THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

ICIPE

Tenth Annual Report 1982

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The International Centre of Insect Physiology and Ecology

Nairobi, March 1983

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Foreword

Searchlight on the ICIPE Scientific Programme

The two years of 1981 and 1982 proved to be a period for reflection, for organising reappraisal of our core scientific activities, and for a clear-cut resolution as to what must constitute the size of the ICIPE. In this respect, it might well be useful to recall the words I wrote last year, in the Foreword to the 1981 Annual Report (Scientific Development: The Trial of the ICIPE Community):

> "One important point that has emerged from a reappraisal of the ICIPE programme during the year 1981 is that the most crucial target of its research effort is the satisfaction of the resource-poor farmer in Africa and other tropical regions in his pest management needs. We are therefore reassessing all of our research activities to ensure that their goals will correspond closely with these needs, and that the research training we are undertaking for Africa is such that it will strengthen the national programmes in this critical area".

One may well ask how far we have taken this exercise.

We believe that we have undertaken this reassessment, and we are now implementing the recommendations arising from this exercise, to the deep foundation level essential for a substantial reorientation of the Institute's scientific activities. Firstly, we have phased out completely two research programmes within the two-year period — the research programmes on African Armyworm and Grassland Termites — we have also trimmed off several projects within existing research programmes: tick physiology (Livestock Ticks), trypanosome *in vitro* culture and trypanosomiasis immunology research (Tsetse), and mosquito projects (Medical Vectors) and we have reorganized the research units dealing with natural products chemistry, biochemistry, and bioassay into one integrated Chemistry and Bioassay Research Unit. Secondly, we have trimmed down our training programme considerably, in this way consolidiating the remaining activities into only six training projects: the Postdoctoral Research Fellowship Programme; the African Regional Postgraduate Programme in Insect Science (ARPPIS); the Finanancial and Administrative Management of Research Projects in Eastern and Southern Africa (FAMESA); the International Group Training Course for Ecologically Sound Pest and Vector Management Systems; the International Training Course in Insect Growth, Development, and Behaviour; and the Staff Development programme (for ICIPE staff only). Thirdly, the scientific programme has adopted two new elements, which will meet a critical need in each case: we have a new research programme on Insect Pathology and Pest Management, which will assist the ICIPE in bridging the gap between fundamental research and operational pest management; and we have established a Biostatistics and Computer Service as a research support service for the proper design of experiments, effective and prompt statistical analyses, a start on computer modelling, and the undertaking of other relevant data processing functions. Finally, we have determined that the institutional size will remain a modest one, described in terms of approximately 44 principal staff-years for the foreseeable future.

These decisions have taken considerable effort of the entire ICIPE community and the Governing Board to reach. It has been a difficult process, often a painful one. But we believe that the ICIPE is now walking a well prescribed locus — and the searchlight we unleashed two years ago will light our new-found way as we confidently take to it in the next several years.

THOMAS R. ODHIAMBO
Director, ICIPE

2nd March 1983

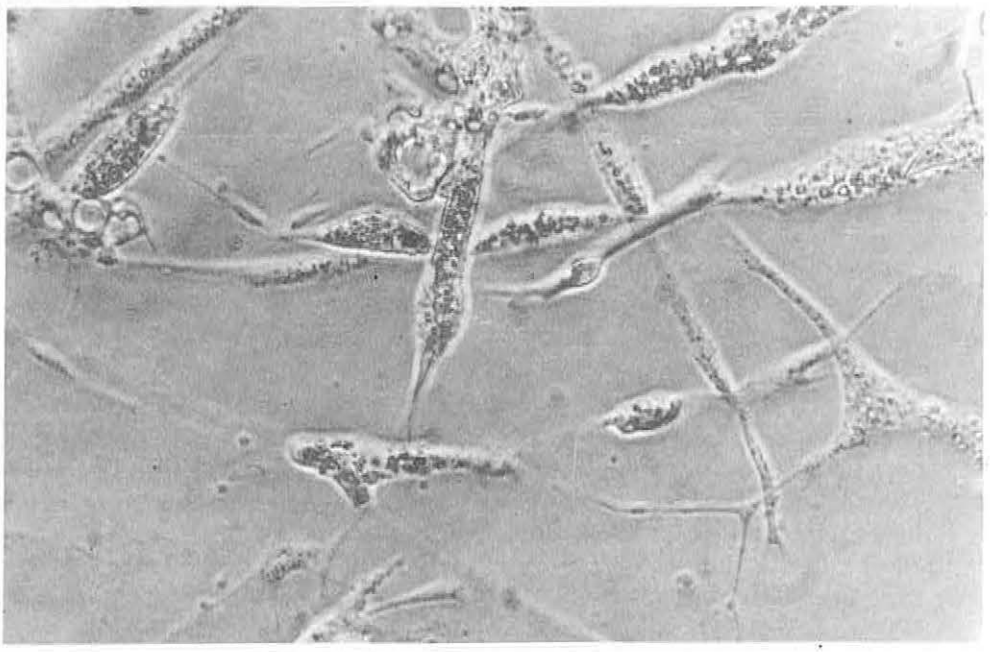


Figure 1. Tick cells of *R. appendiculatus* in culture.
(A report of this research is given on page 13)

Some Considerations of Livestock Ticks in Africa

Throughout the world, but particularly in Africa, ticks and the diseases transmitted by them greatly inhibit livestock production. The only method presently available for the control of ticks is the application of acaricides to cattle in dips or sprays. The use of acaricides to control ticks has many disadvantages, two of the most important of which are (1) Ticks develop resistance to acaricides which necessitates the continuous development of new products by drug firms (The increasing costs of developing these products is becoming probibitive), and (2) It is becoming widely recognized that the close-interval application of acaricides to cattle — which is required for the control of East Coast Fever — produces an inherently unstable situation where the entire cattle population will be completely susceptible to tick infestation and the transmitted disease. If for any reason acaricide control is interrupted, thousands of cattle may die.

In East Africa, the most important tick-borne diseases are Anaplasmosis, Babesiosis, Cowdriosis and Theileriosis. The first three of these occur in many countries of the world, and both vaccines and curative drugs for their control have been available for some time. Theileriosis, or East Coast Fever (ECF), which occurs only in East and Central Africa, is by far the most important of these tick-borne diseases. It is caused by *Theileria parva* and is transmitted by the brown ear tick, *Rhipicephalus appendiculatus.* It affects only cattle and the African buffalo. In indigenous Zebu cattle, mortality is generally confined to calves and varies from 5% to 50% depending on the severity of the challenge to which the calves are exposed. Animals which survive acquire an immunity which is life-long and may become carriers. During the process of acquiring this immunity, however, many cattle are stunted and productivity is severely affected. European breeds of cattle, and improved Zebu originating from non-ECF enzootic areas, are very susceptible, and, when they are exposed to the disease, morbidity and mortality approach 100%. One infected tick can transmit a fatal infection to cattle.

Fortunately, an experimental method of vaccinating cattle against ECF is available and is currently being used in field trials by colleagues at the Kenya Agricultural Research Institute (KARI) Muguga, and also at the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi. Curative drugs for ECF have recently been identified and at least one of them, "Parvaquone", is expected to be marketed in East Africa by Wellcome within the next few months.

Livestock Ticks Research Programme

In view of the foregoing considerations (preceeding page) it seems obvious that a balanced and ecologically sound method of controlling ECF would result if biological control of the tick could be achieved in conjunction with vaccination and drugs. Accordingly, the Tick Programme at the ICIPE has concentrated on producing methods for the control of R. appendiculatus. As has been previously reported, two types of resistance are being investigated, designated Type 1 and Type 2.

Type 1 resistance is acquired by host animals following tick infestation, and is an immune response to the innoculation of antigens in the saliva of the feeding ticks. It has already been demonstrated in a small field trial that Type 1 resistant cattle can control field populations of ticks.

Type 2 resistance is produced when antigens obtained from tick homogenates (not present in tick saliva) are innoculated into host animals. Antibodies against the antigens, ingested by the feeding tick, react with the target antigens and affect the subsequent development of the tick. It is expected that cattle which have both Type 1 and Type 2 resistance will have an enhanced resistance to tick infestation.

During 1982 investigations have continued to improve Type 1 and Type 2 resistance, particularly in cattle, and preparatory work has been carried out to pave the way for large-scale field trials for the integrated control of ECF. Some of these investigations are described below.

Survival of *Rhipicephalus appendiculatus* Ticks under field conditions

This long-term experiment, which started in 1979, was concluded in early 1982. After exposing ticks in field arenas, at three different times of the year, results showed that adults at Muguga could survive up to 20 months, nymphs up to 14 months and larvae up to 8 months. The time of the year when the ticks were exposed seemed important, since those ticks exposed at the end of the dry season survived better than those exposed at the start of the dry season. The final collapse of the population, however, coincided with the dry season. This work is now planned to be done in other areas including the Transmara district of Narok and the Kuja River area south of Nyanza. It will include other species of ticks, as well.

We know that the number of adult ticks found on cattle, or collected from the vegetation, increases markedly soon after the start of the long rains. Engorged nymphs were placed in nylon gauze bags on the surface of the soil, lightly covered with leaf litter, in pasture at the Kenya Agricultural Research Institute (KARI), Muguga. Ticks were exposed in January, February and March 1982. The rainy season started abruptly at the end of March. Identical batches of ticks were observed in the laboratory at 28°C/35% relative humidity (r.h).

In the field most of the adults moulted in 3-4 weeks, and few unmoulted; live nymphs were found after 5 weeks. When each bag was checked any unmoulted ticks were also placed at 28° C/85% r.h. to complete development. Some adults (see Table 1) experienced delays in moulting, especially those exposed in February. After 14 days they fell into two categories and a few did not even complete development in 56 days. Normally, the engorged nymph becomes immobile after a few

Table 1: Mean number of days to moult by engorged nymphs after transfer from the field to a constant 28° C/85% r.h.

Days of exposure	Date exposure started		
	29/11/82	17/2/82	17/3/83
7 males	9.9	10.4	9.1
females	8.4	8.8	8.2
14 males	7.0	7.3*	5.7
females	4.4	5.9*	4.2
21 males	2.4	4.3	2.6
females	1.0	4.7	3.2
23 males	—	—	2.2
females	—	—	3.0
56 males	—	2.0	—
females	—	1.0	—
control 28°/85% r.h., sexes together	11-15	14-16	11-15

*There were also ticks which took much longer to moult; males 14.3 days, females 13.3 days

days, as development proceeds. In the extended pre-moult periods that we observed, however, some engorged nymphs remained mobile for many days, suggesting that early development was arrested.

Individual nymphs were also weighed, placed in separate vials and moulted at 19° and 28° at 44% and 85% r.h. respectively, then checked daily for moulting. There was a consistent difference (Table 2) as expected between the sexes with males taking 13% — 14% longer than females to moult. Development was also slower at the lower r.h. and at 19°C/44% r.h. three males had extended pre-moult periods (42-44 days compared with the modal value of 36 days). There was also a small lengthening of the pre-moult period in all cases correlated with nymphal weight.

Each time that samples of engorged nymphs were put out in the field another 200 were scattered on the ground around them. Later, the moulted adults were counted on the grass each week. At the end of sampling in mid-May, the ticks on the vegetation were collected daily for 5 days, then at weekly intervals, and later monthly until December. The total recovery was 52.0%, 51.1% and 36.0% of those broadcast in January—March respectively; 83% of them were taken in the first 5 days. Assuming that these ticks also took an average of 3 weeks to moult, it was another 3 weeks before the January and February ticks were seen on the

Table 2: Mean (+ S. E.) duration of pre-moult in ticks under controlled conditions, sample size in brackets

19° C			
	Males	Females	
44% r.h.	85% r.h.	44% r.h.	85% r.h.
36.3 ± 1.7 * (84)	35.7 ± 0.1 (87)	31.9 ± 0.2 (14)	31.5 ± 0.7 (12)
28° C			
12.9 ± 0.1 ** (84)	12.9 ± 0.1 (68)	12.0 ± 0.0 ** (15)	11.4 ± 0.1 (31)

Difference between means significant : *P<05 **P<0.01

grass and 2 weeks for the March sample. Thereafter numbers counted each week rose in parallel with the accumulated rainfall. Thus the January ticks had a period of 6 weeks of low activity before they built up to a high level of activity after the rain started. The March ticks were already very active 3 weeks after they moulted as it was raining by that time.

From detailed observations of ambient temperature and r.h. and temperature from four points on the soil surface beside bags of moulting ticks, it was obvious that the ticks were experiencing a very wide range of temperature and r.h. Nevertheless 79% of the January sample moulted, although few survived in the bags until the rain started. For the March sample 90% moulted and most adults were still alive when the observations ended 5 weeks later. Ticks that were free to seek their own hiding places survived better than those confined in bags only a few cm. away. After mid-May very few more ticks ascended the vegetation. We are continuing our observations to see what proportion of the uncollected ticks become active in the 1983 rainy season.

Feeding Performance of Field-derived Ticks Compared to Laboratory Controls

This work was carried out to investigate the possibility that ticks, originating from an area where they had been exposed to resistant hosts over an extended period, might have acquired a resistance to Type 1 resistance in host animals. The feeding performance of Narok-derived *R. appendiculatus* was compared to that of laboratory controls on both resistant and susceptible hosts. Preliminary results have shown that all the three instars of the field-derived ticks had a higher proportion feeding and attained better engorged weights than control ticks fed on resistant rabbits. Table 3 indicates that the Narok-derived larvae had a better proportion of fed ticks ($P<0.01$) and attained better engorged weights ($P<0.01$) than laboratory controls on resistant rabbits. There was no significant difference between field-derived and laboratory-control larvae on susceptible rabbits. Similar results were obtained for nymphs and adults, with field-derived nymphs

Table 3: The number (out of 100) and the mean weight (mg) of larvae feeding and moulting successfully into nymphs: Narok-derived versus control laboratory-derived larvae.

	Narok larvae		Control larvae	
Host	No.	Wt. (mg)	No.	Wt. (mg)
Resistant rabbits				
T386	86	0.51	58	0.38
T388	89	0.49	51	0.35
T389	80	0.48	52	0.40
T390	93	0.52	54	0.34
$\bar{X}$	87.0 ± 5.5	0.50 ± 0.02	53.8 ± 3.1	0.37 ± 0.03
Resistant Cattle				
R64	57*	0.46	39*	0.41
R67	12*	0.39	9*	0.36
Susceptible rabbits				
R458	74	0.52	82	0.54
R459	74	0.57	88	0.50
R477	75	0.50	75	0.52
R478	78	0.55	96	0.49
$\bar{X}$	75.3 ± 1.9	0.54 ± 0.03	85.3 ± 8.9	0.51 ± 0.02

*out of 400 larvae

being significantly more variable in engorged weight on resistant rabbits. Field-derived larvae, however, when fed on a couple of highly resistant cattle, failed to feed significantly better. More "tick feedings" on resistant and susceptible rabbits and cattle are being carried out to establish possible sources of the observed differences.

Induction of Type 2 Resistance in Type 1 Resistant Cattle

Seven cattle with long previous experience of feeding ticks were subjected to our standard tests of feeding 100 nymphs on one ear and 20 ♂♂ + 20 ♀♀ on the other, with identical feeds on pairs of naive control rabbits. The cattle were then immunised with an extract of whole engorged female ticks + Freund's complete adjuvant (FCA). Twenty-nine days later they were tested again and given a booster injection of the same antigen + Freund's incomplete adjuvant (FICA). Tweny-two days after that they were tested again, and further tested in two groups after another 112 and 147 days (the latest tests are still in progress). The results are given in Table 4. The cattle results are also shown as percentages of the controls, since in some cases the rabbits did not give consistent results from test to test.

One animal was outstanding over the first 3 feeds, yielding not a single live larva from the adults feed on it, and only an average of 5 adults per 100 nymphs. Three others allowed larval production of 15%-17% of full potential and 19-28 adults per 100 nymphs. For these 4 cattle the mean weight of the nymphs which engorged on them was only 4.2 mg by the third feed i.e. 45% of the weights from the control rabbits. This ensured that the resultant adults would also have reduced survival and lowered fecundity.

Type 2 Resistance to *Ornithodoros porcinus porcinus* in Rabbits

Preliminary investigations on type 2 resistance to *O.p. porcinus* in rabbits were started using an *in vitro* system. The aim was to establish an *in vitro* system which could also be used to assay antigenic material from *Rhipicephalus appendiculatus* and try to identify common antigens from different tick species.

Groups of rabbits were immunized with materials from ticks using FCA, e.g. whole fresh egg homogenate, mated female reproductive system, day 4 post-feeding midguts, protein fraction from eggs and day—9, 5th instar nymph haemolymph. Boosting was regularly done fortnightly using FICA and bleeding was also done to obtain serum for precipitin tests. Eight-week immune sera showed strong precipitin lines against antigens.

Nymphal and adult ticks were fed *in vitro* on bloodmeal composition of 1:1 pig red blood cells to rabbit serum. Nymphal ticks were observed for moult delay while female ticks were mated, laid eggs and the eggs were observed for hatching and mortality.

Among nymphal ticks fed on bloodmeal fortified with antisera to haemolymph, moult delay of 3 days was observed. Among those fed on anti-midgut serum, moult delay of 3-5 days was observed. Among the female ticks fed on pig red blood cells fortified with immune rabbit serum there does not seem to be any adverse effects on either egg mortality or hatchability following the first feed on immune serum. In a second feed of the same females on immune serum, however, there was seemingly high mortality among the treated females ranging from 25-80% compared to 9% for ticks fed on normal pig blood and 38% for ticks fed on normal rabbit serum.

Type 2 Resistance to *Rhipicephalus appendiculatus*

Experiments are in progress to investigate type 2 resistance to *R. appendiculatus* in both mice and rabbits. Both mice and rabbits have been immunized with B-protein fraction of fresh eggs of *R. appendiculatus*, midgut homogenate and A^1 fresh egg protein fraction. Also 2 groups of mice were immunized with whole homogenate of fully engorged nymphal and larval *R. appendiculatus* in Freund's Complete Adjuvant. They are bled and boosted fortnightly with FICA.

Precipitin lines have been shown with immune sera from both mice and rabbits immunized with B-protein, A^1 protein, larval and nymphal homogenate. Plans are underway to feed ticks on immune animals and observe whether there are any adverse effects on larval and nymphal development, female reproduction, egg hatch, and mortality.

Table 4: Test feeds using 20 females (plus 20 males) and 100 nymphs of *R. appendiculatus*, with fresh control rabbits in each case; cattle results as percentage of controls in brackets

Feed no	hosts	Adult feeds % ♀♀ fed	Mean engorged wt (mg)	Live, LL	Nymphal feeds % fed	mean (mg)	adults[2]
1	7 cattle	53 (68)	244.0 (109)	419 (44)	46 (47)	5.3 (60)	42 (46)
	2 rabbits	78	206.7	946	97	8.8	92
2	7 cattle	55 (85)	123.5 (48)	88 (15)	32 (39)	4.1 (46)	19 (24)
	2 rabbits	65	256.8	585	82	9.0	78
3	7 cattle	45 (75)	170.8 (68)	73 (17)	37 (39)	4.7 (51)	32 (33)
	2 rabbits	60	251.4	427	96	9.3	96
4	7 cattle	55 (62)	139.3 (45)	—	38 (45)	3.0 (43)	—
	4 rabbits	89	307.8	—	85	6.3	—

1. Yield of live larvae per ♀ applied 2. Yield of moulted adults per 100 nymphs applied.

Effects of Tick Infestation on Growth Rate of Cattle

This work has been undertaken with the purpose of establishing basic information on the effect of ticks on cattle under controlled conditions, trying to minimize the influence that other constraints such as management, nutrition and diseases may have on the animals. Thirty cattle were kindly lent by the Director of the Veterinary Research Dept. KARI, Dr. Walter Masiga, for this study including 17 *Bos indicus* type, 6 *Bos taurus* type and 7 *Bos indicus* x *Bos taurus* crosses. Their ages ranged between 6 and 16 months at the beginning of the experiment (May 1982). The animals were separated according to age and size and kept in separate pens in groups of 3 or 4 animals. The animals are being fed with concentrates and hay in measured amounts.

Two months were allowed for the animals to get used to the new feed and handling procedures. They are being weighed and bled two times a week and during the course of the work they have been dewormed and inoculated against Leptospirosis., Black leg, Anthrax, Foot and Mouth Disease and Rinderpest. Several animals showed *Anaplasma marginale* infections and it was decided to treat all animals with imidocarb dipropionate and check blood smears at weekly intervals. Temperature of all animals is checked daily.

Before tick application, the animals were tested for tick resistance against *R. appendiculatus* and *Amblyomma variegatum* by means of the 100 nymphal test (100 NN test) and they were shown to be tick susceptible.

After several different ways of tick application were considered, it was decided to apply the tick *(R. appendiculatus)* every week by means of earbags to be placed on alternate ears. The bags were removed after 48 hours when the viable ticks were attached and feeding. The necessary weekly tick supply was estimated at 4,500 adults and this number is regularly obtained by feeding the equivalent number of nymphae on 10 rabbits every week.

The animals have been divided into 3 groups of 10 animals according to weight and breed.

Group 1: Animals maintained tick-free during the whole experiment
Group 2: Animals infested with 20 females and 20 males every week.
Group 3: Animals infested with 200 females and 200 males every week

(Referred to throughout the report as groups 1, 2 and 3). Ticks have now been applied for 18 weeks. Ticks which do not attach, and therefore are removed with the earbags two days later, are counted. Five days after application those females which drop to the floor of the pens are collected and weighed to evaluate the possible effect of developing resistance in the animals. Earbags are also applied to the control animals for the same period of time as the tick-infested animals to compensate for any effect that the earbags or their application may create.

Food consumption is being measured in the different groups of animals but this cannot be done very precisely. Nevertheless, the concentrates given are calculated as 3% of the bodyweight of the animals in each pen and half bale of hay per pen is added every day, therefore, food is considered to be *'ad lib'*.

Table 5: Mean tick challenge estimated at 48 hours and number of female ticks which were still feeding on the animals 3 days later.

	GROUP 2		GROUP 3	
Date of application ticks	Mean and S.D. of females which stayed on at 48 hours	Mean feeding females 3 days later	Mean and S.D. of females which stayed on at 48 hours	Mean feeding females 3 days later
14.7.82	17.9 ± 1.2	11.7 ± 3.4	168.7 ± 11.5	58.5 ± 34.7
21.7.82	17.2 ± 1.7	11.2 ± 3.7	178.8 ± 21.6	68.2 ± 28.5
28.7.82	16.9 ± 4.2	11.8 ± 4.9	158.3 ± 26.2	43.7 ± 26.5
4.8.82	16.8 ± 2.1	14.3 ± 3.0	145.9 ± 23.9	66.2 ± 30.5
11.8.82	16.5 ± 2.7	9.7 ± 4.1	165.0 ± 11.8	45.5 ± 27.4
18.8.82	16.9 ± 1.9	5.8 ± 2.1	163.2 ± 24.3	23.9 ± 19.4
25.8.82	16.4 ± 1.8	9.7 ± 2.6	158.1 ± 34.1	46.1 ± 27.3
1.9.82	18.2 ± 0.7	8.5 ± 3.9	163.4 ± 10.2	35.5 ± 21.0
8.9.82	15.6 ± 1.8	9.6 ± 3.9	157.4 ± 50.9	40.7 ± 26.4
15.9.82	10.1 ± 3.8	4.3 ± 3.0	127.0 ± 18.8	25.0 ± 14.7
22.9.82	16.3 ± 3.2	13.6 ± 5.0	173.9 ± 11.6	70.2 ± 21.9
29.9.82	13.4 ± 4.6	5.3 ± 3.4	175.6 ± 11.3	50.4 ± 26.3
6.10.82	18.0 ± 0.8	7.5 ± 3.5	161.5 ± 15.3	45.3 ± 26.2
13.10.82	17.8 ± 2.7	7.5 ± 3.5	170.5 ± 12.3	38.0 ± 24.3

The summarized results available after 12 weeks of tick application are shown in Tables 5-9. The data are also being analysed by breed and age, and there appear to be some interesting differences. A considerable body of information on the other parameters namely temperature, blood values, ear condition, lymph node enlargement and weight of engorged ticks dropped by the animals is also being produced. Finally, observations on the grooming and licking behaviour of the animals are being made throughout the experiment.

Table 6: Mean weight and percentage weight gains of group 1 cattle

Date	Mean weight and S.D. (Kgs)	% weight gain after tick application to the other groups*
20.7.82	146.8 ± 23.3	10.21
27.7.82	154.3 ± 23.9	15.84
6.8.82	161.7 ± 25.1	21.40
13.8.82	169.0 ± 26.5	26.88
20.8.82	177.2 ± 27.9	33.03
27.8.82	179.1 ± 28.0	34.46
3.9.82	182.2 ± 28.3	39.04
10.9.82	190.9 ± 29.9	43.32
20.9.82	195.5 ± 30.6	47.00
28.9.82	203.4 ± 31.3	52.70
5.10.82	208.9 ± 31.1	56.83
15.10.82	213.9 ± 29.3	60.58

*Percentages are calculated by considering the mean of the last 5 weighings before tick infestation as zero. This is the same for Tables 7 and 8.

Table 7: Mean weight and percentage weight gains of the group 2 cattle

Date	Mean weight and S.D. (Kgs)	% weight gain after tick application
27.7.82	161.6 ± 28.1	9.04
27.7.82	171.7 ± 30.5	15.86
6.8.82	176.1 ± 30.1	18.82
13.8.82	180.7 ± 30.1	21.93
20.8.82	188.8 ± 26.9	27.39
27.8.82	192.7 ± 28.3	30.03
3.9.82	199.1 ± 28.5	34.34
10.9.82	202.4 ± 27.9	36.57
20.9.82	205.8 ± 27.0	38.87
28.9.82	212.8 ± 28.6	43.59
5.10.82	219.3 ± 31.6	47.97
15.10.82	229.2 ± 28.8	54.65

Note that the first tick application was on the 14.7.82 and from that date, ticks were applied at weekly intervals

Table 9: Mean packed cell volume of the 3 groups of animals before and after tick infestation

Date	Group 1 Cattle	Group 2 Cattle	Group 3 Cattle
6.5.82	34.7 ± 2.6	32.3 ± 5.3	30.4 ± 4.5
2.6.82	35.1 ± 4.6	32.7 ± 4.7	32.8 ± 5.0
22.6.82	32.1 ± 1.8	28.0 ± 4.2	31.1 ± 2.8
20.7.82	36.8 ± 3.8	33.1 ± 4.7	34.6 ± 3.8
27.7.82	39.9 ± 3.0	35.9 ± 5.0	34.6 ± 1.6
6.8.82	36.1 ± 4.6	32.5 ± 6.2	33.1 ± 2.7
13.8.82	36.2 ± 2.3	30.6 ± 10.1	34.0 ± 2.9
20.8.82	40.7 ± 4.3	37.3 ± 3.5	36.3 ± 2.5
27.8.82	37.7 ± 3.9	35.8 ± 4.2	35.0 ± 3.5
3.9.82	38.2 ± 5.4	36.6 ± 3.2	34.3 ± 3.9
10.9.82	37.7 ± 4.0	36.4 ± 4.2	34.8 ± 3.9
20.9.82	39.4 ± 6.8	36.5 ± 5.5	35.9 ± 3.4
28.9.82	42.5 ± 2.9	38.8 ± 4.0	39.4 ± 2.4
5.10.82	42.9 ± 3.7	37.0 ± 3.7	37.1 ± 1.9
15.10.82	39.4 ± 2.0	38.3 ± 4.6	36.4 ± 1.6

Note: Tick application started on 14th July 1982

Tick Cells in Culture

This work was carried out in the hope that target antigens for induction of Type 2 resistance might be produced *in vitro*. Embryonic tick cells have been grown in culture from 21 day-old eggs of *Rhipicephalus appendiculatus*. Cultural conditions consisted of equal volumes of L-15 and RPMI 1640 media supplemented with 20% fetal bovine serum and 10% tryptose phosphate broth. Cell growth occurs at temperature ranges of 29°-37° C but optimum growth has been found to be at 34° C. (See Figure 1, page 6)

Table 8: Mean weight and percentage weight gains of the group 3 cattle

Date	mean weight and S.D.(Kgs)	% weight gain after tick infestation
20.7.82	159.9 ± 28.9	5.13
27.7.82	173.1 ± 30.9	13.81
6.8.82	176.9 ± 32.7	16.30
13.8.82	182.8 ± 30.7	20.18
20.8.82	192.5 ± 37.7	26.56
27.8.82	195.9 ± 34.9	28.80
3.9.82	204.4 ± 35.2	34.38
10.9.82	203.8 ± 33.5	33.99
20.9.82	210.1 ± 37.3	38.13
28.9.82	215.1 ± 36.2	41.42
5.10.82	222.2 ± 36.8	46.09
15.10.82	233.6 ± 39.8	53.58

Morphologically the cells are either epithelial or fibroblast-like or are round-shaped. The cells will be tested in animals for induction of resistance to tick infestation.

Rabbit antiserum against CD tick egg protein

In a double diffusion (Ouchterlony) experiment, rabbit antiserum to *Rhipicephalus appendiculatus* egg protein fraction CD (M.Wt. 1.4 — 2.2 x 10^4) was reacted against X) *R. appendiculatus* egg protein fractions A_4 (M.Wt. 2.1 x 10^6), A_1 (M.Wt. 5.1 x 10^5), B (M.Wt. 1.06 x 10^5) and CD; and Y) *R. appendiculatus* protein fractions A_4, A_1, B and CD. However, the latter were laid by ticks which had been fed on rabbits sensitised against protein CD. The results are summerized below. (Table 10, P. 14).

Antigens 1, 2 and 3, observed in the A_4 fraction are absent in treated eggs. Likewise, A_1 antigens 3, 9 and 10 are not present in treated eggs. However, there is a new antigen, 11 in treated A_1. B appears to be unchanged by the treatment. Antigens 4 and 5 are present in control and treated CD fractions, however, treated fraction CD has two additional antigens, 12 and 13 (Figure 1, P. 14).

Table 10: The reaction of rabbit anti-CD with tick egg proteins, A_4, A_1, B and CD. o — normal eggs; Δ — treated eggs. The number of symbols denotes the intensity of reaction.

Antigens	A_4	A_1	B	CD	Inference
1	o o				Antigens 1 and 2 are exclusive to A_4, but absent from treated eggs.
2	o o				
3	o	o o			Antigen 3 present in A_4 and A_1. However, it could be an A_1 protein contaminating A_4
4			o	o o o	Either present in B and CD or it could be a CD protein exclusively, but contaminating B present in treated eggs
			Δ	Δ Δ Δ	
5				o o	Exclusive CD protein present in treated eggs.
				Δ Δ	
6			o		Antigens 6, 7 and 8 appear to be exclusive B proteins. Perhaps they correspond to the three major proteins of the B complex, observed by isoelectric focusing and ion exchange chromatography.
			Δ		
7			o o o		
			Δ Δ Δ		
8			o		
			Δ		
9		o			Exclusive A_1 antigens. Not present in treated eggs.
10		o			
11		Δ Δ Δ			Present in treated eggs only, suggesting a change in A_1 after treatment.
12				Δ	Exclusively in the CD fraction but only after treatment
13				Δ	

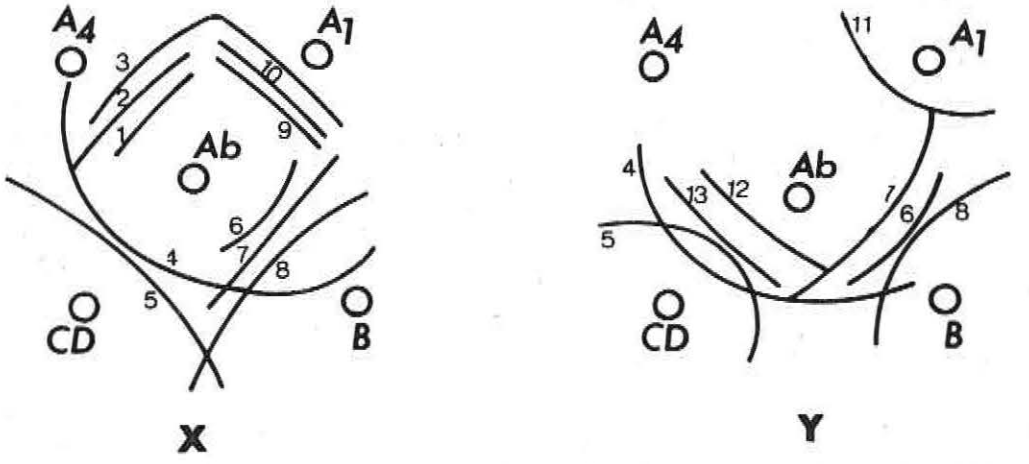


Figure 1. Precipitin lines obtained by doing a double diffusion experiment with *Rhipicephalus appendiculatus* egg protein against the antiserum to the protein.

Tsetse Research Programme

Trypanosomiasis, commonly known as sleeping sickness, is a disease unique to the African continent although two forms of it (Chaga's disease and vivax trypanosomiasis) occur in South America. Sleeping sickness and Nagana are extremely important in Africa because of the problems they pose to public health and livestock development.

The geographical distribution of trypanosomiasis is coincident with that of the vectors of the disease — tsetse, which are found in an area of about 10 million square kilometres of the African continent. The endemic pattern and persistence of the disease is due to the fact that some game animals and some domestic animals are reservoirs of trypanosomes. Due to the broad spectrum of host preferences of the vectors, and to the fact that these reservoirs continuously provide the tsetse with trypanosomes, control measures to date remain inefficient.

Past control schemes have met with varying degrees of success, reflecting the operational and technical constraints encountered. Another major factor has been the incomplete understanding of the ecology and behaviour of the species concerned. In an attempt to throw more light on these aspects of tsetse biology, the ICIPE has placed great emphasis on studies of tsetse ecology and behaviour, and on the epidemiology of the trypanosomiasis as transmitted by tsetse. A sound knowledge of these aspects is a fundamental prerequisite for determining the prospects for successful tsetse and trypanosomiasis control.

Ecology

Tsetse population sampling

For studies on population dynamics of tsetse flies, unbiased estimates of population size and composition are required. Ideally, a sample of tsetse from a particular sampling device would constitute a fixed and representative fraction of the total population — this fraction being the sampling efficiency of the technique. However, catch size is affected not only by the density but also by the activity and physiological state of the population. Moreover, different components of the population, such as males and females, may differ in their activity. This inherent 'activity' bias affects all tsetse sampling techniques. The various sampling devices used add further bias, since components of the population differ in their responses to different attractants. As a result, different sampling methods may not give the same picture of a population. The objective of this research is to develop a methodology by which samples of adult tsetse population *(Glossina pallidipes)* can be translated into true estimates of population size and composition.

Since the biconical trap is presently the most widely used sampling technique, the initial aim has been to determine correction factors for samples using this trapping method. Two approaches have been utilized: firstly to compare seasonal changes in biconical trap catches with mark-release-recapture estimates of population size, and secondly, to release known numbers of marked flies within the attraction range of a trap to see what proportion is recaptured.

Work on the first approach has been carried out from March to December, 1982 in a 600-metre stretch of wooded valley in Shimba Hills National Reserve, Coast Province. Biconical trap samples have been taken at monthly intervals (5—day periods) and mark-release-recapture estimates at two-monthly intervals (12-day periods). Traps were replicated in 3 site types — along the valley

floor, along the edges and 5 metres from the edges. Climatic factors were monitored and flies from some traps were kept for ovarian dissection or fat/haematin analysis. Thus differences in daily catches can be analysed in relation to climatic and physiological factors.

Apparent density of the tsetse population declined from March, as the rainy season progressed, increasing again towards the end of the year. The concurrent mark-release-recapture studies were carried out using flies captured in the same biconical traps, although in November, additional traps were set at 200 metres from the valley edges to investigate dispersal. Flies were marked with oil paint after laboratory studies showed no significant difference in survival rates of marked and unmarked flies. In March a comparison was made of feeding-the-flies with not-feeding them prior to release. Recapture rates were higher when flies were fed, probably because mortality or dispersal of hungry flies was reduced. Subsequently, flies have been offered food before release, with a feeding rate of about 25%. Male recapture rates have consistently been higher than female recapture rates, and there has been cycling of recaptures. This last factor, together with some evidence of atypical dispersal of marked flies, has complicated the analysis and the effort to obtain absolute population estimates for comparison with the apparent densities from biconical traps, but such analysis is now underway.

For the second approach, the initial aim has been to determine the range of attraction of the biconical trap. This has been done by varying the inter-trap distance within groups of four, on the assumption that where the ranges of attraction overlap, the catch per trap will be reduced. Another method used was to vary the distances of traps from the tsetse concentration at the edge of a thicket. Preliminary analysis suggests that 15 — 20 metres is the maximum effective range of attraction for the biconical trap. Trial releases of flies within this range have been carried out at Shimba Hills, but this work will continue at Nkruman in 1983.

G. pallidipes in the Lambwe Valley

In the absence of further tsetse control measures in the Lambwe Valley (see 1981 Annual Report) routine monthly monitoring of the *G. pallidipes* population, using biconical traps, has continued through 1982. Three selected habitats characterised by different vegetational associations were established as monitoring sites: thicket, woodland and coniferous plantation. Following the virtual destruction of the population by the 1981 aerial spraying campaign (over 99% reduction), the population has proceeded to recover. The following aspects were observed:

- Recovery to the pre-spray population density was fully achieved in the thicket on the floor of the valley exactly 12 months after cessation of spraying operations, and the population has remained fairly stable since, at roughly the previous equilibrium level.
- The recovery was evenly progressive in the thicket, but the rate far exceeded that which could be explained on the basis of reproductive potential alone (the population tripled in size each month for 6 consecutive months). Evidently pockets of fly which survived the spraying were being recruited into the area where sampling was being carried out.
- Compared to the thicket population, populations remained very low in adjacent woodland and in a coniferous plantation further away, with no indication of *gradual* increase. Simultaneous with the full re-establishment of the thicket population, however, the woodland population underwent an instantaneous recovery (with a 200-fold increase between two successive months) to its pre-spray level. A similar but much less marked increase also occurred in the coniferous plantation at the same time.
- The recovery was not sustained in the woodland, since the population subsequently fell back and remained at less than half its original, pre-spray level. The plantation has also stabilised at a much lower level than initially.
- It would appear that once the thicket population reached an optimum density, mass movement of flies occurred from thicket to adjacent vacant habitat.

Experiments were also carried out to see the underlying causes of differences in sex ratio and age structure recorded in populations sampled from different localities. Measurements of light incident on traps revealed highly significant, positive correlations between illuminance (related to the degree

of shading vegetation) and both female and non-teneral fly percentages in trap captures. This was subsequently confirmed by a diel activity pattern study in which numbers, sex ratio and age structure of hourly samples were recorded together with corresponding hourly recordings of light intensity, temperature and saturation deficit. The female percentage in hourly captures closely followed the diel light intensity profile, while the percentage of 0 category females (nullipars, less than 10 days old) was inversely related. The male age structure changed little with time of day, except that a younger sample was obtained in the early morning and late evening. Characteristically different male and female activity patterns were recorded, but the total numbers caught hourly—expressed as a proportion of the overall daily catch—was closely related to both temperature and saturation deficit profiles.

Previous data on abortion rates from 24 hr capture samples was suspect on account of the phenomenon of trap-induced abortions. To determine more accurately the true abortion rate, and at the same time to estimate the extent of over-representation in 24 hr samples, rates were determined simultaneously from hourly and 24-hourly captures, in the same locality (thicket), monthly. From 6 months' data, the abortion rate in hourly samples only varied from 1 to 2%, whereas those from 24-hr samples were 3 to 4 times greater. Even the apparent true-abortion rates were somewhat overestimated due to the problem of distinguishing between abortion and normal larviposition in instances of females with an empty uterus and a fully developed egg next in ovarian sequence. The results also indicated that there may be age-specific differences in abortion rate, but contrary to what has previously been supposed, it is young rather than old females that are more prone to abortion; primogravid flies contributed about half to the overall abortion rate of all age classes.

Vector capacity

G. pallidipes in the Lambwe Valley

One year after sequential aerial application of endosulfan aerosol (backed by ground spraying of residual dieldrin and bush clearance in areas of difficult terrain) *G. pallidipes* population in Lambwe Valley, South Nyanza, Kenya, had recovered to pre-spray levels. It was therefore necessary to monitor the reappearance of trypanosome infections in the flies, particularly since the reservoir hosts were not disturbed during the spraying operations. The following trypanosome infection rates were observed: *T. brucei*, 0.4%; *T. congolense*, 2.5%; and *T. vivax*, 14.6%.

T. brucei isolates were frozen and stored in liquid nitrogen. These were later tested for their sensitivity to human serum. Isolates which were found to be insensitive to human serum were further characterized using enzyme electrophoresis on thin layer starch-gel. The enzyme electrophoretic patterns observed were compared with those obtained from human isolates.

Of the 14 *T. brucei* tested so far, five gave enzyme electrophoretic patterns similar to *T. b. rhodesiense* and four similar to *T. b. gambiense*. The remainder gave variable patterns.

Three of the four isolates which gave results similar to *T. b. rhodesiense* cross-reacted serologically (agglutination tests). This suggests that some particular human-infecting serodemes were circulating among the reservoir hosts on which the flies fed. The risk of human trypanosomiasis in this locality is still a very grave one.

G. pallidipes in the Meru National Park

Previous work at ICIPE has shown that *G. pallidipes* from various regions of Kenya are quite different from each other in several physiological aspects. It is very likely that *G. pallidipes* from various populations vary in their ability to transmit trypanosomes. Dissections of approximately 5,000 flies collected from Meru National Park failed to reveal a single *T. brucei*. It is realized that current dissection methodology is not always accurate and it is possible to overlook an infected fly.

The search for the presence of *T. brucei* infection in *G. pallidipes* from Meru National Park used trituration assay in mice. A total of 650 *G. pallidipes* were collected, dissected and examined for trypanosome infection. Twenty-two (3.4%) were found to be infected with *T. vivax*, sixteen (2.5%) had *T. congolense* and one had a midgut infection. No *T. brucei* infection was observed. A total of 425 flies were homogenized and injected into 167 mice.These mice were subsequently examined

for the presence of trypanosome infection. Only *T. congolense* infection was observed. These results, and those of previous studies, very strongly suggest that *T. brucei* is absent from the *G. pallidipes* population in the Meru area. Analysis of saliva from these flies has shown that it is of relatively low protein content, a necessary component for maturation of *T. brucei.* It is therefore possible that the Meru population of *G. pallidipes* has become resistant to *T. brucei* infection. This possibility is being examined.

G. pallidipes in the Nkruman – Rift Valley

In the last report an account was given of experiments with revolving screens for trapping *Glossina pallidipes.* Some of the screens were cylindrical, others were flat revolving at different speeds. There are two main problems being dealt with in these studies. First is the sampling bias experienced in the biconical trap catches, in which teneral and young nonteneral flies are under-represented. The objective is to improve the quality of catches of that trap, and obtain representative samples. A second problem concerns finding a suitable means of sampling low density populations. This involves improv-

Figure 1. Biconical trap with revolving cylindrical screen components

Figure 2. Biconical trap with revolving flat screen components

ing the quantity of trap-catches by enhancing the attraction of the trap.

The revolving screens were specifically developed to improve the quality of catches of *G. pallidipes*. The trap with flat screens making 20 revolutions per minute was found to give the best representative fly sample by age grades. By adding attractive stationary screens to the trap total catches improved.

Reproductive Physiology

(Glossina morsitans morsitans)

Three different lines of investigation have been pursued in the reproductive physiology of *G. morsitans morsitans*. First, assessment of the critical time for precocene action, second, control of parturition and third, changes in cyclic nucleotides during prenancy and parturition.

We have previously reported that precocene treatment of female *G. morsitans morsitans* induces sterility and other abnormalities in ovarian follicle development in the F_1 generation produced by treated females. From these results it was difficult to pinpoint the sensitive stage in the maternal reproductive cycle or developmental stage in the F_1 offspring, when precocene was most effective. Therefore, experiments were designed and carried out to determine the critical stage of precocene action.

Females were treated with precocene either topically, or by contact of treated surface, at a known time after each of the first 3 larvipositions. Treatment ranged from 15 min to 18h for topical application and 2h to 70h for the contact method. Also, precocene was applied topically onto the mother, whose reproductive cycle was in late embryogenesis or 'in utero' first larval instar. Results of the experiments show that the target tissue is sensitive to precocene action during the early stages of embryogenesis. Old embryos and first instar larvae appear to be insensitive. Whether complete sterility or other follicle abnormalities are caused or not depends on the time at which precocene reaches the target tissue, within the narrow range of the sensitive period.

Parturition is a gated response, that normally occurs late in the photophase in our laboratory colony, where both mother and larva participate in determining its precise timing. The role of female neuroendocrine systems in coordinating the birth process, however, is not well understood. Experiments were conducted to determine whether or not a neurohormonal control mechanism operates in the process of parturition. Experiments involved transfusion of hemolymph from donor females onto head-ligated recipient females and transection of medial abdominal nerves.

Results show that parturition can be blocked by neck ligation of the female, thus suggesting a crucial role for the brain. Transection of nerves that innervate the uterine muscles reduces the incidence of successful parturition, and parturition in neck-ligated females can be stimulated by transfusion of hemolymph from females that have recently given birth. The preliminary evidence thus suggests a controlling mechanism operating through neural connections as well as by a hormonal route.

Cyclic nucleotides have been widely demonstrated as important regulatory compounds in most biological systems. We have previously shown that the injection of Adenosine 3′ : 5′ — cyclic monophosphoric acid (cyclic AMP) — or agents that elevate endogenous cyclic AMP—provides a stimulus for ovulation and parturition. The nucleotide levels, however, was not known from these results. In the present study we determine levels of both cyclic AMP and Guanosine 2′ : 3′ cyclic monophosphate (cyclic GMP) in female tsetse in response to events in the pregnancy cycle. Cyclic nucleotides were assayed by using radioimmunoassay with 125_I labelled cyclic nucleotides.

Results show that cyclic AMP and cyclic GMP levels change very little in response to feeding and mating, but during pregnancy and at parturition, major changes can be detected in both mother and larva. In both the female head and larva, cyclic AMP levels reach a peak at parturition. In the abdomen, the pattern of high cyclic GMP closely parallels the activity cycle of the female's milk gland. These findings suggest that there is a link between the rise of cyclic AMP level and parturition. Also, a role for cyclic GMP in regulating secretion in the tsetse milk gland appears likely.

This research was conducted in collaboration with Dr. D. L. Denlinger, a visiting scientist from the Ohio State University, Columbus, Ohio, U.S.A., with a National Institute of Health research grant.

Crop Borers Research Programme

Research on major crop borer species, viz, 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 was initiated in late 1979. Most of the research is carried out at the Mbita Point Field Station (MPFS) and in nearby farmers' fields. The main objective of the Crop Borers Research Programme is to develop environmentally safe and economically feasible pest management practices. The programme has access to international research centres such as ICRISAT, CIMMYT, IITA, IRRI and WARDA, and to national agriculture research programmes in Kenya. Some of the important research activities include: studies on population dynamics of shootfly; incidence and period of activity of crop borer species of maize, sorghum, and rice; mating behaviour and pheromones in cowpea pod borer; pest carry over and wild host plants; yield loss assessment; genetics of sorghum resistance to stem borers; and pest complex within mixed cropping systems of maize, sorghum and cowpea.

Sorghum Shootfly

Atherigona soccata is the most important sorghum shootfly present throughout the year in Kenya on cultivated and wild sorghum, *S. arundinaceum*. Some of the factors affecting shootfly populations are: the availability of host plants of suitable stage, percent survival of first instar larva that can cause desiccation and deadheart in the central shoot, and resistance and susceptibility of the host plant. Population reaches a peak at the end of the rainy season. Moderate temperature (25°C) and high humidity are favourable for egg development and hatching. A few predators and parasites have been identified. Various alternative host plants have been recorded. A number of shootfly resistant lines mainly from ICRISAT have been identified and confirmed. The line IS 2146 possesses strong antibiosis for sorghum shootfly. The local cultivar Serena was found to be highly susceptible to shootfly attack.

Sorghum and Maize Stem Borers

Although the four stem borer species, viz, *Chilo partellus, Busseola fusca, Sesamia calamistis* and *Eldana saccharina* were found damaging, *C. partellus* is the most abundant stem borer species (over 80%) infesting sorghum and maize particularly in the lower altitude areas of Kenya. In the cooler and high altitude areas, however, *B. fusca* was found to be more important and serious than other stem borers. In coastal areas of Kenya *C. partellus* and *C. orichalcociliellus* have been found to be the major stem borer species of sorghum along with *S. calamistis.*

Various alternative host plants of sorghum and maize stem borer species have been recorded. Some of the hosts like *Cenchrus ciliaris, Sporobolus marginatus* (for *C. partellus), Kyllinga* sp. (for *Eldana* and *Sesamia), Leptuous repens* (for *Chilo, Busseola, Eldana* and *Sesamia)* appear to be the new host records. In addition, other hosts such as *Panicum maximum* and *Pennisetum purpureum* for *Chilo, Busseola* and *Sesamia,* and, *Echinochloa haploclada* for *Chilo* and *Sesamia,* were also recorded. Pest carryover studies revealed that larvae of *C. partellus* and *B. fusca* remained in diapause for several months. Therefore, disposal of sorghum and maize stalk and removal of wild

hosts and volunteer cereal hosts appears a desirable management approach.

The following parasites and predators were recorded on the stem borer complex: *Apanteles sesamiae*, a gregarious larval endoparasite of *C. partellus*, *B. fusca*, *S. calamistis* and *E. saccharina; Dentichasmias busseolae*, a solitary pupal endoparasite of *C. partellus* (25% of pupae were parasitized by this parasite). Common predators recorded were black ants, *Componotus rufoglacus* on eggs and larvae of *Chilo*, *Busseola*, *Eldana* and *Sesamia;* lady bird beetle, *Cheilomenes* sp. on eggs and larvae of *C. partellus;* earwigs, *Diaperasticus erythrocephala* on eggs and larvae of *C. partellus*, *E. saccharina* and *B. fusca.*

Based on screening carried out so far some of the sorghum lines which were found to be resistant to both *C. partellus* and *B. fusca* are IS 2205, IS 4337, IS 4660, IS 5480, IS 18427, IS 18479, IS 1044, IS 1082, IS 1151, IS 2122, IS 2146, IS 1855, and IS 10676. Most of these are also resistant to shootfly infestation.

Maize inbred lines found resistant to stem borers are local inbred lines D and G and some CIMMYT materials from populations 25, 27, IDRN. The CIMMYT lines No. 178C (White seed) and 324 (Yellow seed) were found promising.

Rice Stem Borers

Two year results of surveys of rice ecosystems in Kenya revealed that upland rainfed and swamp rice sufferred less damage due to stem borers than low land irrigated rice. Species composition varies with rice ecosystems, but *Maliarpha separatella* (which makes up 80% of the borer population in low land rice) appears to cause only 25% infestation in upland rainfed rice. Upland rice grown in sorghum and/or maize areas was heavily attacked by *C. partellus* and *S. calamistis.* In flooded swamp rice only *M. separatella* was found.

Cowpea Pod Borers

A study on mating behaviour of *Maruca testulalis* was carried out under normal laboratory conditions at MPFS where relative humidity and temperature fluctuate around 50-80% and 24-30%, respectively. It was observed that mating occurs between 2000 hours and 0500 hours, reaching a peak between 0100 hours and 0200 hours. The majority of moth pairs mated for 60-70 minutes. It was also observed that mated moths reached peak oviposition period at the age of 5 days old and a majority of these moths laid eggs in a period of 2-4 days. Of the mated female population observed, 77% laid fertile eggs, 9.5% laid unfertile eggs while 13.5% laid no eggs.

Using Campion pheromone traps with freshly emerged virgin *M. testulalis* females it was observed that female moths produce sex pheromones which attract males from the field at night. The number of males trapped increased with the age of the females in the traps, reaching a peak on the third night of the female emergence.

Crop Loss Assessment

Sorghum

Very little information is available on the actual economic importance of sorghum shootfly on sorghum grain yield. A study was initiated by simulating shootfly attack on plants grown in the field under the protection of an insecticide "umbrella". Preliminary results showed that (simulated) shootfly infestation resulted in a delay of flowering and maturation, however, the total number of harvestable heads did not differ significantly. As a result, there was no difference in terms of grain yield between control and any of the treated plots with 25, 50, 75 and 100% simulated infestation.

Maize

Screenhouse studies on the effect of *C. partellus* infestation on maize yield showed that larval survival was higher at the infestation rate of 5 larvae per plant (76%) compared to 10 larvae per plant (37%). However, grain yields were not significantly different for treatments with 5 larvae/plant, 10 larvae/plant and infestation by the second generation.

Rice

Preliminary studies of irrigated rice under field and cage conditions indicated that the rice plants attacked by *M. separatella* larvae develop subnormal panicles which bear a large portion of unfilled grains. About 10% of yield loss was recorded in cage conditions.

Application of Furadan and N fertilizer gave 20 to 40 percent yield increase depending upon the control methods. It was

observed that crop loss due to early damage, caused by *Diopsis* during vegetative phase, was greater than loss by *Maliarpha* damage occurring at later growth stages. Application of N. fertilizer enhances insect infestation, deadheart and whiteheads, and percent of unfilled grains. Plant population has no effect on insect infestation and rice yield.

Cowpea

A study on the response of cowpea to loss of leaves, shoots, flowers and pods indicated that foliar injury caused the highest reduction in pod and seed production when inflicted at the flowering stage. Differences in computed grain yields, however, were statistically insignificant among treatments. This indicates that cowpea plants compensate to a great extent for foliar injuries.

Genetics of Sorghum Resistance to Stem Borers

Diallel analysis of sorghum resistance to stem borers (particularly *C. partellus)* indicated that the resistance is polygenically inherited. However, the resistance was partially dominant to susceptibility. Combining ability studies showed that resistance to primary damage (deadheart) was governed by both additive and non-additive types of gene action. Secondary damage (stem tunnelling) was governed predominantly by additive genes. Tunnel length 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 local cultivar Serena appears to be tolerant of stem borer damage. Cultivars such as IS 2146, IS 18427 and IS 18489 were found resistant and could be used in resistance breeding programmes. The cultivar IS 18363 was found to be the most susceptible to stem borers.

Inter-cropping and Pest Management

The intercropping experiment included eight possible combinations of monocrops, dicrops and tricrops of maize, sorghum and cowpea. The results indicated that pest colonization processes were interfered with by the cowpea/cereal maize and sorghum combinations, causing delayed stem borer incidence on the maize and sorghum. The productivity per unit area for the various cropping patterns—as expressed by land equivalent ratio (LER)— gave 1.23 LER for maize/cowpea, 1.48 LER for maize/cowpea/sorghum mixed, and 1.33 LER for the maize/cowpea/sorghum intercrop as the best cropping combinations for the 1982 long rainy season. It was concluded that maize and sorghum dicrop is at a disadvantage in land use and productivity, and contributes to stem borer infestation.

Programme on Bases of Plant Resistance to Insect Attack

The main objectives of the programme are to:

- *identify factors that cause susceptibility or resistance/tolerance in crops to specific pests.*
- *study the biochemical, biophysical and/or other factors that cause acceptance or rejection or confer resistance by other mechanisms.*
- *study the causes and mechanisms involved in the development of insect biotypes and investigate means by which this can be suppressed.*
- *devise systems by which insects can be mass produced for resistance screening and other mechanisms of resistance studies.*

Toward these objectives, the programme continued research in 1982 with emphasis on the factors and mechanisms of resistance in (1) maize and sorghum to the stalk borer, Chilo partellus and (2) cowpea to the cowpea aphid, Aphis craccivora and the cowpea pod borer, Maruca testulalis. Other areas of research emphasis were experimental bases for the mass rearing of Maruca testulalis, Busseola fusca, Eldana saccharina and Chilo partellus.

Mechanisms of Maize Resistance to *Chilo partellus* (Swinhoe)

Several maize lines from local and CIMMYT germplasms were screened for resistance to *Chilo partellus* during the 1980–81 seasons. Out of these, five lines showing agronomic eliteness plus resistance to *C. partellus* were selected for studies of the mechanisms of resistance to this pest in 1982. The main emphasis in these studies was larval behaviour and development of the resistant and susceptible lines.

First instar larval behaviour and movement from oviposition to feeding sites

The preferred oviposition site of *C. partellus* is on the lower leaves of the maize plant. Newly hatched larvae have to migrate from there to the leaf funnels to commence feeding. Any factors that influence their migration to the funnels would have a significant impact on the colonization of the plant by the insect.

Studies of the neonate larval behaviour and movement were undertaken using four lines of varying levels of resistance: CMT 324 (high resistance) CMT 33 and Inbred b (moderate resistance) and Inbred A (susceptible). Evaluations were made of the acceptability of the oviposited plant to the neonate larva.

An individual larva was released near the middle of a leaf, two or three leaves up from the ground. Larval behaviour and movement were recorded and timed until (a) it reached the axil or left the plant and (b) it settled in the funnel region or elsewhere on the plant. Behaviour and movement of larvae from egg batches were also studied along similar lines.

Of larvae released on Inbred A plants, 67 per cent followed a high acceptance pattern of behaviour while only 25 and 29 per cent followed this pattern on Inbred b and CMT 324, respectively. Only nine per cent of larvae released on Inbred A rejected the plants outright, compared with 34 and 36 per cent on CMT 324 and Inbred b, respectively.

Larval climbing speed on the stem was similar on all lines (ca. 2cm/min) but total climbing time correlated positively with plant height. Per cent of larvae establishing in the funnels 24 hr after release was highest in Inbred A (54 per cent) and least in CMT 324 (39 per cent). Each maize line was planted, with another line as a background plot and infested with egg batches. After hatching there was a notable larval migration from CMT 324 into the Inbred A background plots. There was very little migration from a susceptible line into one with high resistance.

These experiments indicate a factor on the maize leaf surface is involved in the determination of plant acceptability to newly emerged larva. Different infestation and damage levels frequently observed among the different lines may be accounted for in this way.

Larval feeding behaviour, growth and development

Three-week-old plants of CIMMYT lines 33 and 324 and Inbred lines A, D and b were infested by placing 10 newly emerged larvae of *C. partellus* in the whorl region of the plants. Plant condition was monitored and plants were dissected at one and two week intervals after infestation. All larvae were recovered and their feeding location within the plants was recorded.

One week after infestation 80—90 per cent of larvae recovered were located within funnel regions of all maize lines. After two weeks of infestation only 17 and 25 per cent were recovered from the funnel region of CIMMYT lines 33 and 324, respectively. By comparison, infestation in the funnel region ranged from 39 per cent in Inbred D to 67 per cent in Inbred A. Extensive feeding near the growing point of Inbred A usually caused deadheart. Fifty-five per cent of larvae recovered from the funnel region of Inbred A were older than third instar compared with 31 per cent of those recovered from CMT 324.

These observations suggest a plant reaction (in the resistant material) to damage, resulting in a larval avoidance of the funnel region and poor development of larvae remaining in the region. The observations also help explain the general reduction in deadheart count in the CIMMYT lines reported by Dabrowski and others in earlier ICIPE reports. Material from the funnel, older leaf and stem regions of damaged and healthy plants from these lines have been freeze-dried, lyophilized and incorporated in an artificial diet for more detailed studies.

Mechanisms of Sorghum Resistance to Insects

After the extensive studies on sorghum resistance to *Chilo partellus* in the 1980—81 seasons, the project was extended to cover the multiple resistance mechanisms in this crop to *C. partellus* and the sorghum midge *Contarinia sorghicola* as model pests.

Oviposition preference of sorghum cultivars by *C. partellus*

C. partellus oviposition was monitored and compared under choice and non-choice situations using three different cages, In the first cage (240 x 150 x 100 cm) three non-preferred, three intermediate and three preferred cultivars were planted in a randomised complete block design.

The second and third cages were the same size (20 x 20 x 60 cm). One contained preferred cultivars and the other contained non-preferred cultivars. Three gravid moths were released near the centre of each cage and observations were made during the night in red light. Three days after release all egg batches were counted as well as the number of eggs deposited.

The mean number of egg batches deposited was significantly variable among the cultivars. Three representative cultivars from each group, in both choice and non-choice situations, are given in Table 1.

Effect of sorghum cultivar on *C. partellus* larval growth and development

Selected sorghum cultivars showing different levels of resistance were infested with ten first instar *C. partellus* larvae per plant when plants were 21 and 48 days old. After 14, 28 and 31 days the plants were dissected. The development stage and weight of larvae was recorded.

The weight of larvae from the 21 day-old plants was the lowest in IS 18361, IS 2122 and IS 18479. In the 48 day-old plants the lowest larval weights came from IS 2122

	Cultivar	Number of egg batches per plant	
Choice Situation	IS 18363	2.25	
	IS 4660	2.12	
	IS 2205	1.96	
Non-Choice Situation	IS 18363	0.43	Susceptible
	IS 18319	0.36	
	IS 18361	0.21	
	IS 4660	0.00	Resistant
	IS 1082	0.06	
	IS 2205	0.04	

Table 1. Relative number of egg batches per plant for different sorghum cultivars in choice and non-choice situations.

but not the other two cultivars. In fact, IS 18361, had the second highest larval weight in 48 day old plants. Larval weights were consistently high in IS 18363, IS 18319 and Serena. The results from these experiments correlate well with leaf damage results from previous experiments. They indicate the presence of some factor(s) in the resistant cultivars that influences larval growth and development, and suggest the influence of plant age on the expression of the factor(s).

Evaluation of sorghum cultivars for recovery tolerance to *Chilo partellus*

Sixty sorghum cultivars from the FAO/ Kenya Government Sorghum and Millet Project were planted in single row plots. Each plant was artificially challenged with 10 — 12 *Chilo partellus* eggs at the black head stage. Four types of damage symptoms observed were leaf feeding, deadhearts, tunnelling and broken heads.

The expression of the different symptoms seems to be age-related and tunnelling may result in deadhearts or broken heads. The effects and extent of these damage symptoms are varietal and nodal tillering may result in the case of deadhearts or broken heads. They may also result in chaffy heads or partially filled grains in the case of broken heads. Nodal tillering may result in juvenile panicles at harvest or in smaller matured panicles with smaller grains at harvest. They may also result in two or three of these smaller matured panicles with additive weights greater than that of the primary tiller panicle. Tunnelling may not affect panicle production and size, while broken heads usually result from intensive larval feeding at the peduncle.

Relationships between sorghum and sorghum midge

The sorghum midge, *Contarinia sorghicola*, Coq was studied under field conditions during the main sorghum planting season at Mbita Point, Western Kenya. The developmental period from egg to adult ranged from 17 to 34 days with a mean of 23. On average 3.2 adult midges emerged per spiklet and the ratio of adult females (1,666) to males (1,086) at emergence was 1.53. Adult emergence was bimodel with a major morning and minor late afternoon occurance. The daily relationship between adult emergence and atmospheric temperature was described by the second degree quadratic equation $Y=32.2 + 35.01 x - 0.87 x^2$ ($r = 0.63$; $P<0.05$). The daily relationship between adult emergence and atmospheric saturation deficit was described by the second degree quadratic equation $Y = 21.26 - 1.02 x + 0.03x^2$ ($r = 0.22$; $P<0.05$).

The predator *Diaperasticus erythrocephala*, Olivier *(Dermaptera: Forficulidae)* was observed preying on newly emerged adults. Damage levels varied among 34 sorghum lines, with the size of the panicle ($r = 0.73$; $P<0.05$) and the number of days to 50% flowering ($r = 0.49$; $P < 0.001$) influencing the levels. Damage levels for monthly plantings of the sorghum variety Serena ranged from 1.8 to 97.7 per cent during the months of March through June. From the same period grain yields ranged from 22.3 to 0.9 kg/50 m^2.

The relationship between per cent damage and per cent crop loss was almost perfect ($r = 0.998$; $P < 0.001$). Adult midge counts per sorghum head were successively greater with monthly plantings ranging from a low of 0 to 1 in May and a high of 15 to 20 in July.

Resistance of Cowpea, *Vigna unguiculata* (Walp) to the Pod Borer *Maruca testulalis* (Geyer)

Previous studies on the Biology and behaviour of *Maruca testulalis* have shown that the resistant variety TVU 946 was less preferred for oviposition and also suffered less damage compared to the susceptible variety VITA-1. In 1982, studies on cowpea resistance to *Maruca* concentrated on determining whether the moderate resistance recorded on TVU 946 was due to non-acceptance for oviposition, larval escape, plant tolerance or antibiosis.

Maruca females were forced to oviposit on TVU 946 and VITA-1 potted plants in non-choice situation in large screen cages. After 48 hours of confinement the plants were removed from the cages and the number of eggs on each variety was recorded. There was no difference in the number of eggs laid on each variety. Oviposition preference is only manifested in a choice situation when TVU 946 is grown in plots adjoining the more susceptible cultivars.

Larval development and survival on excised cowpea stems and flowers of the resistant variety TVU 946 and a susceptible variety VITA-1 was studied. Mortality was higher for larvae reared on TVU 946 stems. Larval development was prolonged and pupal weight there was less on TVU 946 compared to VITA-1 (Table 2). This indicates a low level of antibiosis involved in the resistance of TVU 946 stems to *Maruca*.

Maruca larvae were also reared on flowers of TVU 946, VITA-1 and Ife Brown varieties in the laboratory from 1st instar to pupation and adult emergence. Number of pupating larvae, pupal weight and number of emerging adults were recorded. There were no significant differences in larval survival, larval duration, pupal weight and adult emergence, indicating that there may be no antibiosis in flowers. The moderate resitance previously observed on TVU 946 flowers in the field may be due to some other mechanisms.

Biochemical bases of cowpea resistance to *Maruca testulalis*

After determining water and dry matter content, lyophilised cowpea stems of TVU 946, VITA-1 and Ife Brown were incorporated in a synthetic diet (ICIPE Diet 6). The larval survival, larval development, percent of pupation, pupal weights, pupal and adult developmental periods, and adult emergence were monitored.

Results indicate that TVU 946 stems exhibit distinct antibiosis against *Maruca*. Larval survival was reduced and larval development times were all negatively affected.

An extraction procedure yielded hexane, ethyl acetate and aqueous extracts. These extracts and the residue were incorporated in diet 6 and a bioassay was carried out with *Maruca* larvae. Results indicate some antibiotic activity in the ethyl acetate extracts. Thin layer chromatographic (TLC) separation of the ethyl acetate extract resulted in several phytochemicals of varying Rf values, some absorbing or fluorescing under ultraviolet

Table 2. Larval growth and development on resistant and susceptible cowpea lines

CULTIVAR	% PUPATION (from 1st instar)	DEVELOPMENTAL TIME (Average days to pupation) ± SD	AVERAGE PUPAL WEIGHT (MG)
TVU 946	10.01	20.50 ± 1.81	32.25 ± 1.71
VITA-1	18.31	17.80 ± 1.71	35.33 ± 2.80

light. Bioassay of these bands indicates the presence of both Kairomones and allomones in ethyly acetate extract. One band however consistently gave the greatest negative activity against *Maruca* larval survival and development. The structure of this compound is being determined in Chemistry/Bioassay Research Unit at the ICIPE.

Further experiments have confirmed that the resistant TVU 946 stems contain less sugars, amino acids, nitrogen and protein than the susceptible VITA-1 and more phenols, flavonoids and crude fibre.

From the present data the biochemical bases of cowpea stem resistance in TVU 946 to *M. testulalis* is apparently due to both nutritional and allelochemical factors.

Mechanisms of Cowpea Resistance to *Aphis Craccivora*

Anatomical studies reveal that *A. craccivora* feeds mainly form the phloem of the susceptible Vita 1 cowpea cultivar. Laboratory experiments indicate that in comparison with the resistant TVU 310 and 408-P-2, aphids spend less time probing and more time actually feeding.

In a choice feeding situation in the laboratory, Vita 1 stems were preferred over TVU 310 and 408-P-2. In a non-choice situation there was a higher aphid mortality in both resistant cultivars. A higher fecundity was also observed in the susceptible Vita 1.

Analysis of some secondary metabolites shows that quantitatively there are more phenols and flavonoids in both resistant cultivars than in the susceptible Vita 1. A higher concentration of phenols was also present in Vita 1 and TVU 946 stems infested with *A. craccivora* than in the uninfested controls.

There was no significant difference in total amino acids of Vita 1, 408-P-2 and TVu 310 and the total sugars did not correlate with resistance or susceptibility (TVu 310 Vita 1 408-P-2).

Experiments are in progress to segregate any possible biotypes that may exist in the *A. craccivora* population and to develop a bioassay for cowpea plant extracts using C^{14} — Sucrose. These should determine the compounds that are responsible for conferring resistance to cowpea against *A. craccivora.*

Relationships between Rice and *Diopsis thoracica*

Rice cultivars TKM 6, OS 6, Rexero, ADNY 11, IR 28 and IR 579—48—1 were planted under irrigated conditions. Number of eggs thirty days after transplanting (DAT) were recorded and the number of dead hearts were counted sixty days after transplanting. Number of pupae, number of parasites per pupae, site of pupation and site of pupation of parasitized pupae were noted.

Analysis of the data suggests the existence of oviposition preference with two distinct groups among the cultivars. The first grouping is group OS6, IR 28 and TKM 6. These have less than 5 eggs per 15 plants. Group two consisted of Rexero, ADNY 11 and IR 579—48—1 with 10—12 eggs per 15 plants. The percentage of deadhearts (damaged tillers) ranged from 10.8 in TKM6 to 16.9 per cent in Rexero.

There was a fairly strong correlation ($r = 0.66$), between number of eggs (30 DAT) and number of deadhearts (60 DAT). High tillering varieties suffered smaller percentages of deadhearts ($r = 0.74$). Pupation took place on the first four outer leaf sheaths with a clear preference for the first three (96.7%). Healthy tillers or deadheart tillers both had pupae but 90% of the pupae were on healthy tillers. On OS6, with more pupae per healthy tiller (14 compared with 6), there was no significant difference between healthy and deadheat tillers as a pupation site ($x^2 = 3.20$; $P > 0.05$).

Parasitization of *Tetrastichus sp.* (Eulophidae) was affected by (i) site of pupation, with pupae on the first leaf sheaths heavily parasitized; (ii) rice cultivars, with OS6 recording 17.6 per cent parasitization and Rexero 58.8 per cent and an overall of 38.9 per cent (iii) pupal length ($r = 0.68$); (iv) pupal population as indicated by the number of eggs 30 DAT, and (v) number of parasites ($r = 0.79$). Also the number of parasites per pupae was affected by (i) pupal length ($r = 0.90$) and (ii) variety on which pupa was reared.

Distribution of *D. thoracica* eggs on rice

Eggs of *D. thoracica* were laid singly on the leafblades or sheaths of the rice plants. There were 1 - 4 eggs laid per tiller with one-

egg-per-tiller frequency of 91.9 per cent. Similarly, 0 - 7 eggs were recorded per plant. Most of the plants recorded one or two eggs per plant or 33.3 and 24.2 per cent, respectively, and 18.2 per cent of the plants had no eggs.

The second leaf blades from the ground were the most preferred for oviposition followed by the third, first then the leaf sheaths and fourth leaf blade. Expressed as corresponding percentages 43.7, 19.0, 17.1, 12.0 and 8.2 per cent ($x^2 = 60.99$; $P<0.001$). As the plants grow, eggs were deposited almost exclusively on the first and second leaf blades from the ground and also on the leaf sheaths. Eggs started to appear on the leaf sheaths on the seventeenth day of data collection.

There was no significant difference between the numbers of eggs laid on the abaxial (59) and adaxial (80) leaf blade surfaces ($x^2 = 3.17$; $P< 0.05$).

Significantly more eggs were laid on the leaf blades (139) than sheaths (19) ($x^2 = 91.14$; $P < 0.001$) On the leaf blades the eggs were laid 1 — 30 cm away from the axil with a mean distance of 9.6 cm (mean leaf blade length was 43.0 cm). While on the leaf sheaths the eggs were 1.5 - 9.5 cm above the ground with a mean distance of 4.8 cm (mean 'stem' length was 31.2 cm).

The eggs were laid either on the depression or elevation of the midrib depending on whether the eggs were on the abaxial or adaxial surfaces of the leaf blades. Distance from the midrib ranged from 0.1 — 0.6 cm with a mean of 0.25 cm. Significantly more eggs were laid away from the midrib 62.0% with fewer eggs, 38.0% being laid on the midrib ($x^2 = 9.14$; $P< 0.05$).

ICIPE — IRRI Project on Rice Brown Planthopper *(Niloparvata lugens)*

Work on the Rice Brown Planthopper (BPH) was continued in 1982 with more emphasis on cytological variations among biotypes 1, 2 and 3. It was observed that chromosome morphology and behaviour could be used as complimentary indicators for differentiating the BPH biotype complex. Cytological investigations were also made on another closely-related planthopper species, *Nilaparvata bakeri* (Muir) which thrives on a grass host, *Leersia hexandra* (Swartz), commonly found in ditches around rice fields. A population of *N. lugens* was found to co-exist with *N. bakeri* on *L. hexandra*, although *N. lugens* is considered to be specific on rice. Morphological and morphometric examinations of these *Nilaparvata* species indicate distinct differences between the rice-and grass-feeding populations.

Effect of seed oils on some major pests of rice

Laboratory bioassays of the seed oils of neem *(Azadiracta indica* A. Juss) chinaberry *(Melia azedarach* L.) and custard-apple *(Annona squamosa* L.) were found to have some control on certain major pests of rice. Custard-apple oil was the most effective against BPH and the whitebacked plant hopper (WBPH) with 70-100 per cent mortality at doses of about 5 ng/ insect. Neem oils and chinabery oils were similarly effective at doses of about 10 ng/ insect. Custard-apple oil, at a dosage of about 20 ng/insect, killed more of the green leafhopper (GLH) but neem and chinaberry oils had no effect. Similar tests on major predators of these pests suggested that the oils have little or no effect.

These oils appeared to have an antifeedant effect on GLH when sprayed in detergent emulsion on plants. This subsequently suppressed the transmission of rice tungro virus (Figure 1).

Tests of the neem oil on eggs and first instar larvae of the rice armyworm, *Spodoptera mauritia acronyctoides* (Guenee) showed reduced hatchability in the eggs and repellancy to the larvae.

Experimental Bases of Insect Mass Rearing

Legume podborers, *Maruca testulalis*

After formulating several diets, a satisfactory diet based on kabuligram and cowpea flower powder was identified for mass rearing of *Maruca.* The possibility of using artificial oviposition surfaces was also explored. Several artificial surfaces were tested and filter paper was the significant preference for oviposition In the development of the mass-rearing procedure use was made of locally available materials. Larval survival was between 90% and 100%.

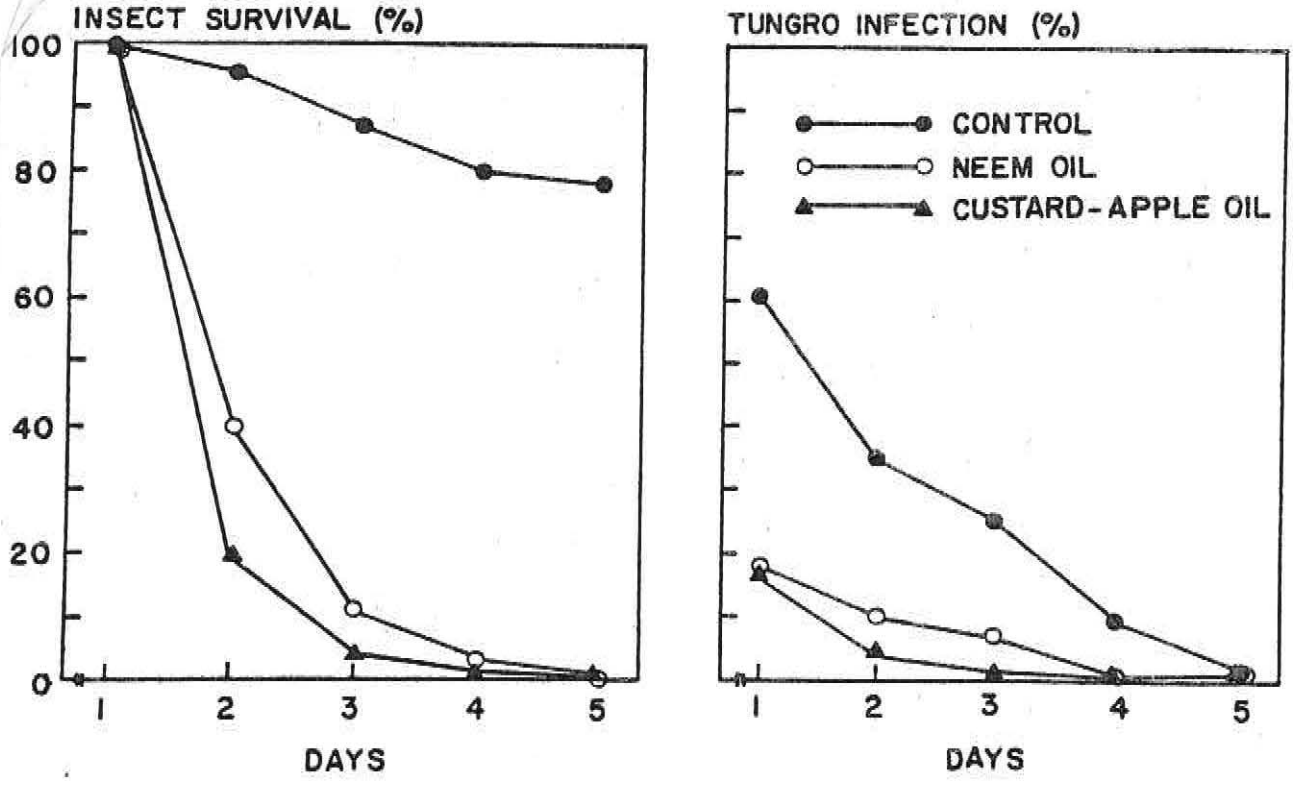


Figure 1. Survival of, and rice tungro virus infection by, *N. viresceus* exposed at different lengths of time on oil-treated TN1 seedlings.

We have tested the manipulation of the production of this insect pest and it has been confirmed that production can be routine and managed such that the requirements of the various scientists can be met.

The protozoan diseases reported in *Maruca* (ICIPE Annual Report 1981) have now been identified by Dr. W. A. Otieno as *Nosema* sp. and *Thelohemia* sp. Viral and bacterial diseases have also been noticed in our *Maruca* culture.

Maize borer, *Busseola fusca*

Rearing of this borer presents the problems of diapause and a very long life cycle. Both these problems increase the exposure-time to chance and contamination. In the last report, it was stated that Pritam Singh's artificial diet showed good result in which the larvae pupated from days 35 onwards. A diet modified from the one used by Seshu Reddy and Davis for *Chilo*, based on beans and sorghum leaf powder, has been found to yield best results to date.

Larval period ranged from 31 to 63 days, pupal period from 9 to 19 days for males and 11 to 21 days for females. A comparison was made between pupal weights of insects raised on diet and those raised on natural diet (stems). Pupal weights ranged between 96 and 329 mg on artificial diet and between 101 mg and 328 mg on stems.

Sugarcane borer, *Eldana saccharina*

The diet described above for *Busseola fusca* has been found to work satisfactorily for *E. saccharina.* The larval development period ranges between 21 and 28 days, pupal weights from 76 to artificial 178 mg on diet, and between 81 and 169 mg on stems.

Spotted stalk-borer, *Chilo partellus*

Rearing performance at 25°C has proven best of all temperatures tested. Because of higher yield and better quality insects, this temperature has been used in developing a mass-rearing procedure. After evaluating various rearing containers, glass bottles have been found most satisfactory. Using 50 larvae per bottle, the percentage yield is 70.

Medical Vectors Research Programme

In 1982 mosquito studies have continued with both anophelini (which are vectors of malaria and bancroftian filariasis) and Culex quinquefaciatus (which is only a vector of bancroftian filariasis). It is now possible for scientists working within the programme to identify the different species of the Anopheles gambiae complex by cytotaxonomy. This has made preliminary observations possible on the distribution of these species throughout Kenya. It has also facilitated studies on their survival and biting rate. These studies started in 1981 on Anopheles merus, a brackish water breeder species of the An. gambiae complex, and have been extended to Anopheles gambiae s.s. a freshwater breeder. Finally, work on C. quinquefasciatus has been dealing with the competition phenomenon between this mosquito and Culex cinereus. It has identified some of the reasons why the latter is not able to displace C. quinquefasciatus in all the areas of the Kenya coast.

Epidemiologogical investigations of leishmaniasis concentrated mainly in the visceral leishmaniasis foci of Machakos, Kitui and Baringo districts and laboratory experiments continued during the year. The disease epidemic itself abated in the Machakos focus as a result of combined control measures taken by the ICIPE against the vectors through sustained use of the sticky trap. Efforts of the Division of Disease Control and Research of the Ministry of Health were in the area of mass diagnosis and treatment. Field epidemiology continued with investigations into the vectors and animal reservoirs.

Mosquitoes

Geographic distribution of *Anopheles gambiae* Complex sibling species and their polymorphic inversions

In East Africa, *An. gambiae* complex consists of three sibling species, namely, *An. gambiae* s.s., *An. merus* and *Anopheles arabiensis.*

An. merus was restricted to coastal villages, accounting for 99.3 per cent (N = 294) of the *An. gambiae* complex in Jimbo shoreline village while in Jego 4 km inland it accounted for only 17.4 per cent (N = 46).

An. gambiae s.s. was predominant in coastal humid hinterland, accounting for 100.0 per cent (N = 22), 56.5 per cent (N = 23) and 55.6 per cent (n = 9) in Msihu, Masheheni and Garashi, respectively.

There were no polymorphic inversions observed in the 296 *An. merus* specimens from the coastal region. Polymorphic inversions 2Rb and 2La were observed in *An. gambiae* s.s. and 2Rb, 2Rbr and 3 Ra in *An. arabiensis.* The 2Rb inversion in *An. gambiae s.s.* was recorded from only one locality (Kisumu) at a low frequency of 28.7 per cent while the 2La inversion was found in all localities, with frequencies ranging from 40.0 per cent to 66.6 per cent. Polymorphic inversion 2Rq and 3Ra in *An. arabiensis* occurred in very low frequencies.

Estimation of the survival and biting rate of malaria vector

The average survival of individuals in a vector population is an important component of the vectorial capacity of the population. It has been conventional to estimate this survival from the proportion of the biting population found to be parous. However, this method has a number of limitations and the survival estimate is often biased.

Recently a computer-oriented method has been used to estimate average survival and biting rates of malaria vectors in Kenya. This method takes into account underlying population dynamics and is more accurate than previous means of estimating. During May/June 1982, time series studies were carried out for *An. gambiae* s.s. in Msihu, a coastal hinterland village in Kenya. The species displayed a higher average survival than *An. merus*, which had been similarly studied in another coastal village, Jimbo, in April/May 1981. Preliminary observations indicate that *An. gambiae* s.s. has a longer expectation of infective life than *An. merus*, and subsequently a higher potential to transmit diseases.

Field studies are being conducted on these species to establish the variation of their survival and biting rates according to location and season. This will facilitate ranking populations by species according to their potential medical importance. These studies will also contribute to mapping areas where, and recording times when the threat of malaria transmission is most serious.

Competition between *C. quinquefasciatus* and *C. cinereus*

In some rural areas of the Kenya coast a non-man-biting, *C. cinereus* mosquito is able to displace man-biting *C. quinquefasciatus* from shared breeding sites. But in 1981 it was observed that such displacement could not occur when domestic detergents were experimentally introduced into breeding sites. In urban areas where *C. cinereus* is rare or nearly absent most of the breeding sites contain detergents. In 1982 a study was made on the yield of the two species reared in water sampled from several urban breeding sites polluted by detergents. In both species a certain proportion of first instar larvae were able to develop to the pupal stage. Still, the yield of *C. cinereus* was always lower than that of *C. quinquefasciatus*. These observations could explain why in urban areas *C. quinquefasciatus* is the dominant mosquito species but they do not explain the absence of *C. cinereus* from most urban breeding sites.

Conclusion

Information collected on chromosomic inversions and distribution of species of the *An. gambiae* complex is still partial. Even so, it already constitutes a basis for any future study on malaria and bancroftian filariasis epidemiology in Kenya. Data is available on the survival rate of two species of this complex. Future research will extend the same type of study to a third species, *An. arabiensis* and to *Anopheles funestus*.

Observations have shown that domestic detergents are an obstacle to the multiplication of *C. cinereus*. In urban environments where most of the breeding sites contain detergent, it is unlikely that *C. cinereus* can compete to reduce numbers of *C. quinquefasciatus*. In rural areas, however, it is recommended to avoid disposing of detergent water in mosquito breeding sites.

Leishmaniasis

Epidemiological investigations (animal reservoirs)

Studies to find out the breeding places of anthropophilic sandflies continued mainly in Machakos and Baringo districts. These studies involved the investigation of termite hills which are one of the natural resting sites of the known vectors of kala-azar. Plastic and earthen cylindrical vials containing fine wet soil were suspended at various depths in ventilation shafts of termite hills. They were lowered into the shafts in the evening and checked for eggs the next morning. The excavation of termite mounds also continued. Soil was collected at the floor of ventilation shafts and was incubated to see if there would be emergence of preimaginal forms of phlebotomine sandflies. The results of these experiments have been fruitless so far. When the vials hanging in ventilation shafts were coated with castor oil, gravid flies were trapped in them. This indicates that the flies were probably investigating the suitability of these containers and probably found them unsuitable.

Vector studies in Machakos

Monitoring of vectors in this previously epidemic focus continued. Phlebotomine sandflies diminished in numbers, particularly those which bite man. *P. martini*, and *S. garnhami* have almost disappeared from houses and termite hills, the main resting sites.

A new *sergentomyia* species has been en-

Figure 1. Sticky sandfly trap

countered in Kenya, appearing first on castor oil traps inside houses and around termite hills (about 5 specimens). Human-baited experiments at the termite hills sites demonstrated that this species bites man (April — June 1982). Larger numbers have since been picked up from the sticky traps in houses, termite hills and animal burrows. The new species was reared to its second generation in the laboratory and its biology and taxonomy studied. The numbers have dwindled since August 1982 and subsequent human baits have not yielded new catches. Because of the possible resurgence of the epidemic, monitoring of the vectors continues in this focus through sticky sandfly trapping to see if the man-biting sandflies will reoccur and what will influence their re-establishment in the area. Furthermore, this would indicate if their re-establishment coincides with the outbreak of kala-azar.

Vector studies in Marigat

Marigat, in Baringo district, is an old active focus of visceral leishmaniasis. It is a priority area as far as the Kenya Government is concerned because the disease appears to be concentrated mainly within the irrigation scheme. Recently a new form of the disease *(Leishmania major)* has been isolated in rodents and could become a threat to the scheme. ICIPE was therefore called upon to collaborate with the Kenya Government ministries concerned to look into ways of managing the disease problem.

Sandfly trapping

Sandflies were trapped using sticky sandfly traps inside houses, around termite hills, inside animal burrows, in thickets and in the open areas. Sandflies were dissected for leishmanial parasite isolation. The overall sandfly fauna was assessed.

During the year, 15 species of sandflies were encountered in Marigat. They included *S. ingrami, S. antennatus, S. bedfordi, P. martini, S. adleri, S. africanus, S. clydei, S. multidens, S. harveyi, S. affinis, S. rossanae, P. duboscqi, S. schwetzi, P. orientalis* and *S. graingeri.* Of these, the known anthropophilic vectors of human leishmaniasis are *P. martini, P. duboscqi* and *P. orientalis. Phlebotomus martini* were caught mainly inside and outside house walls and around termite hills. A few *P. martini* were caught inside rodent burrows.

One female specimen of *P. orientalis* was caught on the outside of a house wall. *Phlebotomus dubosqi* were caught inside rodent burrows where they were the most predominant species of the anthropophilic sandflies.

The various anthropophilic species of sandflies in Marigat appear to be seasonal, although small numbers seem to persit throughout the year. This phenomenon seems to be true even for the subterranean species living inside the animal burrows. Populations steadily increase a few weeks after the onset of rains.

Dissection of sandflies from rodent burrows

Gravid sandflies were dissected and the guts examined for promastigotes. From the rodent burrows a total of 357 sandflies were dissected, out of which 7 flies had promastigotes in the anterior position of the gut. The order of prevalence of phlebotomine sandflies inside animal burrows during the year were: *S. antennatus* (31%), *S. ingrami* (20%), *P. duboscqi* (17%), *S. bedfordi* (16%), *S. africanus* (13%), and *P. martini* (6%). Dissections of gravid female sandflies identified promastigotes in three of the six species. The overall infection rate was 2%. *Phlebotomus duboscqi* had the highest rate of infection (5%) while *S. antennatus* and *S. ingrami* had 4% and 1% respectively.

P. duboscqi has been shown to be a vector of *Leishmania major* elsewhere in Africa. This is a serious cutaneous leishmaniasis disease which has been isolated from rodents in the Baringo area. Our discovery of coexistence of *P. duboscqi* with infected rodents — and consequent isolation of *Leishmania* from *P. duboscqi* — confirms the possible role of this species in the disease transmission among the animals. The identity of the isolates from *S. antennatus* and *S. ingrami* is pending.

In our opinion these findings of leishmanial infection in *P. duboscqi* are very significant. Our studies on the behaviour of this fly in Baringo district indicate their populations are presently limited to the rodent burrows. Female flies do not come out of the burrows and very few males were captured outside the burrows. It appears that the availability of food (rodents) for *P. duboscqi* may explain why *Leishmania major* is not presently a common disease in man in this region. However, if the rodent population is interferred with by the settlers of the irrigation scheme — a distinct possibility as more people settle there — the disease could become epidemic. *P. duboscqi* has already been found to inhabit houses in West Africa where it is the vector of *Leishmania major.*

Dissection of sandflies from houses

A total of 11 species of parous phlebotomine sandflies were captured in houses at Marigat. They included *P. martini, S. ingrami, S. antennatus, S. bedfordi, S. adleri, S. africanus, S. waynnae, S. clydei, S. rosannae, S. schwetzi* and *S. affinis.* The overall rate of infection for all the sandfly species dissected was 1%, with a range of from 1% to 5%. Promastigotes in the gut were encountered in *S. ingrami, S. adleri* and *S. waynnae.*

Dissection of sandflies from around termite hills

Since the known vectors of visceral leishmaniasis in Kenya inhabit termite hill ventilation shafts, dissections were done on all parous females captured in sticky traps set around termite hills during the year (Fig. 1). A total of 932 parous females were dissected and comprised 12 species of sandflies. These were the same species encountered in houses except *P. duboscqi* was additionally found in this environment. The overall infection rate was 1% with a range of from 1% to 5%. The species encountered harbouring promastigotes in the gut were *S. ingrami* and *S. africanus.*

Laboratory investigations of vector-parasite relationship

The objective was to investigate the vectorial capacity of the laboratory-bred sandflies on human leishmanial strains. *Sergentomyia adleri* were artificially fed (through 1 to 2-day-old chick membrane) on rabbit blood infected with *L. donovani.* These preliminary investigations have shown an anterior gut infection rate of 25% in *S. adleri.* This development is a new tool for investigations for vectors of human leishmaniases in the East African region.

Laboratory colony for sandflies

In order to experimentally provide vectorial capacity of vectors of leishmaniases incriminated in the field, it is essential to raise a laboratory colony. Sandflies, however, are

very small insects and very difficult to breed. The programme has now managed to rear 9 species in the laboratory. It is set up to artificially feed these flies using a water-bath heating system and 1 to 2 day-old chick membrane. It takes from 36 to 41 days to produce a generation from egg to adult. The larval diet is still under experimentation. The colony in ICIPE now has the following:

S. schwetzi	— 7th generation
S. ingrami	— 4th generation
S.. adleri	— 6th generation
S. bedfordi	— 5th generation
S. antennatus	— 5th generation
P. martini	— 2nd generation

Taxonomic studies of vectors of leishmaniasis

A problem in identifying the adult phlebotomine vectors of leishmaniases is their morphological similarity between females, although males may be distinct. After a careful re-examination of the cutaneous leishmaniasis vectors from Mt. Elgon, it appears that these species may form a complex of species. Differentiating them by the shape of the aedeagus alone may be inadequate. Similarly, the vectors of visceral leishmaniasis in Kitui district are a complex of three species.

Isoenzymes electrophoresis work has been initiated on the Mr. Elgon vector complex and preliminary results are quite promising. The heads of both sexes and terminalia of the males are preserved for morphological examination while the thorax and abdomen are homogenized in deionized water. These are then applied to prepared gel plates and electrophoresed. About 20 enzyme systems for each species are analyzed. The mouth parts of both sexes and the ovipositor of the females are being prepared for light and electron microscopy studies to determine morphological differences.

Animal reservoir studies

Studies of animal reservoirs in Kitui have been in collaboration with the Division of Communicable Diseases and Research in Kitui district. This effort has resulted in the isolation of leishmanial parasites from mongoose and a genette cat. It is the first time leishmanial parasites have been isolated from wild mammalian hosts and their identity is under study.

Examination of dogs revealed parasites in tissues obtained from popliteal glands of 3 dogs in Tseikuru focus, Kitui district, and 3 from Kibauni focus of Machakos district. Animals examined from Machakos included: 194 dogs, 208 lizards, 9 mongoose, 5 gennette cats, 1 hyrax and 13 bush baby. Other parasites seen were: Trypanosomes and *Microfilaria spp.* from several lizards.

From Masinga focus, 2 dogs, 12 gennette cats, and 2 mongooses were examined. No parasites were seen.

From Kitui focus 6 gennette cats, 3 mongooses, 3 squirrels and 52 lizards were examined. Leishmanial parasites were observed in one mongoose and one gennette cat. From Marigat focus, Baringo district, 4 mongooses, 21 rats and 3 lizards were examined (tissues). Leishmanial parasites were observed in splenic smear of one rat *(Avicanthis* spp.).

Experimental infection of dogs with human *L. donovani*

Leishmania donovani has been previously isolated from domestic dog in the Kala-azar endemic areas of Kenya. As it is the causative organism of human visceral leishmaniasis, it is imperative to determine the course of the infection. Studies in experimental infection were initiated by the innoculation of eight domestic dog pups with *L. donovani* from an infected human. Four of these dogs (which died during the course of investigations) did not harbour parasites in spleen, liver and blood. The others are still under investigation.

Uptake of promastigotes of different leishmanial isolates by mice and hamster mononuclear phagocytes

Animals were injected intraperitoneally with *in vitro* raised parasites. Peritoneal exudate cells, peritoneal fluid and peritoneal blood were examined at specific time intervals for parasites. It was possible to show that lizard leishmanial promastigotes could infect BALB/C mice microphages and transform to amastigotes. Other isolates which infected the mice macrophages were human strain, and an isolate from a sandfly from Makueni. This type of peritoneal fluid examination may prove an additional diagnostic tool in the leishmanial isolates identification.

Insect Pathology and Pest Management Programme

This programme was established in April 1982. Its objective is to assist the ICIPE crops programmes by designing integrated pest management systems. The goal is improving food production and health in rural farming communities in the tropics by utilizing pathogens, parasites, predators and new cultural practices.

Initial emphasis has been on field work. On the vector side, an attempt has been made to map the distribution of virus-like particles in coastal tsetse fly populations and to identify these particles. A study has been carried out in the laboratory to learn how protective immunity against bacteria can be induced into tsetse flies. An earlier study on Coelomomyces indicus (a fungus and potential control agent for mosquitoes) was terminated in 1982 when ICIPE research on mosquitoes disbanded.

The main effort of the IPPM Programme has been on the crop pest side. Although the programme is still in the building stage, research is carried out at Mbita Point in close cooperation with the ICIPE crop pest programmes. During the later half of 1982 extensive regular surveys have been carried out in mono- and integrated-crops at the station and in farmers' fields in the area. In addition to disclosing possible pathogens and parasites which can be used for pest control, these surveys supply life table data. Work in 1982 has been directed toward two crops. sorghum and maize, and involving two stemborers: Chilo partellus and Busseola fusca.

Monitoring of Stemborer Populations

This study aimed at mapping the distribution of stemborers and their natural enemies in maize and sorghum crops. It also sought to determine the role of left-over maize stalks in farmers' fields as a source of infestation for the next crop.

In new sorghum crops (Serena) the first batches of *Chilo partellus* eggs were detected three days after germination. First instar larvae were collected six days after germination. Thus, first infestation of *Chilo partellus* may be expected within a week of germination (roughly 3- to 4- leaf seedling stage) in areas with a large endemic population and with weather conditions similar to those at Mbita Point.

Two hymenopterous parasites, *Pediobius furvus*, Gah. *(Eulophidae)* and *Dentichasmias busseolae*, Heinrich *(Inchneumonidae)* were collected from farmers' fields during the main crop season (April - July). *P. furvus* is a pupal endoparasite which, at Mbita Point, has produced up to 216 adults from one single pupa of *Busseola fusca*. *D. busseolae* is a solitary pupal endoparasite and was recovered from several *Chilo partellus* pupae and *Busseola fusca* pupae in the same field.

Earwigs *(Dermaptera)* were observed to feed on young *Chilo partellus* larvae when confined with larvae in petri dishes in the laboratory. It is not yet established whether they can prey on larvae within stem tunnels in the field.

So far, the locally occurring natural enemies have shown little impact on borer population build-up both at the Field Station and in farmers' fields. Infestations have been observed of up to six *Busseola fusca* larvae per sorghum plant in farmers' fields and up to five *Chilo partellus* larvae per maize plant at the station. In one field at Gingo more than 90 per cent of the sorghum plants were infested by *Busseola fusca* and *Chilo partellus*

larvae. However, if the simultaneous emergence of a large number of *P. furvus* adults from a single pupa turns out to be due to polyembryony, this opens scopes for mass rearing and possible use of the parasite as a control agent.

So far no pathogens have been observed to cause heavy mortality in the pest populations monitored. In addition to fungi and bacteria, what appears to be a virus has recently been found on *Chilo partellus*. This observation needs to be confirmed and the virus isolated before anything can be said about its potential as a control agent.

Busseola fusca and *Chilo partellus* larvae persisted after harvest in living stalks of sorghum for more than five months. Field-collected larvae of *Busseola fusca* have been maintained in fresh stems in the laboratory, actively feeding and moulting for more than six months, without pupating. *Chilo partellus* larvae maintained in the same way, however, pupate and emerge as adults. No hibernating *Busseola fusca* larvae have been recovered from dried up maize stems in the field. This indicates that *Busseola fusca* populations may carry over from one cropping season to the next in sorghum stems left in the field after harvest even in the absence of wild alternative hosts. It also indicates that growing sorghum and maize together may increase the pest pressure on the maize crop, an observation confirmed by intercropping trials carried out at Mbita Point.

The fact that *Chilo partellus* larvae (recovered from post-harvest sorghum stalks) pupate and emerge, coincides with results from pheromone trap catches that indicate *Chilo partellus* does not have any hibernating period similar to that of *Busseola fusca*. Alternative hosts and/or volunteer sorghum shoots seem to be necessary to support residual *Chilo partellus* populations between cropping seasons.

Virus-Like Particles in the Tsetse Fly, *Glossina pallidipes*

The microbial control potential of the virus of *Glossina pallidipes* is its ability to cause abnormalities to both male and female reproductive systems, and partial or complete sterility of infected hosts. Similar viruses are known to occur in two other tsetse species, *G. morsitans centralis* and *G. fuscipes*. The present study aimed at determining the distribution and nature of virus particles that are associated with the salivary glands of *Glossina pallidipes*. Field surveys have shown that enlargement of the salivary glands occurs in 2 to 15 per cent of the coastal populations of *G. pallidipes* but does not occur in the two other species occupying the same ecological environment, *G. brevipalpis* and *G. austeni*.

To isolate and purify the virus, tsetse flies were trapped in the field. Salivary glands of infected flies were removed by dissection. These were subjected to standard purification procedures and biochemical tests and the virus particles were examined in an electron microscope. An extremely sensitive method was found to be suitable for surveys in the field. This method, the enzyme-linked immunosorbent assay technique (ELISA) depends on the production of antibodies in a suitable host. An enzyme alkaline-phosphatase is linked to the gamma-globulin and this is used to test for the antigen in the unknown extract. This method was sensitive enough to show the presence of the purified virus down to 2 ng.

The tsetse virus was found to be rod shaped (size 533 by 4mμ to 1,011 by 4mμ) with DNA as the basic nucleic acid. It therefore resembles vaculoviruses although it is larger and does not occur within a protein sheath.

Induction of Protective Immunity

If pathogens are to be used as biological control agents it is important to know whether or not the target pests can develop defense mechanisms against them. The present study is an attempt to investigate if tsetse flies can develop immunity against bacteria and to determine the nature of this immunity.

In the 1980 ICIPE Annual Report the interaction between *Bacillus cereus* and tsetse hemocytes was presented and it was shown that the bacteria were cleared from the hemolymph through phagocytosis by plasmatocytes. Further experiments have now shown that injection of small doses (1×10^3) of live *Escherichia coli* into *Glossina morsitans morsitans* induces a protective immunity. When challenged with a higher lethal dose, the mortality rate is much lower for vaccinated than non-vaccinated flies.

In three separate replications, groups

of 56 newly emerged female *G. m. morsitans* were used. In each experiment, one group was injected with 1 x 10^3 live *E. coli* from a three-month-old nutrient broth culture. A second group was injected with saline to act as a control. Twenty-four hours later, each fly was challenged with 1 x 10^5 live *E. coli* from a one-day-old culture and mortality was recorded for 12 consective days. Pooled data from these experiments shows a highly significant difference in mortality rate between the control and the vaccinated groups. Six days post-challenge, only 10 per cent of the vaccinated flies had died compared to 40 per cent in the control. In the vaccinted group 12 days post-challenge overall mortality was still 10 per cent while mortality in the control had reached 50 per cent and was still increasing.

The nature of this immunity is now being investigated. We are trying to establish whether the immunity is cellular or humoral, specific or non-specific and to determine its maximum duration.

Influence of Copepod Density on Infection Levels

Certain species of cyclops serve as intermediate hosts of the fungal pathogen *Coelomomyces*. Earlier research has shown that control of mosquitoes by *Coelomomyces* must utilize cyclops as an intermediate host, so factors that affect cyclop survival and infection must be considered.

The present study was carried out to determine the relationship between density changes in cyclop species and *Coelomomyces* infection levels in mosquitoes. Along the Kenya coast *Anopheles gambiae* breeding sites contain five different species of cyclops. Of these, only *Microcyclops minutus* appears to serve as an intermediate host of *Coelomomyces indicus* which attacks the larve of *A. gambiae*. The occurence of *M. minutus* is not very consitent, however, and densities differ both over time within a pool and from one pool to another.

A semi-permanent and temporary set of pools were sampled weekly for 20 weeks. Cyclops were counted and the species identified, using the morphology of mature females. *Coelomomyces indicus* infection of mosquito larvae was also recorded.

Two species of cyclops predominated in the pools: *M. minutus* and *Mesocyclops leukarti*. In the semi-permenent pool, the densities of *M. minutus* were high immediately after the rains, while those of *M. leukarti* were low. *M. minutus* density then suddenly decreased while the numbers of *M. leukarti* increased. By the 20th week there were no cyclops in the water sampled. Infection in this pool occurred only at the beginning of the sampling period when *M. minutus* density was high. In the temporary pool there was no defined pattern except that *M. minutus* occurred most of the time while *M. leukarti* did not. Infection in this pool was present most of the time.

In our field study a definite relationship existed between cyclop densities (particularly *M. minutus)* and *Coelomomyces* infection. High copepod densities appear to favour *Coelomomyces* infection although in the laboratory it has been shown that too high a copepod density retards fungal development. In the field, where pathogens, predators, poor environmental conditions and poor nutrition are at work, copepod densities probably do not go as high as those artificially formed in the laboratory. Our highest field-recorded *M. minutus* density was 2 cyclops per 1 ml of water and this was not encountered frequently. In the field, as in the laboratory, there is probably a maximum level of density which will result in high infection rates.

The Crustacean copepod, *Microcyclops minutus:* An Intermediate Host of the Fungus *Coelomomyces indicus*

The main deterrent to the use of *Coelomomyces* as a biological control agent has been our inability to obtain consistent laboratory infection so that large quantities of the pathogen can be stockpiled for field releases. A significant aspect of the life cylce of *Coelomomyces* is that copepods are obligate intermediate hosts of the pathogen. In our laboratory we have now successfully demonstrated that the cyclopoid, *Microcylops minutus*, is the intermediate host of *Coelomyces indicus*. This has facilitated the transmission of *C. indicus* to larvae of *A. gambiae* in the laboratory. Thus we have managed to maintain a culture of *M. minutus* and *C. indicus* in *Anopheles gambiae* larvae.

Chemistry and Bioassay Research Unit

Chemistry and Biochemistry merged with the Bioassay Unit on January 1st and for the first time took on its own Special Project with International Atomic Energy Agency (I.A.E.A.). This is to investigate ligno-cellulose degradation by enzymes from microorganisms associated with termites. This meant creating a microbiology laboratory in the little space available and engaging another biochemist. Hence 1982 has been a crowded year in more sense than one, but the influx of funds meant that at least we could re-equip the unit with a new capillary Gas Cromatograph (GC), a modern High Performance Liquid Cromatograph (HPLC) and a data acquisition system for the Gas Cromatograph/Mass Spectrometer (GC/MS). The equipment has at last arrived and will be operational in 1983.

Synthetic chemistry, started last year, has really begun to make strides not only in formulation of further analogues of "pallidilure" (the tsetse sex-stimulant) but in the making of urgently needed precursors which would take too long to order from abroad. Starting materials were required for new routes of synthesizing potential antifeedants and anti-JH analogues. Isolation of new and known phytochemicals continued to be exciting because their screening in new and already established bioassays turned up hoped-for pesticidal properties. The collaborative venture with IRRI on brown planthopper control has led to the cotton stainer now being used routinely to test the efficacy of many of the natural products we have isolated from African plants. Also, our microbiologist has arranged a new battery of tests for bacteriocidal compounds that is already proving most useful.

The list of ten papers accepted for publication since the last report was written, attests to the good work that continues to come out of the revamped Chemistry and Bioassay Research Unit.

Fungal Enzymes

A novel screening method has been devised whereby presence of the enzymes capable of hydrolysing azure-cellulose can be established in cultures of fungi obtained from *Macrotermes michaelseni* and *Odontotermes montanus* mounds. The carbohydrase complex in the conidiophores of *Termitomyces* spp. collected from fungus combs of *M. michaelseni* has been purified using CM and DEAE-sepharose columns. Cellobiosidase and CMC-cellulase properties have been studied and compared with enzymes from other sources.

The arrival of the HPLC means that the enzymes in microorganisms capable of degrading ligno-cellulose can now be studied. Suitable model substrates, along with soluble and native grass lignin, have been prepared for what promises to be the central problem of the Special Project assignment — the rendering of agro-industrial biomass into fermentable, degradable components. Equipment and isotopically labelled substrates expected from I.A.E.A. will allow even more sophisticated enzyme studies to be carried out with bacteria already isolated from termite workers.

Insect Hormones and Pheromones

The discovery of ecdysteroids in the ovaries of pregnant tsetse nearing term (day 7 of the cycle) has stimulated research into the cause and effect of allatostasis in *G. m. morsitans*. Topical application of JH III reversed the effect, as expected, of feeding 20-hydroxyecdysone at the beginning of pregnancy. Professor Heinz Rembold of Max-Plank Institute (Munich) now assists us in determining JH titres during pregnancy so that we can explain the function of ecdysteroids released

into the haemolymph from the ovaries precisely when the uterine gland ceases to synthesize "milk". The identity and titre of ecdysteroids in the egg, embryo and larva of tsetse are also being determined.

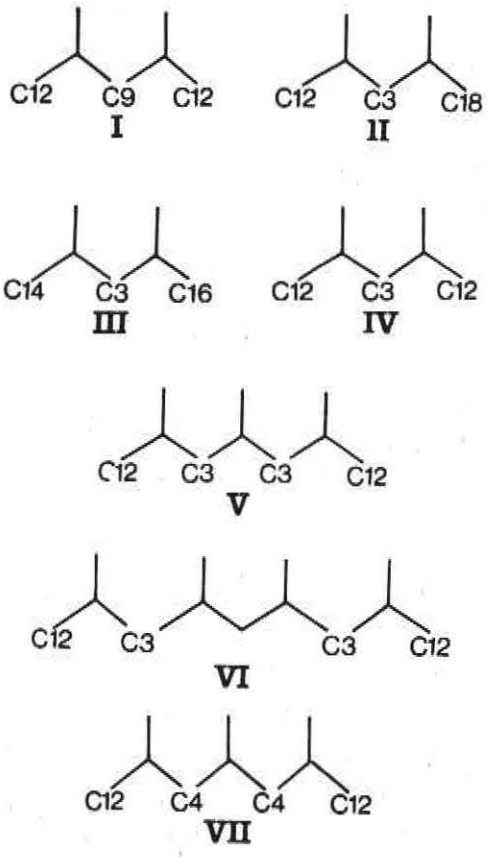
A new synthesis of grandisol, a component of the sex attractant of the Boll weevil *(Anthonomus grandis)*, was attempted with Professor Norin in Stockholm, but we cannot yet report how successful the method will be. In collaboration with Crop Borers and Sensory Physiology, isolation and testing of *Maruca testulalis* sex pheromone is in progress, while the oviposition deterrent of the sorghum shootfly continues to pose microchemical problems relating to activation of this semiochemical.

Last year (ICIPE Annual Report 1981) we reported synthesis of 3 isomers of "pallidilure", the tsetse sex-stimulant. Examination of new mass spectral data (obtained from the active fraction of female cuticle extract) allowed us to conclude that the predominant alkane was indeed 13, 23 — dimethylpentatriacontane (I) and not the 13, 17—(II) or 15, 19—isomers (III) as earlier supposed.

It is noteworthy that the sex-stimulant for *G. m. morsitans* ("morsilure") is also a symmetrically substituted alkane (trimethylheptatriacontane). Therefore it came as a surprise to find that the non-symmetrical isomer II (which has only one 13-methyl substituted alkyl group) is twice as active in laboratory bioassay as the natural pheromone I. Isomer II and the much-less-active III share a common feature with "morsilure" in having a 1, 5-dimethyl structural unit. In order to investigate the relative importance of symmetry, chain length and the presence of the 1, 5-dimethyl unit in conferring aphrodisiac properties to these hydrocarbons, we have undertaken synthesis of IV, V, VI and VII and bioassays are in progress. In addition, the stereoisomers of I and II are being synthesized by Professor Kenji Mori (our scientist in residence for 1983). Hopefully the results of these structure — activity studies will throw some light on the mode of action of the pheromone at the receptor site.

Allelochemicals

At long last collaboration with Bases of Plant Resistance Programme has begun in earnest. Volatile substances from cultivars of maize partially resistant to attack by *Chilo*



partellus are being analysed by GC prior to testing by Sensory Physiology. A sorghum variety has yielded a compound which is active in retarding growth of *C. partellus* when extracts and Thin Layer Chromatogram (TLC) elautes were added to an artificial diet developed at the Centre. We only need a harvest of the crop to confirm our tentative identification of the active substance.

Natural Products Chemistry

An oil with insecticidal properties against Brown Plant Hopper *(Niloparvata lugens)* was previously isolated from a plant by Dr. R. C. Saxena at International Rice Research Institute. In this laboratory the oil was prepared by steam distillation and then fractionated (vacuum distillation) into four fractions, of which two, containing sesquiterpenoids, were active in the bioassay.

Micropreparative separation of these components on u-porasil by HPLC provided sufficient material for bioassay and identification of the most active compound. Confirmation of the structure by FT-NMR and synthesis is underway. Ten to twelve analogues are also being synthesized for evaluation of their insecticidal properties.

Table 1: Antibacterial activity of three Kauranoid diterpenes from *Aspilia pluriseta* leaves

Bacteria	Gram Reaction	Activity (min. inhibitory concentration ug/disc.)		
		X	XI	XII
Escherichia coli	–ve	50–100	50–100	100–200
Bacillus mycoides	+ ve	>150	100–200	100–200
Macrococcus luteus	+ ve	75–100	>200	>200
Bacillus subtilis	+ ve	>300	>200	>100
Staphylococcus aureus	+ ve	>300	>200	>100
Xanthomonas pelargonii	–ve	75–100	>100	>100

Bioactive natural products

Milletia thonningii

A new isoflavone (VIII) has been identified in the seeds of this plant which also contained a known isoflavone (IX) not previously found in nature.

Their structures were determined by Mass Spectrometry and NMR spectroscopy before and after derivation (methylation and acetylation).

Aspilia pluriseta

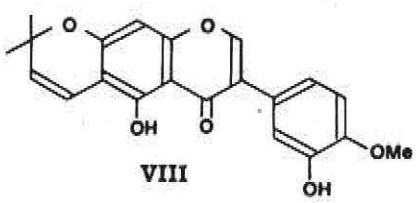
Aspilia pluriseta leaves are used medicinally as an antiseptic and an antifungal agent. Three kauranoid diterpenes have been isolated and identified as active against the bacteria shown in Table 1.

Teclea trichocarpa

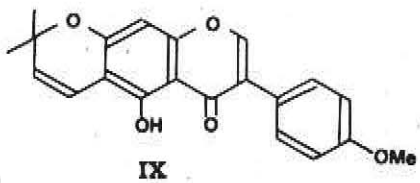
Three 9-acridone alkaloids (tecleanthine, its 6-methoxy derivative and Melicopicine) were isolated from bark. They showed activity against the fungus *Cladosporium cucumerinum* and the bacteria *Bacillus subtilis* (>200 µg).

Tovomita mangle

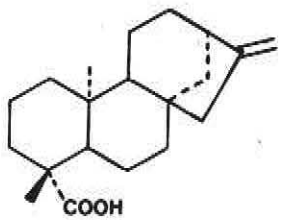
Two new benzophenones named tovophenone A and B have recently been isolated from the bark. Prior to testing their antimicrobiol activity, we have confirmed their structures by derivation and spectroscopy.



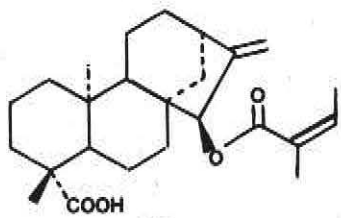
VIII



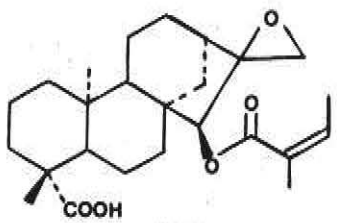
IX



X



XI



XII

Sensory Physiology Research Unit

The Senosry Physiology Research Unit provides a means for understanding the neuroal and sensory basis of pest behaviour. Investigations on target insects of ICIPE are undertaken in collaboration with the research programmes, using electrophysiological and behavioural methods.

At the moment attention is focused on acoustic and chemocommunication in tsetse and on chemocommunication in stem borers and ticks.

Tsetse

Acoustic communication

Sound is suspected of playing an important role in the behaviour of tsetse. Sounds produced during feeding behaviour of the tsetse *Glossina m. morsitans* Westw. were recorded and analysed.

Male and virgin female flies very seldom sang before feeding, but mated females sang in more than 20% of the cases before taking a meal. Sound production during engorgement was negligible among all three groups of flies. After feeding, sounds were commonly produced, mated females singing significantly more than virgin females and both of these groups more than males. The mean duration of the postfeeding sounds did not differ between the sexes and did not change with age.

Oscillograms of the songs indicated that they differed between the sexes. The songs contained frequencies up to 50 kHz with the exception of the male prefeeding songs, which did not contain any ultrasonic components. The most dominant frequency was between 1.5 and 2.5 kHz. Peaks were also observed between 0.5 and 0.8 kHz and around 5 kHz and 9 kHz. Prefeeding songs were of a weaker intensity than postfeeding songs.

Sounds associated with feeding behaviour may play a role in attracting hungry flies to suitable hosts. The low frequency components, having higher intensities (30—40 dB) than the ultrasonic ones (10 dB), most likely carry the most important part of the acoustic information. Studies are also in progress to investigate the songs produced during mating and larviposition. The behavioural responses of tsetse flies to these songs are being investigated.

Chemocommunication

Studies are in progress to investigate the suggestion that the female contact sex pheromones in Glossina are perceived by receptors on the legs of males. In collaboration with the Chemistry and Bioassay Research Unit a method was found to dissolve the highly non-polar pheromones in electrolytic solutions, so that electrophysiological recordings could be made from taste hairs on stimulation with the pheromone. So far, no pheromone receptors were found on the legs. However, very sensitive mechanoreceptors and thermoreceptors were found.

Investigations on the sensitivity of the antennal olfactory receptors to various chemicals are also in progress.

Stem Borers

Studies are in progress to determine the stimuli which induce oviposition and feeding behaviour. These will investigate the sensitivity of the olfactory receptors on antennae and the taste receptors on the mouthparts, legs and ovipositor of stem borer moths and larvae.

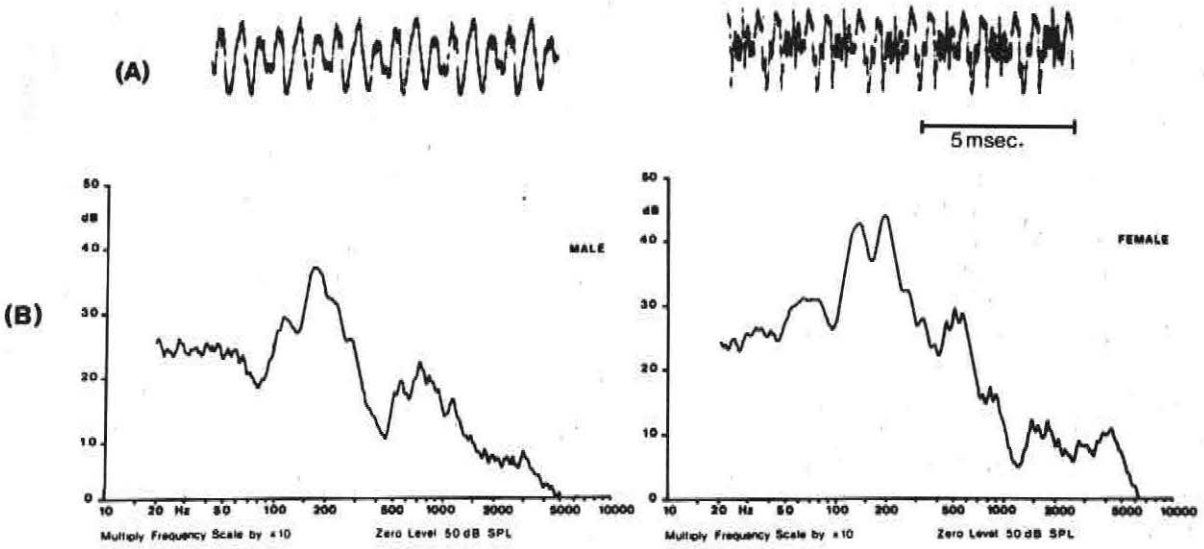
Various plant volatiles, including geraniol,

linalool and citronellol, induce olfactory responses from the electroantennogram recordings. In addition, it was shown that among the mass of several hundred hairs at the distal end of the ovipositor of *Chilo partellus*, four of them are sensitive to chemical and mechanical stimulation (bending). In contrast, the ovipositor of *Eldana saccharina* is equipped with several of these hairs. Chemomechanosensitive hairs have also been found on the tarsi of the moths. Various organic substances are now being tested to determine the chemical stimuli influencing oviposition behaviour.

Stimulation of the taste sensilla of larvae of *C. partellus*, *E. saccharina* and *Maruca testulalis* with various plant substances have revealed that in the maxillary sensilla styloconica of these species, water and sucrose sensitive cells are present. In *C. partellus* larvae an additional chlorogenic acid-sensitive cell has been discovered. The *C. partellus* and *E. saccharina* sugar cells are more sensitive to sucrose than those of *M. testulalis*. These studies are now being extended using various other plant substances. In addition, other important pest stem borer species *(Busseola fusca, Sesamia calamistis)* are being tested.

Ticks

Electrophysiological recordings from tarsal olfactory sensilla in the ixodid ticks *Rhipicephalus appendiculatus* and *Amblyomma variegatum* were made to determine their sensitivity to the phenolic compounds 2, 6—dichlorophenol, p-cresol, phenol and salicylaldehyde, reported to act as sex pheromones of various ixodid ticks. Response patterns of cells in sexually mature and immature males on stimulation with these substances appeared to be similar, but response levels were higher in sexually immature males. Hexane washings of adult and larval ticks contained very potent odorous stimuli, which produced responses similar to 2, 6—dichlorophenol. The chemical identity and behavioural significance of these stimuli are under investigation.



POSTFEEDING SONGS OF MATURE FLIES

(A) The wave forms as shown by oscillograms. (B) The frequency components of these songs.

Histology and Fine Structure Research Unit

The biology of insects varies from species to species. As a prerequisite to the study of the biology of target insect pests, therefore, it is important to understand fully the structure and function of the vital organs of each insect in question. The reproductive process and development in general are under the influence of the neuroendocrine system. Consequently, structural and functional studies of this system lead to a better understanding of the physiology and behaviour of these insects. Some of the target insects are vectors of important human and animal diseases. Structural and functional studies of the salivary glands and digestive systems of these vectors can tell us a great deal about parasite-vector relationships, in general, and the biology of the parasite within the vector, in particular. Finally, in their quest for food, mate and shelter, insects are constantly interacting with their immediate environment. The structural identification of the sensory receptors that enable insects to perceive various environmental stimuli, whether chemical or physical, provides valuable information concerning their biology and behaviour.

Studies on Neuro-Endocrine Systems of Target Insects

The corpus cardiacum (CC) has long been known as the major nuerohaemal organ from which the material from the nervus corporis cardiacum (NCC) is released into the haemolymph. In *Glossina*, do other neurohaemal organs exist? What is the relation of the time of release from the axonal endings to the cyclic nature of tsetse reproduction? In insects the corpus allatum is known to produce the juvenile hormone (JH), important for the regulation of development in juvenile stages. In the adult female the same hormone plays a major role in regulating reproduction (egg development). It was, therefore, interesting to examine the ultrastructural changes that may take place in the corpus allatum during the unique pregnancy cycles in *Glossina*.

Wall of the aorta

Upon leaving the brain, the NCC nerve comes to lie on the ventro-lateral walls of the anterior aorta. Axons branch off from the NCC nerve and migrate into the area between the two stromal sheaths of the aorta. The aorta wall, very thin before the entry of axons, now enlarges, and the swollen axonal endings become the main feature of the aortal wall.

These swollen axonal endings, fully loaded with neurosecretory material (NSM), are observed on the wall of the aorta throughout the life of the insect. There are no particular peak periods of release of neurosecretory material from the axonal endings.

Corpus allatum

Further posteriorly, several axons branch off from the NCC nerve and penetrate into the unpaired corpus allatum (CA). Axonal endings loaded with NSM are observed both in the intercellular spaces and within the cytoplasm of individual CA cells.

The main features of CA cells are an abundance of polyribosomes and free ribosomes, numerous mitochondria and microtubules which have a varied orientation within the cytoplasm. The nucleus is round or oval in shape with large nucleopores and dispersed chromatin. Stacks of membranous structures resembling smooth endoplasmic reticulum occur as a unique feature in the cytoplasm.

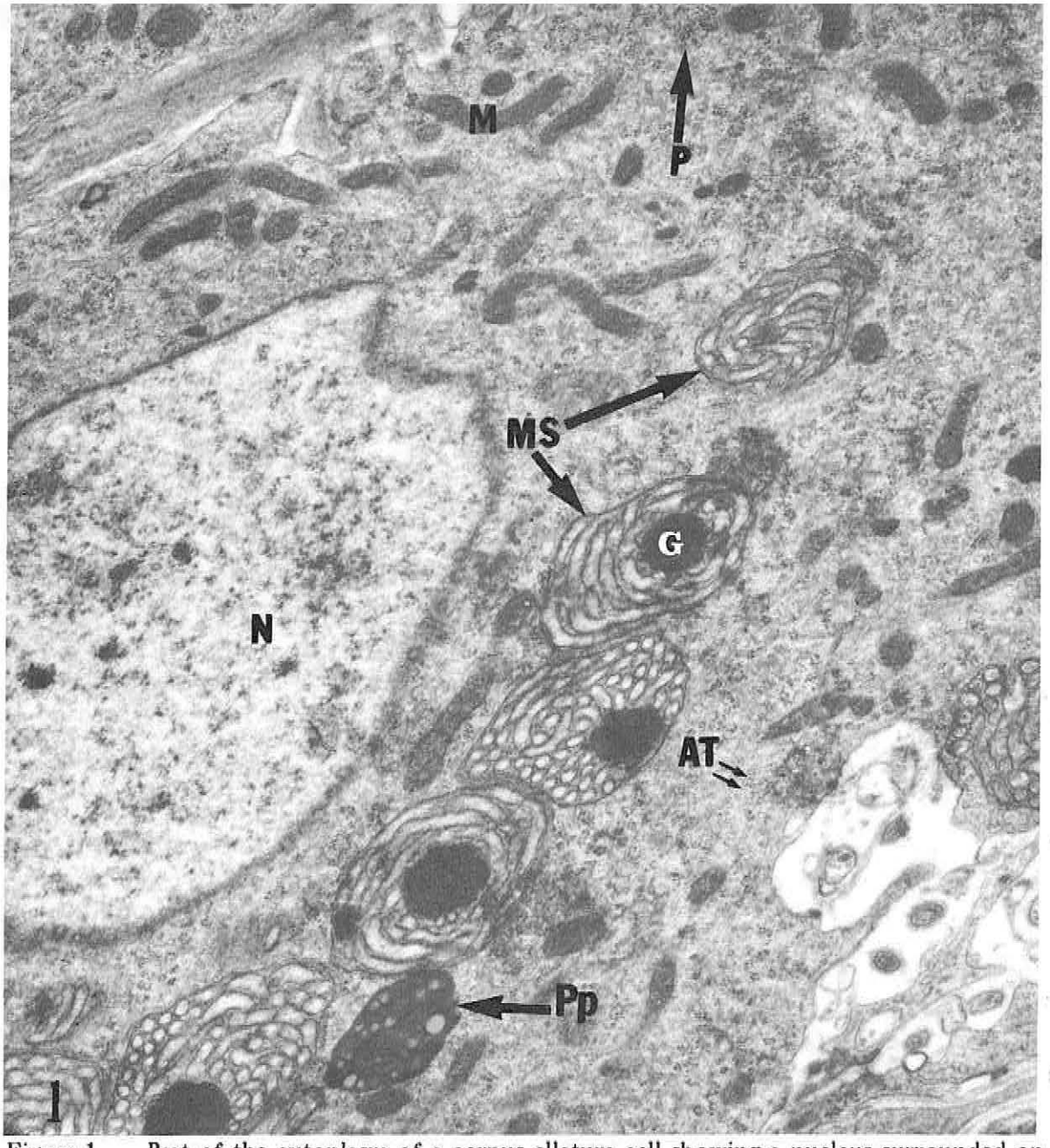


Figure 1. Part of the cytoplasm of a corpus allatum cell showing a nucleus surrounded on one side by a group of membranous structures. The rest of the cytoplasm is rich in mitochondria, polysomes and granular structures which are probably involved in the formation of the ergastoplasmic membrane system (endoplasmic reticulum). N, nucleus; MS, membranous structures; P, polysomes; M, mitochondria; AT, agranular tubules, Pp, polyphagosome; G, granule.

In the initial stages of their formation, each stack occurs individually and has a dark granule in the centre. Often several stacks align themselves around the nucleus (Figure 1).

There is evidence that in the CA cell cytoplasm of mated pregnant flies the stacks merge into each other to form a mosaic system of membranes. In virgin flies the stacks are either isolated in the cytoplasm or group together, but do not seem to merge.

Search for and Identification of Sensory Receptors

Moths of stemborers like *Chilo partellus* and *Eldana saccharina* live for 3-6 days. Their behaviour during that period plays a crucial role in their reproduction and in subsequent crop-damage caused by their offspring. Since olfactory and gustatory stimuli are important factors influencing moth behaviour, it is

appropriate to identify the various types of moth sensilla. In addition to morphological studies, electrophysiological tests are carried out to identify the stimuli detectable by the moth's various sensory receptors. This information enables us to identify the media through which the moths perceive and interact with their environment.

Preliminary scanning electron microscope (SEM) results indicate that there is more than one type of sensilla on the antenna of *C. partellus*. Those identified include thermo, gustatory, olfactory and mechano-receptors. Transmission electron microscope (TEM) studies are in progress.

Salivary Glands and Digestive System

The salivary glands of tsetse flies provide a suitable environment for the development of infective forms of *Trypanosoma brucei*. It has been observed that infected glands tend to be larger than normal ones. To find out whether *Trypanosoma* in tsetse affects longevity of the tsetse vector, research is underway in the ultrastructural nature of the secretory cells of trypanosme-infected salivary glands of *G. morsitans*, and the histochemistry of the secretions in the glands.

Ultrastructurally, infected glands contain trypanosomes in the epithelium, with flagella extending into the basal plasma-membrane. The lumen of such glands fill up with trypanosomes and these give positive reactions with some histochemical reagents. Normal glands have a relatively small epithelium surrounding the lumen. The homogeneous secretion in the lumen of uninfected glands gives similarly positive results.

One commonly used method of confirming virus-like particle infection in tsetse is by dissection. Electron microscopy grid serology is used for the detection of tsetse virus-like particles in saliva and extracts of salivary glands of *G. austeni*, *G. brevipalpis* and *G. pallidipes*.

The virus was purified and an antiserum prepared in rabbits. The antiserum was used to develop the enzyme-linked immunosorbent assay (ELISA) for virus detection. The results showed the method to be sensitive, but a number of false-positive results were recorded. To clarify the unexpected results, transmission electron microscope (TEM) studies were employed to check for presence of virus-like particles in the ELISA positive tsetse extracts. There was a specific response following negative-staining of virus suspension after incubation of virus suspension with specific antiserum.

Reproductive Organs of Target Insects

In *Glossina morsitans*, the reproductive system of the male produces and delivers spermatozoa to the female to fertilize the eggs. The spermatozoa leave the tsetse via the vasa deferentia. Associated with the vasa deferentia is a pair of accessory reproductive glands which lead into the ejaculatory duct and aedeagus. The male accessory gland material and sperm are carried across the ejaculatory duct during mating. Studies continue to determine the functional significance of the ejaculatory duct in the physiology of reproduction of *G. morsitans*.

The morphology appears to be intricate in the anterior ejaculatory duct, where the paired vasa deferentia and accessory glands merge. The epithelium is made up of a single layer of cuboidal cells. In electron micrographs, the apical plasma-membrane of the cells have deep infoldings with close mitochondrial association. The basal plasma-membrane is infolded, and such infolds penetrate into the cytoplasm forming tubular channels and densely lined sacs. These are comparable to pinocytotic vesicles, described in a number of insect tissues. The surface of the epithelium facing the lumen is covered with a cuticular intima, probably representing the epicuticle. The apical surface commonly encloses spaces of variable dimensions (Figure 2).

The lateral boundaries of the cells follow a tortuous course, and are closely apposed only over small portions of it. Septate desmosomes are formed, closing off intercellular spaces of variable width. The cytoplasm of the ejaculatory cells contain ribosomal clusters, scattered profiles of rough endoplasmic reticulum, Golgi, mitochondria and microtubules.

The fine structural studies of the ejaculatory duct of *G. morsitans* reported here suggest that this organ may be involved in active transport of ions and water. Physiological studies on the ejaculatory duct are underway to confirm this suggestion.

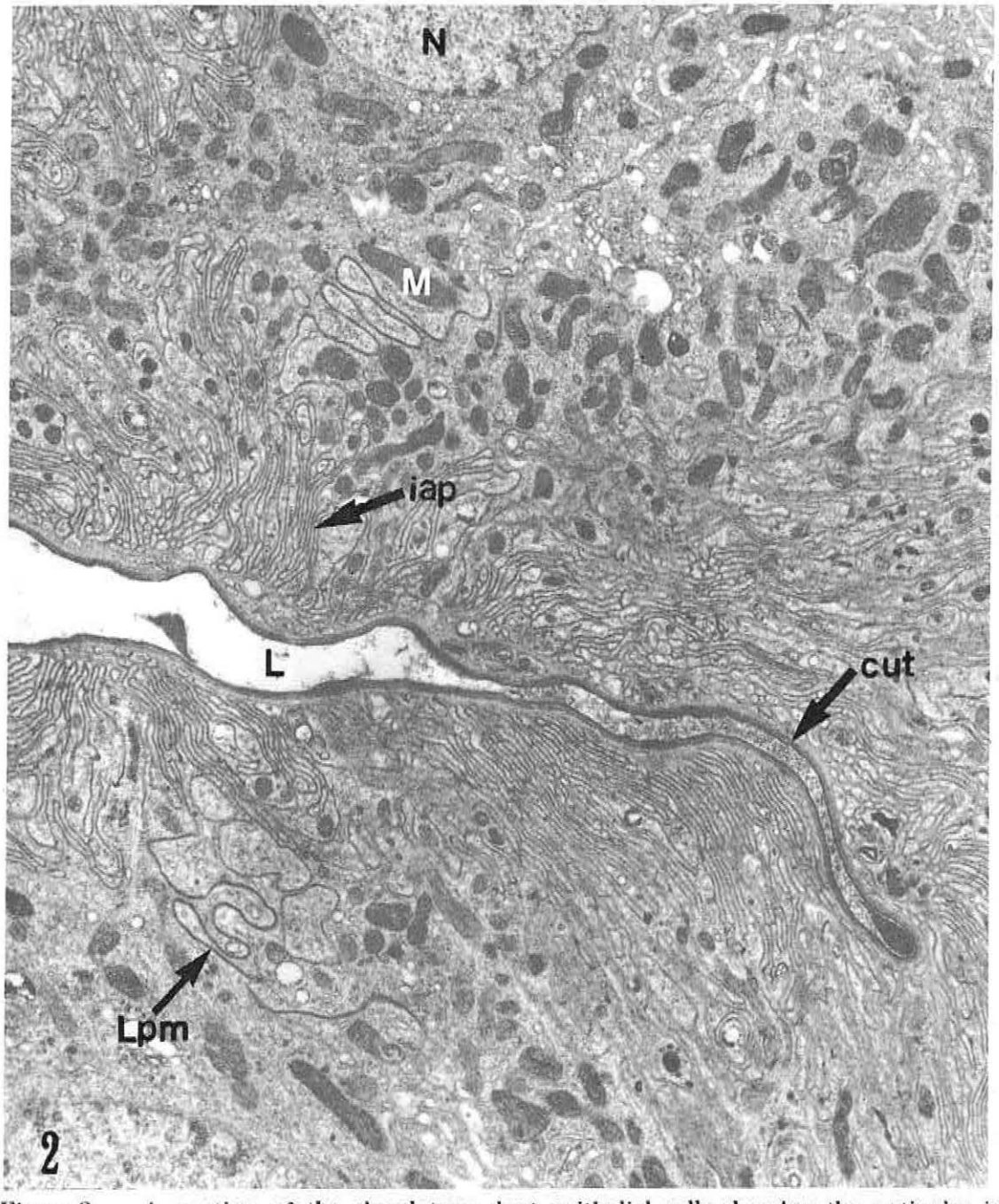


Figure 2. A portion of the ejaculatory duct epithelial cells showing the cuticular intima (cut) surrounding a lumen (L) with secretions. Infoldings of the apical plasma-membrane (iap) are closely associated with mitochondria (M). Lpm, lateral plasma membrane; N, nucleus.

Insect and Animal Breeding Services

The Insect and Animal Breeding Unit aims to centralize and streamline the breeding and supply of the target insects and laboratory animals required at the ICIPE.

Glossina morsitans morsitans

During 1981 a total of 29,201 ♂♂, 30,035 ♀♀ and 5492 pupae were supplied for experimental use, an increase of more than 100% on the previous year. This abundant situation continued until late January. A new diet had been introduced for the host rabbits, one which proved to be toxic to tsetse flies.

The net result was the loss of 2/3 of the colony in 12 days and very high mortalities in teneral and emerging flies. The colony never recovered and by July it was necessary to obtain pupae from outside the ICIPE to re-establish *G. m. morsitans.*

The colony is building up rapidly and at the time of writing stands at 3000 mated ♀♀. The Unit started supplying tsetse for expertimental use in October and intends to meet all the demands of the ICIPE scientists early in 1983.

Chilo partellus

The Chilo colony was reared on a small scale in 1981 (about 1500 larvae of all stages at any one time) with plans to transfer it to Mbita Point Field Station when facilities for mass rearing become available there. Since these facilities are not yet complete the colony has been expanded at ICIPE. It can now produce up to 5000 late instar larvae when the demand arises.

Spodoptera exempta

In 1981 the armyworm programme drew to a close and the demand for insects declined. The colony was moved in March 1982 from cramped conditions to a larger insectary and is housed along with other insects used for bioassy work. It continues to perform well on a natural maize leaf diet. The colony produces an excess of about 400 insects per month.

Insects for bioassay

The Bioassay insects were incorporated into the Insect and Animal Breeding Unit in March 1982. These insects are bred specifically to the requirements of the Chemistry and Bioassay Research Unit and are left at low population levels until required for experiments, when numbers are increased. All these insects are reared on artificial diets.

Rabbits

Rabbits are supplied to the Tsetse and Tick Programmes as arthropod hosts; to the Tsetse Programme for the maintenance of pathogens and to the Medical Vectors Programme as a source of blood for culture media.

At the start of 1982 the colony was able to meet the requirements of the tsetse colony, the ICIPE scientists at Chiromo and for the first time ICIPE scientists at Muguga received a total of 90 — 100 rabbits per month.

Supplies of rabbits for use as hosts for tsetse had to be halted in February 1982 after the unknowing use of rabbit diet contaminated with a toxin. However, no effect from the toxin was observed on the rabbits kept on the toxic diet for 15 days.

Table 1. Primary users of insects and mammals reared by the Insect and Animal Breeding Unit	CROP BORERS RESEARCH PROGRAMME	LIVESTOCK TICKS RESEARCH PROGRAMME	MEDICAL VECTORS RESEARCH PROGRAMME	PROGRAMME ON BASES OF PLANT RESISTANCE TO INSECTS	TSETSE RESEARCH PROGRAMME	CHEMISTRY AND BIOASSAY UNIT	HISTOLOGY AND FINE STRUCTURE UNIT	SENSORY PHYSIOLOGY UNIT	OTHER (outside research universities etc)
TSETSE *Glossina morsitans morsitans*					X	X	X	X	X
STEM BORER *Chilo partellus*	X			X				X	
WAX MOTH *Galleria Mellonella*						X			
MEAL WORM BEETLE *Tenebrio molitor*						X			
HOUSEFLY *Musca domestica*						X			
MOSQUITO *Aedes aegypti*						X			
COTTON STAINER *Dysdercus fasciatus*						X			
AFRICAN GRASSHOPPER *Gastromargus africanus*						X		X	
ARMYWORM *Spodoptera exempta*						X			
RABBITS		X	X		X				
RATS			X		X				
MICE			X		X				
HAMSTERS			X						

Their appearance and behaviour was normal and no effect on reproduction was observed, either while they were on the diet or since ceasing to use it. In rabbits kept on the toxic diet for a prolonged period, no adverse effects were observed until 3½ months, when the first mortalities were observed. Death was preceded by agitated movement and muscular spasms, but the cause of death was not determined. Some rabbits were kept on the toxic diet for 6 months without showing any abnormalities.

Over the years the original rabbit colony had been mixed with local rabbits of uncertain origin. The history of the colony could not, therefore, be defined. With a view to replacing the existing colony, 40 does and 2 bucks of New Zealand white rabbits have been obtained from a reputable supplier.

Rodents

The mating and rearing of rodents does not present a problem to the Unit. The only limiting factor to greater production of rodents is space. Since early in 1980 the Unit has been geared to produce in excess of 75 rats and 100 mice per month although the demand fluctuates considerably.

One innovation to rodent - rearing was the introduction of hamsters early in 1982. The colony is established and hamsters are supplied, again, according to the fluctuating demand.

Library/Documentation Services

The increase in library stock was modest in 1982, due largely to the ever rising cost of publications. There were 120 new book accessions, 108 of which were purchased and the rest received as gifts.

No significant changes occurred in periodical subscriptions apart from higher charges. Assistance from several organizations, notably the Swiss Academy of Sciences, reduced the hardships. It is still not possible to obtain all required journal literature locally. As is the case with many research institutions in developing countries, ICIPE has to buy photocopies of many relevant periodical articles from abroad especially from the British Library. This year about 200 such monographs were procured.

Publications

The ICIPE publications list for the year is by no means exhaustive. Supplements are recorded as information on new publications is received. Note the Supplement to the 1981 Publications List included in this year's report. Requests for these supplements should be addressed to the Library/Documentation Services, ICIPE, P. O. Box 30772, Nairobi, Kenya.

By far the most significant publication of the year was the long-awaited ICIPE sponsored monograph: Physiology of Ticks; ed. F. D. Obenchain and R. Galun. The book can be purchased through book agents or from the Publishers: Pergamon Press, Headington Hill-Hall, Oxford OX3 OBW, England. It is the first of a new Pergamon series: "Current Themes in Tropical Science" which is edited by Prof. T. R. Odhiambo. Insect Science and its Application, the ICIPE sponsored quarterly journal, moved into its third year. The quarterly newsletter, Dudu continues to provide information about ongoing activities of the ICIPE.

Mbita Point Field Station Library

Mr. Eric Ndegwa took up his post as Librarian for Mbita Point Field Station in May, 1982. Previously he worked with the Kenya National Library Service. Undaunted by the teething problems of Mbita, Mr. Ndegwa has launched a library service at the station, operating from a classroom at the International School. It is hoped that he will have a proper library when the new administrative block is completed. The stock is growing slowly but steadily. A British Council book donation forms a major part of the collection.

Cooperation with other institutions

ICIPE continued to cooperate with many libraries around Nairobi for mutual benefit. The libraries most commonly interfacing with ICIPE are those of the University of Nairobi and the Kenya Agricultural Research Institute (KARI). At Mbita, ICIPE is cooperating fruitfully with the Kenya National Library Service libraries at Kisumu and Kisii, and the British Council Library in Kisumu.

Address requests for the Supplement to the 1981 Publications list to:

Library/Documentation Services
I. C. I. P. E.
Post Office Box 30772
Nairobi, Kenya

Major Seminars

Dr. J. B. Kaddu ICIPE, Kenya	—	Vector parasite relationships of leishmania and Kenyan phlebotomine sandflies.
Dr. C. A. Mcfoy ICIPE, Kenya	—	Induced inhibitors from plants and their multiple role in the defence mechanisms against plant pathogens and insect pests
Dr. A. Alghali ICIPE, Kenya	—	Some studies on the relative susceptibility of ten rice varieties to *Diopsis thorecica* West
Dr. J. O. Olobo ICIPE, Kenya	—	Evaluation of different methods for identifying leishmanial isolates
Mr. S. Okech ICIPE, Kenya	—	Mechanisms of resistance to brown planthopper, *Nilaparvata lugens* in rice
Professor M. F. Claridge University College, United Kingdom	— —	Brown planthoppers: a variable pest on rice Acoustic signals and biological species problems in Homoptera
Dr. D. A. Otieno ICIPE, Kenya	—	A new synthetic approach to Grandisol, the Boll Weevil sex attractant
Mr. J. E. Okiri ICIPE, Kenya	—	Does your job motivate you?
Dr. C. C. Payne Glass House Crops Research Institute, United Kingdom	—	The role of insect viruses as biological control agents
Dr. L. R. S. Awiti ICIPE, Kenya	—	Neuroendocrine mechanism involved in pupal colour dimorphism in the Swallow-tail Butterfly *Papilio Xuthus* L.
Mr. Pieter Cuperus Groningen University, Netherlands	—	Sensory organs on the antannae of small ermine moths (Yponomeuta spp.) : A comparative electron microscopial study
Mr. T. K. Golder ICIPE, Kenya	—	Separation of subpopulations of bloodstream forms of *T. brucei* by cationic exchange chromatography
Dr. B. Amoako-Atta Ghana Atomic Energy Commission	—	Observations on the pest status of alcidodes leneogrammus (striped bean weevil) on cowpea under intercropping systems in Kenya
Dr. J. O. Ampofo ICIPE, Kenya	—	The role of certain mutant characters in plants on insect — plant relationship; The cotton plant as a model
Mr. S. M. Othieno ICIPE, Kenya	—	The biology and trapping of sorghum shootfly at ICRISAT
Dr. D. Denlinger Ohio State University, Ohio, U. S. A.	—	Diapause: the physiology of escaping time

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Training

In 1982 training at the ICIPE continued in the area of staff development, research training in pest management and training for practitioners. The ICIPE moved to optimize its resources in pest management research training aimed at generating a nucleus of high calibre research scientists in the developing world. The emphasis has been mainly on postdoctoral training as a means to tap and channel talented young researchers into the area of pest management and also on postgraduate research training. One major project in training was the African Regional Postgraduate Programme in Insect Science (ARPPIS). Considerable effort was devoted to its planning during the year.

African Regional Postgraduate Programme in Insect Science (ARPPIS)

Following the planning conference held at Bellagio in 1981, at which the communique establishing the ARPPIS was signed, ICIPE management devoted great efforts to preparations for launching the programme. Since ARPPIS is a collaborative programme of African universities, research institutions and the ICIPE, the most important task was to devise a mechanism for formalization of the relationship between the ICIPE, as the executing agency, and the other institutions.

Four Professors from the University of Ibadan led by Professor B. L. Fetuga, Dean of the Postgraduate School, signed a communique outlining the participation of their institutions in ARPPIS. Since then a number of other institutions and universities took steps to formalize their participation in the same way.

The Interim Committee, a caretaker group appointed at the Bellagio Conference, wound up their duties in August at their fourth meeting. They have now passed-on their task to the Academic Board which was formed and held its first meeting in August. The board held its second meeting at the ICIPE in December. The present membership of the board consists of the following:

Professor Thomas R. Odhiambo,
Director, ICIPE — Chairman

Professor Faysal Abushama
University of Khartoum

Professor J. R. Mainoya,
University of Dar es Salaam

Professor A. Lutalo-Bosa,
Makerere University

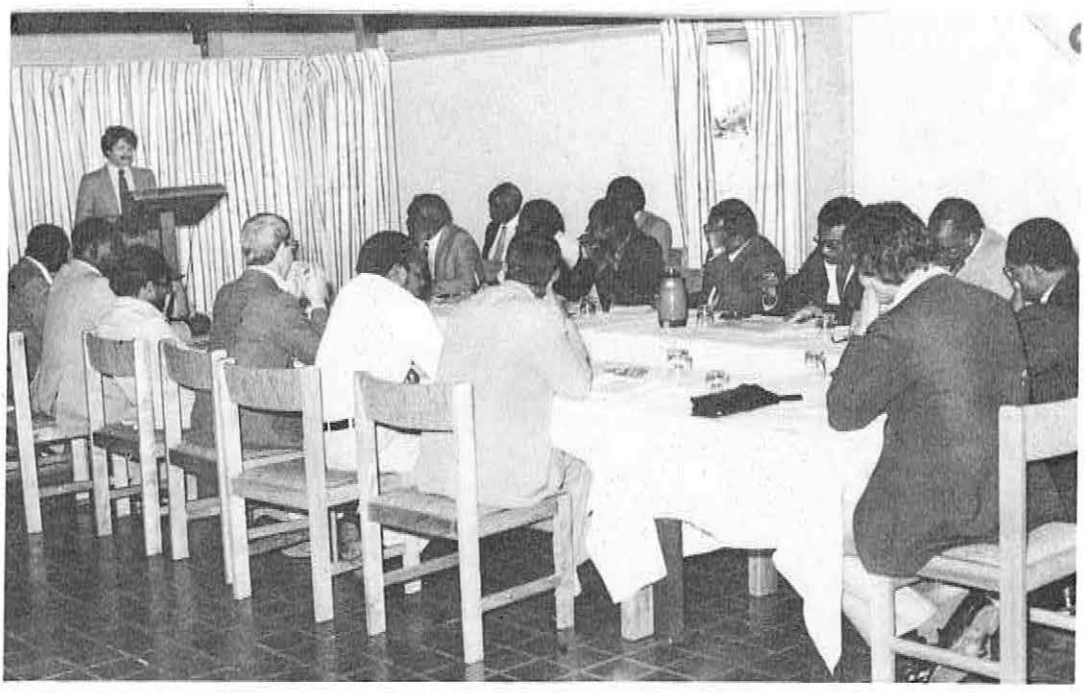
Professor W. Z. Coker,
University of Ghana (at Legon)

Dr. Teferi Gemetchu,
Addis Ababa University, Ethiopia

Professor R. Kumar,
Rivers State University of Science and Technology, Port Harcourt, Nigeria.

At its December meeting the board also appointed Professor John Okedi the first Academic Coordinator. He is also to serve as Secretary to the Board.

The programme (which will be launched in March 1983) is intended for Ph.D. students only. The MSc programme is expected to



Mr. Robert Bruce-Scott (standing), Regional Director of the International Development Research Centre, IDRC (Canada) opening the Planning Workshop on Financial and Administrative Management of Research Projects in Eastern Africa (FAMESA).

start in 1984. The selection of candidates was done by the Academic Board at its second meeting. The selected candidates, who are registered with participating universities include:

Mr. Getachew Tikubet,
Addis Ababa University

Dr. J.H.P. Nyeko,
Makerere University

Mr. Okeyo-Owuor,
University of Dar es Salaam

Miss Suliman W. Forawi,
University of Khartoum

Mr. S. Kyamanywa,
Makerere University

Mr. Abdullahi Latif Ibrahim
University of Khartoum

Mr. S. H. O. Okech,
University of Dar es Salaam

Mr. B. C. Njau,
University of Dar es Salaam

The teaching staff of ARPPIS will be drawn from ICIPE research staff, academic staff from participating universities, visiting academicians from overseas and African universities. Although the level of funding for the programme is low at present, it is hoped that ARPPIS will attract many donor agencies, cognizant of the necessity for Africa to develop a high-level scientific manpower base for tackling development-oriented research. Already a number of such agencies, including the Australian Development Agency Bureau (ADAB), UNESCO and the German Academic Exchange Service (DAAD) have made commitments to fund ARPPIS.

Financial and Administrative Management of Research Projects in Eastern and Southern Africa

In yet another important development, a programme for training in research management was started at ICIPE. From January 18th to 22nd, a planning workshop on financial and administrative management of research projects in Eastern and Southern Africa, attended by key science admini-

Participants in the International Group Training Course listen as Dr. A. M. Alghali, Postdoctoral Research Fellow at the ICIPE Mbita Point Field Station, South Nyanza, explains work on resistance of sorghum lines to stemborers.

strators and policymakers in the subregion, was held at the ICIPE. The workshop recognized the immense gap between research efforts and their delivery systems, especially at the national level and therefore resolved to formulate a number of activities leading to the improvement of the management of research institutions. Dubbed FAMESA — Financial and Administrative Management of Research Projects in Eastern and Southern Africa—the action programmes recommended were as follows:

- to create awareness and understanding of the importance of Research and Development (R & D) process in government policymakers
- to improve institutional policy formulation
- to improve communication among all those involved in the R & D process
- to sharpen planning and financial skills of managers
- to improve required skills among research support staff.

The ICIPE was appointed the executing agency and it is expected to implement the recommendations and the workplan for the programme. The International Development Research Centre (IDRC) of Canada is expected to cooperate fully with the ICIPE in this venture as it has from the beginning. Other agencies have shown keen interest and, hopefully, will contribute to the programme.

Training for Practitioners

The sixth ICIPE/UNEP International Group Training Course for Ecologically Sound Pest and Vector Management Systems was held from July 18th to August 6th, 1982. A total of 27 trainees participated from 17 developing countries including Sudan, Ghana, Zambia, Zaire, Colombia, Lesotho, Malaysia, SierraLeone, Malawi, Israel, Nigeria, Tanzania, Egypt, Uganda and Kenya. From 1977, when the first course was held, the total number trained is now 159, representing 34 countries of Africa, Southeast Asia, Middle-East and South America.

Many new features including lectures and field trips were introduced into this course. The most notable introductions were

Participants in the International Group Training Course viewing a sticky sandfly trap at the ICIPE Outreach Research Station, Kalawa, Machakos District — which is a major focus of Kala-azar (leishmaniasis) in Kenya.

the two field trips — one to Limuru on pest management under agroforestry situations, and the other to the ICIPE Mbita Point Field Station, South Nyanza. There participants observed the research ICIPE is undertaking on pest management in rural farming conditions.

Table 1. Training Output (Man-Years) at the ICIPE, 1982

Type of training	Output (Man-Years)
1. Staff Development Training	
. Research Training	2.5
. Technical Training	8.5
. Mangement Training	1.75
. Training in Communication Skills	0.75
2. Pest Management Research Training	
. Postdoctoral Research Fellowships	10.0
. Postgraduate Training	24.0
. Research Associateship	1.0
3. Training for Practitioners	
. Group Training Course in Pest Management	1.56
Total	50.06

Communications

The Communications department provides support in areas of editorial services and publications, photographic and graphic art, organization of seminars, conferences and workshops as well as visitor service.

During the year the ICIPE organized and hosted two International Study Workshops on crop-borers and termite research. A four day Crop-borers Research Workshop was held at the Mbita Point Field Station from the 14th to the 18th of June 1982. It attracted over 40 prominent scientists from different parts of the world. The 38 papers presented covered a wide range of topics including pest management in the tropics, crop loss assessment, intercropping and pest incidence. The workshop reviewed current and potential pest management practices in the tropics. Collaboration between the ICIPE and international and national research institutions was also on the agenda. The venue of the workshop (Mbita Point Field Station) afforded the participants the opportunity to view ICIPE research in the field. A tour of the experimental fields and farmers' fields gave workshop participants the opportunity to acquaint themselves with various problems facing area farmers.

From 7 to 12 November 1982 a number of distinguished scientists in the field of termite research spent a busy week at the Duduville International Guest Centre attending a Termite Caste Differentiation Workshop. Over 25 papers were presented under the chairmanship of Dr. J. A. L. Watson of the Division of Entomology CSIRO, Canberra, Australia. There were wide-ranging discussions and presentations under the workshop theme, including pathways of caste development in principal termite groups, mechanisms of caste differentiation and caste differentiation in other social arthropods.

The event was highlighted by the unveiling of a statue erected in honour of professor, Martin Luscher, performed by Mrs. Noemi Luscher. Professor Martin Luscher was not only one of the world's most distinguished termite research scientists, but a long term friend and supporter of ICIPE.

The 12th ICIPE Annual Research Conference was held in April, where three programmes were reviewed in depth. A poster review was organized to depict those research programmes not scheduled for in-depth review. ICIPE's Governing Board conducted an on-site review of research work underway at the Mbita Point Field Station.

Visitor Service

In 1982, the ICIPE received a number of distinguished guests including Hon. Alhaji Gusau, Nigerian Minister for Agriculture, who visited facilities in Nairobi and at Mbita Point Field Station. He explained that the purpose of his visit was to learn the nature and extent of research at ICIPE and to establish how his government and the ICIPE might collaborate in pest management.

ICIPE was also honoured by visits from his Excellency Mr. Paal Bog, Norwegian Ambassador to Kenya, Dr. R. K. Cunningham, Chief Natural Resources Advisor of the Overseas Development Administration (ODA), United Kingdom, and Mr. J. R. Goldsack, Agricultural Advisor at the British High Commission in Nairobi. ICIPE also received His Excellency P.I. Jaccaud, the Swiss Ambassador to Kenya.

The University of Ibadan, which for many years has collaborated with the ICIPE,

sent a delegation in connection with the proposed Post-Graduate Programme in Insect Science (ARPPIS). The delegation included Professor B. L. Fetuga, Dean of the Post-graduate School, Professor G. Babatunde, Dean of Agriculture and Forestry and Professor Antony Youdeowei, Head of the Department of Entomology.

Among the larger parties ICIPE recieved was a delegate of 15 from the World Food Council Regional Consultation for Africa. This delegation was led by Dr. Oscar Valdez, President of the World Food Council and Minister for Agriculture, Republic of Mexico, and accompanied by Professor Eugene Bortei Doku, the Minister of Agriculture in Ghana. Various groups of delegates visited the ICIPE at different times during the meeting of the Governing Board of the United Nations Environment Programme (UNEP). The visitors included the late Professor Carroll Wilson, first Chairman of the Board of ICIPE who was in Nairobi to receive the Tyler Award being presented him during UNEP's 10th Anniversary.

Special Tribute to Professor Martin Lüscher

Professor Martin Lüscher was a close friend and staunch supporter of the ICIPE from its ineception. He is remembered for his outstanding leadership and contributions to the support and advancement of scientific research, not only as Director of Research at the ICIPE but throughout his lifetime. Until his death on 10th August 1979, Professor Lüscher was Head of the Department of Zoophysiology at the University of Bern, Switzerland, where he maintained his ties with the ICIPE by training scientists and technicians.

The statue of the late Professor Lüscher pictured here was unveiled on the grounds of the ICIPE International Guest Centre at Duduville in Nairobi.

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Mr. W.O. Ogallo	Planning Officer
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Dr. G.M. Binta	Associate Scientific Officer
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Mr. K.C. Wainaina	Subordinate Assistant
Mr. J.N. Ndungu	Subordinate Assistant
Mr. M.C. Ngumah	Junior Technician

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Mbita Point Clinic

Dr. J.B. Odhiambo	Doctor-in-Charge
Mr. C. Munafu	Laboratory Technologist
Mr. E.O. Kirowo	Pharmaceutical Technologist
Miss Z.N. Macharia	Staff Nurse
Mrs. L.A. Abuya	Janitorial Assistant

Kajiado Field Station

Mr. J.M. Ole Kobaai	Watchman

Coastal Field Station — Mombasa

Mr. M.M. Moinde	Senior Administrative Officer
Mrs. C. Rarieya	Assistant Secretary
Mr. S.M. Kibati	Security Assistant
Mr. B.M. Mbuthia	Mechanic
Mr. S. Abdalla	Watchman

Duduville International Guest Centre

Mr. J.A. Achilla	Business and Catering Controller
Miss S.M. Kagondu	Secretary
Mrs. E. Kwach	Telephonist/Receptionist
Mr. J.E. Mwangi	Head Cook
Mr. A.I. Okapesi	Assistant Head Cook
Mr. A. Lweya	Cook
Mr. P.H. Opondo	Kitchen Assistant
Mr. G. Gichuru	Kitchen Assistant
Mrs. J.A. Musiga	Housekeeper
Mr. A.M. Mutwoli	Room Steward
Mr. C.B. Oyieyo	Room Steward
Mr. A.E. Mulae	Room Steward
Mr. B.O. Randiga	Assistant Launder
Mr. H.M. Kibisu	Assistant Launder
Mrs. P.A. Osoro	Room Steward
Mr. J.O. Mukhobi	Janitorial Assistant