and simulation modelling. In addition, computer services are to be offered to the library and the administrative and finance divisions and Word processing facilities are provided for the whole centre.

The computer system comprises two Wang computers, a main frame VS80 and a 2200VP, situated at Duduville International Guest Centre, and an OIS word processor with terminals at Chiromo.

Experimental design and data analysis

R.D. Dransfield

Early in 1983, the available statistical and graphics packages for the 2200VP were tested exhaustively and by March were ready for use by the scientists. Later in the year some statistical programmes were also available on the VS80. Over 80% of ICIPE scientists have now made use of the Unit's expertise for experimental design or data analysis.

In the Livestock Ticks Research Programme, statistical advice and assistance was given to Dr. J. De Castro on analysing the effects of different loads of *Rhipicephalus appendiculatus* on growth rates of cattle. A significant interaction was found between treatment and time indicating that although growth rates were initially reduced by high tick loads, this was largely offset by higher compensatory growth rates later.

Effects of dipping on growth rate of cattle were analysed and a stepwise multiple linear regression suggested that *R. appendiculatus* was primarily responsible for depressed growth rates. Data on population trends of ticks in pastures were analysed (Dr. R. Newson) and advice given on the design of experiments to test the effect of different radiation doses on tick egg production. Analysis of variance was carried out on data on the effects of feeding ticks on rabbits injected with tick antigen (Dr. C. Mango).

For the Tsetse Programme, the recovery curve of the *Glossina pallidipes* population in thickets in Lambwe Valley after spraying (Dr. D. Turner) was fitted to a logistic function enabling the rate of increase to be estimated. Data from Shimba Hills (Dr. R. Dransfield) were analysed to determine the range of attraction of the biconical trap for tsetse and to relate the apparent density of *G. pallidipes* to absolute density as determined by mark-release-recapture. Analysis of the latter was done by Jolly's method using a programme modified by Mrs. W. Ssebunnya. Assistance was given to Dr. R. Tarimo on estimation of the probability of a tsetse picking up a trypanosome infection from a blood meal and to Dr. T. Golder on analysis of data on effects of sublethal doses of insecticide on *Trypanosoma brucei* infected *G. morsitans*. Advice on experimental design and analysis of multiple latin squares was given to Mrs. M. Owaga and Mr. I. Onweluzo who were investigating the effects of different trap types on tsetse catch size.

Advice on experimental design was given to scientists in the Medical Vectors Research Programme (Dr. M. Mutinga, Dr. J. Kaddu and Mrs. L. Rogo) working on sandflies and leish-

maniasis and a trip is planned in early 1984 to some experimental sites. Analysis was carried out on data from mosquito research previously done in Mombasa. The displacement in the field of *Culex quinquefasciatus* by *C. cinereus* was investigated by fitting logistic functions to the data to estimate rates of increase and decrease (Dr. R. Subra) whilst laboratory data on competition were subjected to analysis of variance. The same technique was used on survival rates and vectorial capacities for filariasis of *Anopheles gambiae* sibling species, which indicated that *A. merus* is likely to be a poor vector (Dr. F. Mosha).

Mbita Point Field Station was visitied in March to see the field conditions and to advise scientists working there in the Crop Pests Programmes on experimental design. The importance of keeping detailed records each year of the locations of experimental plots was stressed. Techniques for key factor analysis of pest populations were discussed with Dr. G. Oloo, and analysis of variance of data from field trials has been done for several scientists (Drs J.O. Ampofo, A. Alghali, J. Suh and Mr. G. Masina) in part utilizing a programme for combined split plot designs written by Dr. H. Magalit.

As well as the research programmes, both Sensory Physiology and the Chemistry and Bioassay Research Units have made use of the Unit's facilities and expertise. The Unit has been involved in both design and analysis of experiments on bioassay of *G. morsitans* pheromone and odours; antifeedants to lepidopterous larvae and EAG responses in relation to temperature (Drs C. den Otter, R. Saini, and S. waladde). Advice has also been given on the bioassay of isomers of the *G. pallidpes* sex pheromone (Dr. P. McDowell).

Computer operations and programming

Dr. H. Magalit, Mrs. W. Ssebunnya

The computers were installed at the end of 1982 and early in 1983 the system and available packages underwent extensive testing in order to explore the full potential of the system. Programmes were later written for both scientific and management usage.

For the scientists, original programmes were written for analysis of multiple latin squares and combined split plot designs. Three programmes from Biometry by F.J. Rohlf were rewritten and tested including ones for nested analysis of variance and tests of homogeneity of variances. Seventeen programmes from Computer Programming by R.G. Davies were rewritten and tested. These included Jolly's stochastic method for estimating population size, a model II regression technique and a test of Fit to a negative binomial distribution.

For management, several programmes were written for the payroll data, and the payslips and letters to the banks are now prepared within the Unit. Initial plans on computerization have been made with the Financial Manager and the Acting Administrative Manager. Most ICIPE secretarial staff underwent training within the Unit on use of the word processing facilities, and these facilities are now used extensively.

Future plans

One of the priorities for the Unit is to make computing facilities available at Mbita Point Field Station. Two Wang Professional Computers which can operate as a stand alone installation and can also be linked up to the main system will be installed there in 1984. Similar computers will be installed in Chiromo for scientific staff and at ICIPE House to improve terminal accessibility. An operator/programmer will be assigned to Mbita Point to assist there on data and word processing.

At Duduville, the main frame VS80 will be upgraded and a scientific programmer will be hired to strengthen the Unit in the areas of statistical programming and simulation modelling. Special programmes will be written to enable all data from long term projects to be stored on the computer, or diskets greatly facilitating subsequent analysis and graphical representation.

Insect and Animal Breeding Service

Haematophagus insects

During 1983 we maintained a healthy colony of 6,000 mated female *Glossina morsitans morsitans* The colony produced an average of 600 pupae daily, out of which we harvested an average of 500 tenerals which adequately served our experimental needs. In February 1983, a *G. pallidipes* colony was started at Mbita Point Field Station. A viable colony of 1,000-2,000 mated *G. pallidipes* females was maintained throughout the year. We also maintained a small colony of about 600 adult *Aedes aegypti* for bioassay research.

Phytophagus insects

During 1983, the following phytophagus insects were supplied mainly to the Chemistry and Bioassay Research Unit for bioassay tests.

C. partellus

An average of 6,000 all-instar larvae were maintained at any one time.

S. exempta

A small colony of moths producing an average of 2,000 all-instar larvae was maintained. The larvae were fed on natural maize leaf diet. Small colonies of *Galleria mellonella, Tenebrio molotor, Dysdercus fasciatus* and *Gastromargus africanus* were also maintained.

Laboratory mammals

Rabbits

Loppers are here! On 29 June 1983, we started a small colony of lop-eared rabbits and the colony is now increasing. Our usual breeds of California white and New Zealand white cross-breeds are still thriving. Although our space is too limited to breed enough rabbits, we supplied 60-80 rabbits every month for research purposes, during the year.

Rodents

We supplied 50 rats, 10 hamsters and 200 mice per month to various programmes. Lack of adequate breeding space sometimes forced us to supplement supplies by buying from elsewhere.

Communications and Information

Annual Research Conference

ICIPE's 13th Annual Research Conference was held at Duduville International Guest Centre from 17 to 22 April 1983. As in the past, the conference was attended by a cross-section of the scientific community, both local and international, as well as science administrators, representatives of donor agencies and governments and members of the ICIPE Governing Board.

Two programmes were reviewed in depth: Tsetse Research Programme and Crop Borers Research Programme. All the other programmes were reviewed by poster exhibition. Professor M.D. Bentley of the University of Maine who was, at the time, Visiting Scientist with the Chemistry and Bioassay Research Unit, gave the annual public lecture on "Chemical Ecology of Mosquito Oviposition".

Workshops

An International Study Workshop on Tsetse Epidemiology was organized from 23 to 29 October 1983. Papers were presented by participants from both national and international research organizations. The wide range of topics covered the following aspects, among others: tsetse sensory physiology, reproduction, population ecology, sampling techniques, parasite/vector/host interactions and the impact of recent advances in insect ecology and vector control strategies.

Proceedings of this workshop are being published in a special issue of the journal, *Insect Science and its Application.*

Publications

Major publications during 1983 included: ICIPE 9th and 10th Annual Reports, ARPPIS calendar, brochure and first report and 4 issues of the Dudu. In conjunction with the International Development Research Centre (IDRC) the proceedings of a FAMESA seminar were published in the IDRC Manuscript reports series (IDRC — MR87e).

The journal, *Insect Science and its Application*, has since 1982 been diversified in content. Apart from mini-reviews and original research papers, the journal also publishes book reviews. Since 1983 a New Patents Section has been introduced. We have during 1983 published a special issue (of volume 4) on "Crop Borers and Emerging Strategies for their Control" based on an International Study Workshop on Crop Borers held at ICIPE in 1982. The total pagination for Volume 4 was 427 pages, compared to 296 for Volume 3. We are close the anticipated full pagination of 500 for the annual volume. Circulation has tripled since 1980, Africa leading in subscriptions, followed by the Americas.

In the book series, "Current Themes in Tropical Science", we have continued to receive assistance from guest editors on particular volumes. The second volume, *Natural Products for Innovative Pest Management*, edited by D.L. Whitehead and W. Bowers, was published. This book, as well as the first volume, *Physiology of Ticks*, edited by F.D. Obenchain and R. Galun, are available for sale at ICIPE. Volume 3, entitled "Caste Differentiation in Social Insects (with emphasis on termites)", edited by J.A.L. Watson, B.M. Okot-Kotber and Ch. Noirot, is in press and should come out in 1985.

ICIPE scientists continue to publish in international journals. A list of publications appears in this report. Other than editorial and conference services the department also provides graphic arts and photographic services to the centre.

Library and Documentation

The Library acquired 141 new books in 1983. Of these 121 were bought and 20 received as

gifts or on exchange. There was a drop in journal subscriptions from 140 to 104 titles due to increased costs.

Mbita Point Field Station Library

The building which houses the library at Mbita Point has been completed. New furniture is being acquired and the library should be fully operational soon. A new librarian—Miss Margaret Mathai, appointed towards the end of the year — has already taken up her duties. With these developments, it is anticipated that a basic reference collection of books and journals will be provided as well as current awareness service.

The ICIPE Library continues to cooperate with other libraries around Nairobi and beyond for mutual benefit by way of inter-library loans and exchange of information.

An agreement was reached with FAO for ICIPE to issue a monthly bibliography on tropical insect pest management based on FAO documentation, AGRIS, and ICIPE's own publications. Details of this are being worked out.

Study tours and visits

Mr. W.E. Umbima, the Chief Librarian, spent three months in Europe studying modern methods of information handling with emphasis on tropical entomology. Useful contacts were made for cooperation. The tour was sponsored by the Canadian International Development Research Centre (IDRC) who are giving some support to the ICIPE library and documentation services. For some time now, ICIPE has been trying to set up a tropical insect pest management documentation centre. It is still very much at the planning stage, and it is hoped that as a prelude, a workshop will be organized to map out a plan of action.

Visitors

Outstanding among the distinguished visitors to ICIPE were: the Australian High Commissioner to Kenya, H.E. A.G.D. White, Ambassador Yang Ke-ming of China, and Mr. G.L. Pennachio, UNDP Resident Representative to Eastern Africa.

1983 Publications

Alghali, A. M., Relative susceptibility of some rice varieties to the stalked-eyed fly *Diopsis thoracica* West. *Insect Sci. Appl.* 4 (1/2) 135-140.

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1983 Seminars

Dr. D. Arn Swiss Federal Research Station for Fruit-growing, Viticulture and Horticulture Switzerland	—	The insect antenna as a detector in the chemical identification of pheromones
Professor M.D. Bentley University of Maine, USA	—	Studies of spruce budworm feeding deterrents
Dr. D.E. Bignell University of London, UK.	—	Structure and function of the gut in soil feeding termites
Dr. M. Boppré University of Regensburg West Germany	—	On the biology, physiology and ecology of pharmacophagus insects
Dr. D. von Brentano FAO, Kenya	—	The current food crisis in Africa.
Dr. M.F.B. Chaudhury ICIPE, Kenya	—	Effects of precocenes on tsetse
Dr. T.S. Dhadialla ICIPE, Kenya	—	Juvenile hormone regulation of vitellogenin gene expression in the African migratory locust, *Locusta migratoria*
Dr. C.J. Den Otter ICIPE, Kenya	—	Prey detection by the Panamanian bir-spider *Sericopelma rubronitens*
Professor S. Friedman University of Illinois, USA	—	Trehalose metabolism in insects
Mr. A. Goldman Clark University USA	—	Pest control decision-making by small-scale farmers in Kenya.
Dr. A. Hassanali ICIPE, Kenya	—	What are feeding deterrents?
Dr. H. Hirumi ILRAD, Kenya	—	Recent progress in cutlivation of the African trypanosome *in vitro*
Professor N.J. Holliday University of Manitoba Canada	—	An assessment of carabid beetles for biological control of orchard pests in Canada.

Dr. M. Kaib University of Bayrenth West Germany	—	Defense strategies of termites. A review exemplified by *Schedorhinotermes lamanianus*
Dr. J. Kawooya University of Chicago USA	—	Purification and properties of microvitellogenin and apolipophorin III of *Manduca sexta*
Dr. P. Kevenhoerster DSE West Germany	—	DSE approaches and programmes.
Professor J.H. Law University of Arizona USA	—	The biochemistry of insect camouflage
Professor H. Lipke University of Massachusetts USA	—	The tanning controversy. A contribution to confusion
Dr. W.W. Page KARI, Kenya	—	Armyworm moths. Behaviour and pheromones
Mr. R.K. Saini ICIPE, Kenya	—	Acoustic communication in tsetse flies
Dr. D.M. Robinson National Institute of Health Bethesda, USA	—	A molecular basis for some temperature effects on living systems.
Professor D. Schneider Max-Planck Institut (West Germany)	—	Insect senses and orientation
Professor R.A. Steinbrecht Max-Planck Institut, West Germany	—	New trends and recent advances in biological electron microscopy

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