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Twelfth Annual Report 1984

International Centre of Insect Physiology and Ecology

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ICIPE

International Centre of Insect Physiology and Ecology

The prime concerns of the International Centre of Insect Physiology and Ecology (ICIPE) are research in integrated control methodologies for crop and livestock insect pests and other related arthropods, as well as insect vectors of tropical diseases crucial to rural health in the tropics (especially in Africa); and the strengthening of scientific and technological capacities of the developing countries in insect science and its application through training and collaborative work.

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Foreword

The ICIPE Scientific Comes of Age

The first Triennial Review of the ICIPE was carried out in March/April 1983 by a team comprising international senior scientists and institutional management experts selected by the Sponsoring Group for the ICIPE (SGI). The terms of reference of the review team included a review of the scientific programme of the ICIPE, as well as its governance and management. This review was probably the most thorough analysis of the Centre's achievements since its beginning in April 1970, and provided a searchlight on its management and long-term future. The team's report was positive, and it led to three major strategic definitions: the re-focussing of the ICIPE mandate; the restructuring and rationalization of the scientific programme, and the refining of the role of the SGI in the long-term development and responsibility of the Centre.

The mandate as spelt out clearly by the Governing Board when adopting the Triennial Review Team report, states that "The prime concerns of the International Centre of Insect Physiology and Ecology (ICIPE) are research in integrated control methodologies for crop and livestock insect pests and other related arthropods, as well as insect vectors of tropical diseases crucial to rural health in the tropics (especially in Africa) and the strengthening of scientific and technological capacities of the developing countries in insect science and its application through training and collaborative work". While the goals are clearly practical ones, the means by which these goals may be achieved are varied — and surely include fundamental research, strategic research, applied research and development, and any other form of research that can provide a bank of knowledge specifically relevant to the target problem area, and define and direct the necessary problem-solving applied research and development which would lead to the desired technological innovation. The ICIPE is beginning to achieve the balance required to tackle the very difficult tropical pest problems which presently lack the type of knowledge base so essential for long-range and continuing pest control.

The core research programme is tightly organized under four subject-headings. Crop Pests Research Programme, Livestock Ticks Research Programme, Tsetse Research Programme, and the Medical Vectors Research Programme; all being supported by four basic research support units on Chemistry and Bioassay, Histology and Fine Structure, Sensory Physiology, and Biostatistics and Computer Service. The objectives and workplans for each of these programmes is sharply focused, and has been stated in a three-year strategic plan, 1984-1986, making it possible for the Centre and the SGI to determine achievements, indicate gaps, and identify changing needs and complementarities. The ICIPE is poised for a major scientific thrust, and the following two to three years should give abundant evidence of this prognosis.

The 8th meeting of the SGI held at the World Bank headquarters in Washington D.C., on 3 November 1984, gave great encouragement to the Centre's Strategic Plan (1984-1986), its performance over the last year or so, and the critical role the ICIPE is poised to play in integrated pest management in the tropics, and especially in Africa, a continent presently engulfed in a monumental crisis.

THOMAS R. ODHIAMBO, *Nairobi, 31 March 1985*

Crop Pests

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Crop Pests Research Programme

Research on insect pests of crops at the ICIPE was previously being carried out under three programmes: Bases of Plant Resistance to Insect Attack, Crop Borers, and Insect Pathology and Pest Management. From January 1984, following the recommendations of the Triennial Review Team, all research on crop pests has been brought under one programme designated as the Crop Pests Research Programme. This programme is divided into four sections: 1. Plant Resistance to Insect Pests (PRIP), 2. Bionomics and Applied Ecology (BAE), 3. Biological Control (BC), and 4. Insect Mass Rearing Technology (IMRT). The section on Plant Resistance to Insect Pests deals with aspects and bases of plant resistance to insects. The BAE section is concerned with studies on crop loss assessment, population biology, physiology, behaviour, intercropping and other cultural practices in relation to pest attack. Biological Control section is involved in investigations on bio-control agents while the IMRT section is concerned with the development of mass-rearing techniques and supply of insects to all the other sections of ICIPE for various experiments.

The programme is aimed at developing strategies for the management of certain key insect pests of sorghum, maize and cowpea by methods which would be environmentally safe and economically feasible for subsistence farming conditions in developing countries. The insect pests currently under investigation include the sorghum and maize stem borers, Chilo partellus, Busseola fusca, Sesamia calamistis, *and* Eldana saccharina; *the sorghum shootfly,* Atherigona soccata; *the cowpea pod borer,* Maruca testulalis; *aphids and thrips. The studies on plant resistance to insect pests have identified additional maize genotypes which are resistant to stem borers. The mechanisms of resistance in sorghum, maize and cowpea to their borer pests have also been elucidated, employing the methodology developed during the year. Additional information on the genetics of resistance in sorghum and cowpea has been obtained. Behavioural manipulation of the sorghum shootfly by using its ovipositional stimulants, extracted from its host plants, has been tested for its management. Tests on intercropping have confirmed that the sorghum/cowpea combination is the most effective in keeping the stem borer population down, whereas the sorghum/maize combination is the least effective in this respect. Certain bio-control agents, particularly parasitoids, have been reported to attack the stem borer pupae almost equally in both monocrop and intercropping systems. The relationship of these parasitoids, as well as of certain insect pathogens e.g., bacteria, fungi, nematodes and protozoans, with the stem borer population has been examined. The techniques for mass rearing of the target insect pests with improved biological performance for the above-mentioned research have been refined.*

The research under this section is geared to generating information on certain essential aspects of bionomics and ecology of the target pests of crops, and to utilize this information for developing methods to interrupt the build-up of the pest populations. The results of the work done during 1984 are briefly presented below.

Population patterns of stem borers on sorghum

K.J. Seshu Reddy, G.C. Ommiman

In order to develop methods for the management of stem borers of sorghum and maize, it was first necessary to have knowledge of the pattern of their population fluctuations from time to time.

During the long rainy season of 1984 at field 5 of Mbita Point Field Station (MPFS) the incidence of stem borers, *B. fusca* and *C. partellus*, on sorghum (cv. Serena) started at 5th week after seedling emergence, while *S. calamistis* and *E. saccharina* started at 8th and 9th weeks respectively. A maximum of 95% infestation by *B. fusca* was recorded as opposed to 35% by *C. partellus*.

The *B. fusca* population (larvae and pupae) from 40 sorghum plants varied from 2 at 5th week to 164 at 14th week just before harvest, and for *C. partellus* it ranged from 7 at 5th week to 32 at 11th week. A maximum of 9 larvae and pupae of *E. saccharina* was obtained from 40 plants, while for *S. calamistis* it was only one larva.

Studies conducted on farmers' fields in Rusinga Island during the same season have revealed that *B. fusca* was the predominant stem borer species on sorghum. Starting 6 weeks after the emergence of the crop, 79-100% of the sorghum plants were infested with *B. fusca*. *B. fusca* larvae per stem ranged from 2.4 to 5.7. Infestation by *C. partellus* was very low, ranging from 0-9% only. No other stem borers were recorded.

Population patterns of stem borers on maize

M.T. Bosch-Ico

Distribution of the immature stages of C. partellus *on the maize plant.* Maize plants (Katumani composite B) were infested with first-instar larvae of *C. partellus* at the time of tasselling (30 days after emergence). The larvae were found to aggregate over the maize stalks (Table 1). Using the Duncan's new multiple range test, the maize stalks were divided into sections in a descending order of area of larval aggregation. The larvae tend to aggregate in the tassel, ears and the internodes bearing the ears.

Table 1. Mean number of *C. partellus* larvae, pupae and pupal cases per internode, tassel and ear and the percentage of ears present per group of internodes

	Tassel and ear	Internodes 6, 7 and 8	4, 5 and 9	3, 10, 11 and 12	1 and 2
% ear present		89.8	10.2	0	0
C. partellus	0.74	0.51	0.42	0.22	0.03

The aggregation of the larvae around the tassel, ears and internodes bearing the ears is responsible for loss in yield even with late attack. Attack on the ear stalk before or immediately after fertilization was found to cause failure of grain formation. However, tunnelling of internodes bearing the ears and ear stalks after the milking stage did not seem to affect grain yield.

C. partellus *survival and establishment on the maize plant.* The survival rate of *C. partellus* is higher (76%) at initial infestation level of 5 larvae per plant than at infestation level of 10 larvae per plant (37%) (Table 2). The differences observed between *C. partellus* establishment on maize plants at initial infestation of 5 larvae per plant (3.8 larvae per plant) and 10 larvae per plant (3.7 larvae per plant) and the infestation by the second generation from the two treatments (3.9 larvae per plant) were not significant ($t = 0.18$; $P>0.05$). The mean establishment of *C. partellus* on maize plant under screen-house conditions was found to be 3.8 larvae per plant.

Population fluctuation of the stem borer complex on maize. The stem borers found on maize are *C. partellus* (Swinhoe), *E. saccharina* (Walker), *B. fusca* (Fuller), and *S. calamistis* (Hampson). *B. fusca* and *S. calamistis* appeared in very low numbers showing peaks of less than 0.17 larvae and pupae per plant, while *C. partellus* and *E. saccharina* were found in high numbers and showed population fluctuations with peaks of 3.67 larvae and pupae per plant for *C. partellus*, 8 weeks after plant emergence, and 3.9 larvae and pupae per plant for *E. saccharina*, 16 weeks after plant emergence, during the short rainy season (October-January). Pest populations were found to be very low during the long rainy season (April-July).

The differences in number of immature stages of *C. partellus* counted per plant per week during the short rainy season were found to be significant. Three population peaks were identified. The first was a small peak (1.86 larvae and pupae per plant) occurring 4-5 weeks after emergence. The second (2-73 larvae and

Table 2. Number of larvae, pupae and pupal cases per plant and the survival and establishment of *C. partellus* per plant after 35 days of infestation.

Initial infestation level per plant	Larvae, pupae and pupal cases recovered after 35 days	Percent survival	*C. partellus* established per plant
5 larvae	3.8	76	3.8
10 larvae	3.7	37	3.7
Uncontrolled infestation by 2nd generation	3.9	--	3.9

pupae per plant) occurred 7-8 weeks after emergence. The third peak (2.51 larvae and pupae per plant) occurred 11-12 weeks after emergence. *E. saccharina* on the other hand was found in the maize plant 9 weeks after emergence and its population kept rising, reaching maximum population at harvest.

Development and reproduction of *B. fusca*

G.C. Unnithan

Preimaginal development. Under laboratory conditions (24.5-28.5°C and natural photoperiod) the incubation period of *B. fusca* eggs was 5-6 days. When reared under these conditions on young sorghum stems (Serena), post-embryonic development was completed in 42.8 ± 3.5 (mean ± SE) days. There were seven larval instars. The duration of the larval instars were; 3 days each for first and second instars, 2.75 days for third instar, 3.1 days for fourth instar, 3.15 days for fifth instar, 5.75 days for sixth instar and 8.9 days for seventh instar. The pupal period lasted for 13.3 days.

Eclosion. In the laboratory, under natural photoperiod, eclosion of the pupae starts between 1830 and 1900 hours and reaches a peak between 1900 and 2000 hours. Out of a total of the 372 adults, 172 (52.6%) were males and the remaining (47.4%) were females.

Reproduction. Mating starts within a few hours (6-8 h) after eclosion and takes place between 0300 and 0600 hours. Maximum number of pairs mated between 0400 and 0500 hours. The pair remained in copula for 95.3 ± 2.3 min. Males mated repeatedly with virgin females. Fifty three per cent of the pairs mated before they were 1 day old, 77% when they were 1 day old, 58% at 2 days old, and 50% at 3 days old.

The fecundity of *B. fusca* reared on Serena and fed on sucrose solution or distilled water alone was studied in the laboratory. The results showed that *B. fusca* had a very high fecundity with an average of 636 eggs per female on sucrose solution and 677 eggs per female on distilled water alone. Maximum number of eggs were laid on the second day after emergence.

Effects of mating and delayed mating on fecundity and fertility. Experiments were conducted to study the effect of mating and delayed mating on the longevity, fecundity and fertility of *B. fusca*. Virgin females laid only very few eggs, if any. Mating within few hours after eclosion (before 1 day) reduced the longevity and preoviposition period and increased the fecundity. A delay in mating for two or three days resulted in a decrease in fecundity and fertility (Table 3). It is possible that if mating is delayed or disrupted in the field it will have negative effect on the fecundity and fertility of the moths, thereby reducing the infestation.

Alternative host plants of stem borers

K.V. Seshu Reddy, G.C. Unnithan

Surveys in search of different natural hosts of the sorghum and maize stem borers were conducted at Mbita Point and nearby fields on the shores of Lake Victoria, during the period June to September 1984. Several species of graminaceous host plants which include *Hyparrhenia rufa, Pennisetum macrourum, Phragmites mauritianus, Sorghum arundinaceum* and *S. verticilliflorum*, were found to harbour *Busseola* sp., while *Sesamia* sp. were found to infest *Cyperus articulatus* and *C. papyrus* (Cyperaceae) and *Typha latifolia* (Typhaceae). Of these plants, *H. rufa, P. macrourum* and the wild sorghums showed 20 to 56% infestation by *B. fusca*.

Estimation of loss in cowpea due to insect pests

K.V. Seshu Reddy

Among the various methods of quantitative assessment of losses due to insect pests, comparison of yields under protected and unprotected conditions was used to estimate the loss in cowpea due to a range of insect pests. The cowpea variety used was Ex-Luanda. Two treatments – unsprayed and sprayed – were replicated six times. Furadan was applied to treatment 2 at planting time and after emergence of seedlings. Rogor was applied five times at 14-days interval.

Observations were taken on 10 random plants in each treatment at weekly intervals. The matured pods were harvested and threshed. The cowpea seed damaged by pod borers and pod bugs was separated from healthy seed and weighed. The results are summarized in table 4.

During the course of the observations, several insect pests were recorded, including; aphids *(Aphis craccivora)*; thrips *(Megalurothrips sjostedti)*; five species of grasshoppers; flower beetle *(Mylabris* sp.); pod bugs *(Nezara viridula, Riptortus dentipes, Anoplocnemis curvipes, Agonoscelis pubescens, Dysdercus nigrofasciatus, Acanthomyis tomentosicollis, A. horrida)*; and pod borers, *(M. testulalis, Heliothis armigera)*, and lycaenids (unidentified).

The avoidable loss in yield of cowpea grain caused by various insect pests was 98.7%. However, even in the treated plots, the protection was not complete as a result there was 10.5% loss in yield.

Table 3. Influence of mating and imaginal age at mating on female longevity, fecundity and fertility in *B. fusca*

Female age at mating	Longevity (days; $\bar{X}$ ± SE)	Preoviposition period (days; $\bar{X}$ ± SE)	Fecundity (eggs/female) ($\bar{X}$ ± SE)	Fertility (percent eggs hatched) ($\bar{X}$ ± SE)
Virgin female	8.0 ± 0.9 ab	4.1 ± 1.0 ac	97.5 ± 42.1 a	-
0 day (8 h)	5.9 ± 0.7 a	1.0 ± 0.0 b	802.8 ± 84.7 b	93.6 ± 1.3 a
1 day	6.8 ± 0.6 ac	2.2 ± 0.1 bc	699.1 ± 87.2 b	89.4 ± 4.1 a
2 day	7.9 ± 0.5 bc	3.0 ± 0.0 bc	562.3 ± 121.0 bd	91.9 ± 5.03 a
3 day	7.8 ± 0.6 bc	4.1 ± 0.1 bc	288.3 ± 57.1 cd	65.0 ± 9.5 b

Notes
Fed on distilled water alone
Means followed by the same letter are not significantly different from each other

Table 4. Avoidable loss in yield of cowpea caused by insect pests

	T1 (unprotected) Total	T1 (unprotected) $\bar{X} \pm SE$	T2 (Protected) Total	T2 (Protected) $\bar{X} \pm SE$
Total pods	573	95.5 ± 40.34	23496	3916 ± 647.19
Healthy	38	6.33 ± 3.53	2374	295.67 ± 92.90
Damaged	535	89.17 ± 37.11	21122	3520.33 ± 555.92
Weight of grain (g)	181	30.16 ± 14.91	13778	2296.3 ± 503.06
Healthy grain (g)	133	22.17 ± 11.99	12325	2054.2 ± 469.36
Damaged grain	48	8.0 ± 2.99	1453	242.2 ± 36.69
Total grain yield	7.1 kg/ha			540.3 kg/ha
Healthy grain	5.2 kg/ha			483.3 kg/ha
Damaged grain	1.9 kg/ha			57.9 kg/ha

Intercropping in relation to insect pest infestation

E.O. Omolo

Effect of different crop combinations. The best combination (sorghum/cowpea), and the worst combination (sorghum/maize) in terms of crop borers control, productivity and crop loss, have been tested and confirmed in three different ecological zones; MPFS (erratic rainfall and intensive pest population), Ogongo (one reliable rain season with normal pest population), and Rongo (highlands with both reliable rain season and low pest population).

In an attempt to explain the reasons why some crop combinations are better than others, both growth rate and pattern of each crop within different cropping patterns were monitored in terms of the dry matter turnover at every two-week interval throughout the growing season. Indications are that sorghum and cowpea or maize and cowpea in both cases differ in their use of growth resources.

On the other hand, sorghum and maize are more or less similar. Sorghum is able to complement cowpea and so make better use of resources than when grown separately. This complementary action occurs much more between sorghum and cowpea than between maize and cowpea. This type of complementary action is said to give a better 'temporal' use of resources.

Maize grew faster in terms of total dry matter content in pure stand than when intercropped, while sorghum did equally well in both pure stand and intercropping. In the cowpea, however, the situation was reversed and cowpea grew much better when intercropped than in monocultures. Maize, therefore, is much more suited to monoculture, sorghum is quite happy under both cropping systems, while cowpea is much more adapted to mixed cropping. This may explain why sorghum and cowpea turned out to be the best intercropping combination.

Genotype identification. The behaviour of a crop in mixed stand cannot be predicted from its behaviour in pure stand. This has been shown in the present studies. It is therefore important that genotypes which are eventually going to be used in a given intercropping situation should be evaluated in that situation. So the need to identify suitable genotypes in intercropping has been emphasized and it is likely that the use of resistant/tolerant cultivars in intercropping may offer an even wider scope for pest management.

Preliminary evaluation conducted at Rusinga Island during the major cropping season of 1984 indicated that there were significant interactions between sorghum genotypes, pest populations, cropping systems and grain yields. Before conclusions are drawn, these experiments will have to be repeated in Rusinga Island and other different ecological zones.

Intercropping and its relation to plant pests and weeds

A. Dixsemond

Preliminary testing of sorghum, maize and cowpea with different crop combinations and spacings showed certain differences in crop borer attack. The sorghum/cowpea combination harboured the smallest number of stem borers whereas sorghum/maize dicrop combination had the highest level of infestation. The sorghum shootfly and aphids were obviously not influenced by the cropping pattern.

Plant diseases are not important at MPFS and the infection occurs late in the season and spreads slowly. Intercropping sorghum, maize and cowpea, however, did not have any significant influence on disease development.

A weed-smothering effect of intercropping cereals with legumes was observed both for weed yields and area covered by weeds.

Ecological factors governing the bean flower thrips, *Megalurothrips sjostedti* (Tryhon) in cowpea maize mixed crop system

S. Kyamanywa

Three field experiments have been conducted at Mbita Point Field Station, to study the effect of intercropping cowpea (Ex-Luanda) with maize (Katumani composite) on the population density and activity of flower thrips and also on predators in general. Temperature and humidity variation between the mixed and single crop systems have also been monitored using thermohygrographs.

The data from the three experiments indicate that crop mixture reduced the population density and activity of thrips significantly. Future experiments will examine the possible cause of reduction of thrips in maize/cowpea combinations as compared to pure-cropped cowpeas.

Trapping and pheromone biology of *B. fusca* and *C. partellus*

G.C. Unnithan, K.N. Saxena

The objective of this research is to develop efficient trapping techniques which can be used to monitor and suppress stem borer populations.

The efficiency of water traps without any lure (blank) or with virgin females, mated females or synthetic *B. fusca* and *C. partellus* pheromone has been examined.

B. fusca virgin females were more than two times as efficient as synthetic pheromone in attracting males. Mated *B. fusca* females did not attract any males, which indicates that they had ceased to produce the sex attractant. No males were caught in the blank traps.

In the case of *C. partellus* also, synthetic pheromone and blank traps attracted only very few males, 4 and 7%, respectively, compared to 89% by virgin females. Even after mating and oviposition, *C. partellus* females continued to attract males, although not as many as the virgins.

Sorghum shootfly oviposition stimulant and its application for pest management

G.C. Unnithan, K.N. Saxena

Atherigona soccata is a monophagous insect which usually attacks only plants of the genus *Sorghum*. The susceptible hybrid sorghum from India (CSH 1) has been found to be highly preferred for shootfly oviposition. Screen-house and field studies were conducted to determine whether CSH 1 seedlings possess any oviposition stimulant which can be used for the manipulation of the behaviour of the pest as a strategy for its management.

CSH1 seedlings were extracted first in hexane followed by acetone and the acetone extract was assayed for oviposition stimulant activity in the screen-house and in the field using the non-host plant maize as an oviposition substrate. Shootflies laid most eggs on maize plants sprayed with the acetone extract, and less on control maize, CSH 1 or Serena plants used as control (Table 5). Results of these experiments clearly showed that CSH 1 seedlings contained an oviposition stimulant which lured the flies to lay eggs on non-host plants such as maize and cowpea. It should be noted that maize and cowpea are often intercropped with sorghum and shootfly eggs laid on maize and cowpea would not survive. The potential application of the oviposition stimulant for pest management is being investigated. Work on the isolation and chemical characterization of the oviposition stimulant from CSH 1 is being carried out in collaboration with the Chemistry and Bioassay Research Unit of ICIPE.

Socioeconomic aspects of pest management

W.P. Conelly

Research on the socioeconomic aspects of pest management was begun in early 1984. The goals of this research are to:

- increase our understanding of the socioeconomic context in which farmers' decisions about pest management are made;
- identify what farmers perceive as their most serious pest problems and the existing pest control practices of farmers; and
- identify socioeconomic factors that may influence the ability of farmers to adopt recommended pest control practices.

Fieldwork and a formal survey of a sample of farmers were conducted in South Nyanza District, Western Kenya. The survey covered low potential single-crop areas near the lake shore as well as medium-high rainfall areas farther inland that support many small-scale commercial farms producing two crops per year. We report below on preliminary findings.

Farmers' perceptions of pests. A large majority of farmers interviewed identified stem borers as the most common insect pests found on maize and sorghum and were able to give accurate descriptions of their feeding habits and the type of damage they caused. Farmers'

Table 5. Shootfly oviposition on sorghum and on non-host plants (maize and cowpea) sprayed with acetone extract of CSH 1 seedlings

Substrate	Percent plants oviposited upon ($\bar{X}$ ± SE)	Percent eggs ($\bar{X}$ ± SE)	Eggs/infested plant ($\bar{X}$ ± SE)
Maize, sprayed with acetone extract	92.5 ± 4.9*	84.1 ± 3.0**	4.7 ± 2.6 NS
Maize, control	47.5 ± 10.6	15.9 ± 3.0	0.9 ± 0.3
Maize	18.3 ± 16.4	6.7 ± 6.4	0.6 ± 0.6
CSH 1	100**	93.3 ± 6.4**	5.2 ± 1.3*
Maize, sprayed with acetone extract	92.5 ± 5.3NS	52.6 ± 4.6	4.4 ± 0.9NS
CSH 1	91.2 ± 6.1	47.4 ± 4.6	4.5 ± 0.8
Maize	2.5 ± 2.5	0.2 ± 0.2	0.1 ± 10.0
Serena	100**	99.8 ± 0.2**	10.2 ± 1.7**
Maize, sprayed with acetone extract	100 NS	72.7 ± 6.0**	10.7 ± 1.4*
Serena	97.5 ± 2.5	27.3 ± 6.0	4.0 ± 0.8
Cowpea, sprayed with acetone extract	46.0 ± 16.9NS	92.0 ± 8.0***	3.2 ± 0.3***
Cowpea, control	16.0 ± 16.0	8.0 ± 8.0	1.0 ± 0.0

* **Significantly different at P<0.01 and P<0.001 respectively
NS Not significant

knowledge of other insect pests of grain crops, however, especially the sorghum shootfly and the cowpea pod borer was much more limited. Many farmers reported serious insect problems with their vegetable and fruit crops as well as infestation of grain crops by post-harvest storage pests, especially weevils.

Farmers' assessment of the yield loss caused by insect pests varied between locations within the district. On the mainland, both in low and high rainfall environments, farmers indicated that, though potentially serious, insects in most years were not a major constraint to grain production. Other pest and environmental problems including erratic rainfall, weeds (especially *Striga hermonthica* and *Digitaria scalarum*), wild animals, and birds were ranked as more serious. On Rusinga and Mfangano Islands, however, insects are seen as a more serious threat to farming. Here sample farmers ranked only rainfall and animal pests as more important problems than insects. Table 6 shows the overall results from all three survey areas of a question asking farmers to rank various constraints to production in terms of the yield loss in most years. Overall, it is clear that from the farmers' point of view, insects should not be viewed in isolation, but rather should be seen as only one of a complex of crop pests and environmental constraints that limit yields.

Farmers may be unwilling to adopt new farm practices designed to reduce insect pests if these ignore or exacerbate other serious constraints to production.

Table 6. Farmers' ranking of constraints to production in South Nyanza, Kenya (all localities, n = 48)

	Most serious				Least serious		Mean score
Constraint	5	4	3	2	1	0	
Rainfall	24	7	5	5	6	1	3.73
Animals	9	12	11	4	7	5	2.94
Weeds	6	11	8	15	4	4	2.75
Birds	6	5	13	6	10	8	2.31
Insects	1	9	7	13	13	5	2.10

Traditional pest control practices. Intentional control of insect pests by traditional methods is not widespread in the district. A few farmers are aware of traditional herbal 'insecticides' but these are no longer widely used. Some farmers hand pick insects and destroy heavily infested plants, but this does not seem to be done systematically.

None of the farmers report using pesticides to control insects on their maize and sorghum crops, though some do employ chemicals to protect vegetables destined for the market. The intercropping of legumes with grain crops, which may play a significant role in reducing pest infestation, is widespread. Though not intentionally employed to control insects, this traditional agronomic practice shows much promise as a cultural control method.

Recommended cultural control practices. The existing socioeconomic conditions in the district make it unlikely that farmers will be able to adopt some commonly recommended cultural control practices without modification. As elsewhere in Africa, the burning of the maize and sorghum residue after the harvest is not feasible because of several important alternative uses. These include use of the residue as livestock fodder, building material, fuelwood, and as mulch in banana and coffee orchards. Likewise, early and simultaneous planting is problematic because of erratic rainfall, variation in soil conditions, and the fact that many poorer farmers without a plough or animal must wait to rent or borrow a plough team before they are able to prepare the soil. Further research is needed to assess more fully the potential of these and other cultural control measures.

PLANT RESISTANCE TO INSECT PESTS SECTION

The main objectives of this section are to elucidate the principles which impart resistance or susceptibility to insect pests in different plant species or varieties with a view to: provide appropriate information to plant breeders in various national or international agricultural centres for developing cultivars which incorporate resistance-imparting characters or eliminate susceptibility-imparting characters, cultivation of such cultivars serving as an important component of an integrated pest management programme; and to develop methods of manipulating the resistance or susceptibility-imparting plant characters in such a way as to interrupt pest populations build-up and thus contribute to its management.

To achieve the above objectives, this section is engaged in evaluating and confirming resistance in germplasms of the target crops received from different national or international agricultural centres against their key insect pests; investigating the bases of resistance or susceptibility in these plant materials; and studying the genetics of their resistance to the target insect pests. The results of the work done on the above-mentioned aspects during 1984 are outlined briefly below.

Evaluation of germplasm from other centres (USA)

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

During this year, additional maize genotypes, received from certain centres in USA, were evaluated for resistance against the stem borers at Mbita Point Field Station:

Group I: MP 701, MP 702, MP 704, (developed by Drs. Paul Williams and Frank Davis in Mississippi and reported to have resistance against *Diatraea grandiosella* and *Spodoptera frugiperda;*

Group II: CI 31A, B 75, B 86 and OH 43, orginating from the USDA Corn Insects Research Laboratories at Ankeny, Iowa, and reported to be resistant to the European corn borer *Ostrinia nubilalis* (Hubner), and B 85 reported as susceptible.

Group III: ICZ1-CM (referred to as CMT 33 in previous publications from ICIPE and derived from CIMMYT population 27) and ICZ2-CM (referred to as CMT 324 in previous publications of ICIPE and derived from CIMMYT population 22). These maize genotypes have previously been reported to show resistance to *C. partellus* at Mbita.

Group IV: Inbred A and Katumani, originating from the Kenya germplasm and previously reported to be susceptible to the stem borers, were used as the susceptible checks.

Methodology. Evaluation of resistance by maize germplasm to lepidopteran pests, like many other crops, is generally conducted through natural infestation of the plants in the fields or through artificial infestation with larvae, in the field or the screen-house. During the course of the present work, it was considered important to evaluate the different maize genotypes by artificial infestation not only against the larvae but, also, against the adults because it is the latter which initially select or reject the plants for laying eggs.

A special technique was, therefore, developed during 1984 for evaluating the resistance of different maize genotypes against adult infestation. For this, the above-mentioned 12 maize genotypes were grown in radial rows, 2 m long each, along the circumference of a circle of 2 m diameter. All the plants were enclosed in a cage (6 x 6 x 2.5 m) of nylon net to avoid contamination from the natural population of the stem borers. Twenty ovipositing females were released in the centre of the cage and could select plants of any of the 12 genotypes in radial rows with equal chances for laying eggs. The percentages of eggs laid on different genotypes were recorded on alternate days, removing the eggs after each recording and replenishing the moths once every week.

Side by side with the above-mentioned tests on the adults, linear rows of the same 12 genotypes were artificially infested with the larvae of *C. partellus*. The foliar damage to these plants was recorded on a 9-point scale. At the time of harvest the borer tunnels within the stems were also measured.

Results. The susceptibility or resistance of the 12 maize genotypes to adult oviposition is clear from table 7. Egg-laying was heaviest on the susceptible Katumani and the resistant ICZ2-CM, medium on Inbred A (susceptible check), OH 43, B 86, MP 702, CI 31A, MP 704 and lowest on MP 701, B 85, B 75, and ICZ1-CM.

Table 7. *C. partellus* oviposition on different maize genotypes presented in radial rows along a circle within a cage in the field

Genotype	Origin	% eggs laid* (mean ± SE)
Inbred A	Kenya	10.8 ± 1.8
Katumani	Kenya	15.2 ± 3.3
ICZ1-CM	CIMMYT	2.5 ± 1.4
ICZ2-CM	CIMMYT	17.9 ± 4.3
CI 31A	Iowa	6.9 ± 1.4
OH 43	Iowa	10.7 ± 2.3
B 75	Iowa	4.2 ± 0.9
B 85	Iowa	4.4 ± 3.1
B 86	Iowa	9.8 ± 3.4
MP 701	Mississippi	4.5 ± 0.6
MP 702	Mississippi	8.2 ± 1.4
MP 704	Mississippi	6.8 ± 3.3

***On the basis of total number of eggs laid on all the genotypes from 5 to 9 weeks after plant emergence.**

With reference to larval infestation, Inbred A, CI31A, B 75 and B 86 were most susceptible, ICZ1-CM, ICZ2-CM, OH 43 and B 85 were moderately resistant, and BP 701, MP 702 and MP 704 were highly resistant (Table 8). Larval establishment and development was best on B 85 and poorest on MP 704, indicating thus that this cultivar has a high level of resistance among the additional genotypes tested for *C. partellus*.

Table 8. *C. partellus* larval primary damage and damage expression on different maize genotypes

Genotypes	Mean foliar lesions rating	% dead hearts	Stem tunnelling (cm)	% stem breakage
MP 704	2.90 a	0.0	22.8 a	14.3 a
MP 702	2.60 a	0.0	28.2 ab	16.0 ab
MP 701	3.35 a	0.0	24.8 a	30.3 ad
ICZ2-CM	3.45 ab	2.5	37.2 b	17.8 abc
B 85	5.08 cd	0.0	35.7 ab	34.4 bcd
ICZ1-CM	3.48 ab	0.0	38.4 b	27.5 abc
Katumani	3.10 a	0.0	40.6 bc	40.4 cde
B 75	6.73 ef	37.5	39.0 b	23.5 abc
B 86	6.35 de	12.0	38.0 b	31.0 ad
OH 43	4.80 bc	0.0	39.9 b	37.5 cde
Inbred A	8.25 g	57.5	53.9 c	48.7 de
CI 31A	7.88 fg	45.0	•	54.5 e
Std. error	0.35	NA	4.46	4.53

Means followed by the same letters are not significantly different at the 5% level according to the DMRT.

•CI31A plants were completely destroyed before harvest.

Evaluation of germplasm from other centres (IITA, Nigeria)

E.O. Omolo, K.V. Seshu Reddy

A maize population, TZBR, resistant to *S. calamistis*, was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and was crossed with the ICIPE lines resistant to *C. partellus*. The resultant crosses and lines derived from them were categorized into 5 groups and then screened under natural infestation for multiple resistance to *C. partellus* and *E. saccharina* at Mbita Point Field Station.

The parameters observed were: tunnelling, number of borers per plant, percentage of plants infested by *C. partellus*, *E. saccharina* or both, percentage of ears damaged, and grain yield. The importance of grain yield in this study was to facilitate identification of mere sources of resistance (lines with high level of resistance and low yield) from the lines with acceptable levels of grain yield which could be used in breeding programmes to develop commercial varieties. From group II lines, 13, 18, 22, 24, 33, 41, and 45 had the least damaged ears and therefore realized high grain yields. The ears were damaged by a complex of crop borers, the predominant species being *E. saccharina* followed by *C. partellus*. Line 5 from group V had almost no attack from both stem borers. The best lines in terms of grain yield and resistance to both *C. partellus* and *E. saccharina* were the group IV lines: line 26, with grain yield of 55.95 q/ha; 25, with grain yield of 52.36 q/ha; and 30 with a grain yield of 50.07 q/ha. Line 68 from group II also gave 51.49 q/ha grain yield. The fact that one of the parents of these lines had previously been screened for *S. calamistis* resistance and had also shown a good level of resistance to both *C. partellus* and *E. saccharina* implies the existence of cross resistance or resistance of a broad-base type.

Thus, in this study, new sources of multiple resistance to *C. partellus*, *E. saccharina* and *S. calamistis* have been identified, and crosses between them would obviously offer a wide scope in multiple resistance approach to pest management.

Bases of resistance or susceptibility to insects

A. N. Saxena

The approach followed for this study has been the same as explained before. The susceptibility or resistance of a plant to an insect pest is reflected in the insect's establishment on the plant. Under otherwise identical environmental conditions, differences in an insect's establishment on different plant species or varieties are determined by an interaction of the insect's responses to the plants, and the plant characters governing these responses.

An insect's colonization of a plant is governed by the following six main responses operating in as many stages of its establishment on the plant: 1. Orientation, involving attraction or repulsion and resulting in its arrival on or avoidance of different plants. 2. Feeding, involving stimulation or inhibition of food intake by different plants. 3. Metabolism of ingested food, involving its utilization by the insect and determining its nutrition. 4. Development of the insect, if in the larval stage. 5. Egg production, if in the adult stage. 6. Oviposition, which may be stimulated or inhibited by different plants.

The plant characters governing the above-mentioned responses include: 1. Distance-perceivable stimuli (visual, hygro, olfactory) determining the insect's orientation to the plants. 2. Contact-perceivable characters (chemical, mechanical) determining the insect's feeding and oviposition. 3. Nutritional and toxic constituents, determining the insect's metabolism and thereby its survival or mortality.

In view of the above, our present study is being taken up in two parts. The first part involves a comparison of the above-mentioned responses of the target insect to the susceptible and resistant genotypes of its host plants. The second part involves the study of the role of the plant characters in determining these responses. Below, we summarize methods developed to measure and compare these responses.

Measurement of ovipositional responses to whole plants in the field or screen-house. These methods have been so designed and the experimental conditions so controlled that differences in the insect's responses to the plants would clearly be due to the plants' internal characteristics and not to any outside factors.

Tests were conducted in a single-compartment chamber (150 x 120 x 120 cm) with wire-net walls except at the base which was provided by the floor of the screen-house or the field bearing the test plants. The plants of one cultivar were arranged in a row inside the chamber along one end-wall and the other end left blank (no-choice situation) or had a row of the other cultivar (2-choice situation).

Ten ovipositing females, released in the centre, were able to orientate and oviposit either on the plants or elsewhere within the chamber. On the basis of the total number of eggs laid in 48 h, the percentage of eggs laid on the test plants was calculated and served as an indicator of the ovipositional response.

Measurement of ovipositional responses to distance-perceivable characters of the plants. A specially designed triple-compartment chamber (210×80×80 cm) was used. The plants or other test materials were presented just outside the wire-net all at one or both ends so that the ovipositing females within the chamber could perceive their distance-perceivable characters without coming in contact with them.

The percentages of eggs laid by the females on the wax paper strip (serving as ovipositional substrates) at each end would reflect the insect's preference or non-preference for the distance-characters of the plants viz., visual (colour, pattern etc.,) olfactory (volatiles), hygro (water vapour). Techniques have been devised to test the role of each of these types of stimuli by excluding or including one or the other at one or both ends of the chamber.

Measurement of responses to the volatiles from the plants or their extracts. The oviposition response to the volatiles from the plants or their extracts were tested in a specially designed rectangular twin-compartment chamber (20×15×6 cm) with a closed base and open top. A sheet of perforated wax-paper (serving as the ovipositional substrate) was stretched across the top of the chamber and was covered with a wire-net (20×15×2.5 cm) within which an ovipositing female was released. Leaves of one cultivar, or its extracts in the desired quantity, were kept in one compartment and the other compartment was left empty or filled with the leaves or extracts of another cultivar. Differences in the percentages of eggs laid overnight on the perforated wax-paper segments above the 2 compartments would reflect the ovipositional preference for the volatiles tested.

Measurement of ovipositional responses to contact-perceivable characters. These are measured in a circular test chamber consisting of a base (11.5 cm dia, 3.5 cm ht) and a cover (11.5 cm dia, 1.2 cm ht), each having a wire-net across its top. The cover rests on the wire-net of the base across which are placed one or two test materials, e.g. plant leaves or their extracts, on glass plates.

Single ovipositing females are released within the cover and the percentage of eggs laid on the test materials compared. In such a chamber, the insects are in contact with one or the other test material throughout the experimental period so that differences in the egg laying on them would be due to their contact characters.

Measurement of larval orientation, feeding and development on resistant and susceptible cultivars. Methods have also been developed for measuring the orientation of the larvae in terms of percentages arriving or departing from the plants, feeding, in terms of area of leaves consumed, and development in terms of percentages reaching the pupal or adult stage, during a given period, on the test plants in the fields or screen-house.

Basis of maize resistance or susceptibility to *C. partellus*

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

The following three maize genotypes were used to compare the above-mentioned responses of *C. partellus*: Inbred A, ICZ1-CM and ICZ2-CM. Some observations on the responses of *C. partellus* adults and larvae to Inbred A, and ICZ2-CM were given in the *ICIPE Eleventh Annual Report, 1983*. During 1984, further work on these genotypes was carried out and a third genotype, ICZ1-CM, was included for comparison. The results are briefly presented below.

Oviposition on whole plants. Oviposition is the first step in a lepidopteran's colonization of a plant. We compared *C. partellus* ovipositional response to the maize genotypes mentioned above in order to find out whether this response bears any relationship with the plant's susceptibility or resistance to this insect. When the three genotypes were presented to the insect in a no-choice situation within the single-compartment test chamber, the rate of oviposition was almost equally high on all of them (Table 9). In a 2-choice situation with susceptible Inbred A and one or the other 2 resistant genotypes, ICZ2-CM attracted almost as much oviposition as Inbred A, whereas oviposition on ICZ1-CM was much less. There was clear non-preference for ICZ1-CM.

Table 9. *C. partellus* ovipositional response to certain susceptible and resistant genotypes in 2-choice as well as no-choice situations within the single compartment test chamber

		% eggs laid (mean ± SE)	
A	B	A	B
Inbred A	Nil	92 ± 2	8 ± 2
ICZ1-CM	Nil	85 ± 3	15 ± 3
ICZ2-CM	Nil	95 ± 3	5 ± 3
Inbred A	ICZ1-CM	63 ± 7	37 ± 7
Inbred A	ICZ2-CM	46 ± 7	54 ± 7

Role of distance-perceivable plant characters. On presenting the plants outside the wire-net wall of the 3-sector test chamber at only one end (no-choice situation), the insects within the chamber laid almost twice as many eggs at the end facing any of the three genotypes as at the blank end. As the insects were not in contact with the plants, the oviposition would be in response to certain stimuli from the plants which could be perceived at a distance. However, under a 2-choice situation, presenting Inbred A with ICZ1-CM or ICZ2-CM, response to both the resistant genotypes was almost equal to that of the susceptible one. Selective presentation of different types of plant stimuli revealed that the visual (colour, pattern) did not influence oviposition. Aqueous vapours serving as hygro stimuli as well as the non-aqueous volatiles serving as olfactory stimuli from all the three genotypes elicited equally high egg laying.

Thus, distance perceivable volatiles would not account for ovipositional preference or non-preference for the plants.

Role of contact characters. On allowing the insects contact with the plants within the circular test chamber, egg laying on the lower surface of the leaves was about 8 to 9 times that on the upper leaf surface, for each genotype, when given a choice between the two surfaces. Even on the upper surface, the oviposition on the basal portion of the leaf was much higher than on its terminal portion. On the other hand, on the lower surface, the basal portion of the ICZ1-CM leaf elicited more egg laying than its terminal portion whereas there was no significant difference in oviposition on the two portions of Inbred A and ICZ2-CM. In all the 3 genotypes, the terminal portions of the leaves, though less preferred than the basal, elicited oviposition at a high level when offered as a choice against a glass surface, which by itself is quite suitable for egg laying.

When presented in a choice situation, oviposition on the resistant ICZ1-CM was much less than on the susceptible Inbred A, for the upper as well as lower surfaces of the basal and terminal portions of the leaves. Although the lower surface of the terminal portion of ICZ2-CM received less eggs than Inbred A, the difference was not as much as for ICZ1-CM. Removal of hairs (trichomes) on the upper leaf surface of ICZ1-CM increased egg laying, which suggests that hairiness can inhibit ovipositional response. However, since the lower surface of the leaves without hairs is preferred for oviposition, it is likely that certain other contact characters are responsible for the ovipositional differences among the three genotypes.

Larval orientation. On emergence, larvae begin their orientation and either stay on the same plant and move to the feeding site or leave the plant in preference for others. Continued stay on a plant or arrival on it depends on the plant's attraction to the larvae. Comparisons of such responses of the neonate *C. partellus* larvae to the three maize genotypes revealed that the susceptible Inbred A attracted these larvae more than the resistant ICZ1-CM or ICZ2-CM. A greater percentage of neonate larvae settled on the susceptible than on the resistant genotype.

Larval feeding. The area of the leaves in the whorl consumed by the neonate larvae was greater on the susceptible Inbred A, less on the resistant ICZ2-CM and least on the resistant ICZ-CM. Thus, the larval feeding non-preference for these two genotypes is part of their resistance mechanism.

Larval development. The larvae released immediately after emergence on the test genotypes developed better on the susceptible Inbred A than on the resistant ICZ2-CM. The percentage of larvae developing to the last pupal instar in 28 days was 34 on Inbred A and 18 on ICZ2-CM. Such a difference in larval development on the two genotypes is related, at least partly, to feeding as reported above. Thus, non-preference resulting from contact, inspite of initial ovipositional attraction, larval orientation and feeding non-preference causing poor development, all contribute to the resistance in ICZ1-CM and ICZ2-CM.

Basis of sorghum resistance or susceptibility to *C. partellus*

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

Two sorghum cultivars have been initially taken up for this study: susceptible IS 18363 and IS 2146 which is

relatively resistant. *C. partellus* responses to these plants and the role of plant characters are being studied in the same way as described above. Our observations last year showed that differences in certain responses of the insect to the two cultivars accounted for those in their relative susceptibility or resistance. The ovipositional response for the resistant IS 2146 was lower than that of the susceptible IS 18363 because of lower attraction by the former's volatiles while the contact characters of both were equally effective in eliciting this response. The larvae emerging from the eggs on the oviposited plant showed equal attraction to and arrest on both the cultivars. But, larval feeding and development was higher on the susceptible cultivar. Further study of these responses and the plant characters involved has been carried out during 1984 and the results are briefly presented below.

Oviposition on whole plants. The tests in the single-compartment chamber showed that *C. partellus* lays less eggs on IS 2146 than on IS 18363 in no-choice as well as 2-choice situations. During 1984, this conclusion was tested in a larger arena, in the screen-house. IS 18363 plants in a block (3 x 2 m, 5 rows of 15 plants each) were grown in one corner of the screen-house while at the diagonally opposite corner was a similar block of IS 2146. The wind blew at right angles to these blocks of plants without passing from one to the other. This arrangement ensured that the distance stimuli were not mixed-up within the screen-house. Twenty ovipositing females were released in its centre and the percentage of eggs laid on each cultivar was recorded after 2 nights. The results showed that, out of the total number of eggs laid, 58% were on the susceptible IS 18363 and 42% on the resistant IS 2146. Thus, the insect's ovipositional non-preference for the latter, relative to the susceptible IS 18363, was observed even in a test arena larger than the single-compartment chamber.

Role of plant volatiles in oviposition. It has been previously shown that with *C. partellus*, the ovipositional responses to the volatiles of the sorghum leaves in relation to their quantity can be measured in the twin-compartment chamber in the laboratory. It was also reported that with 4 times as many IS 2146 leaves in one compartment as the susceptible IS 18363 leaves in the other compartment resulted in almost an equal ovipositional response to the volatiles of both.

Further studies on the role of these volatiles have been conducted during 1984. Firstly, it was observed that the leaves of both the cultivars, when air-dried, continued to emit volatiles which elicited oviposition in the twin-compartment chamber. However, when equal amounts (3 g) of the dried leaves of the two cultivars were taken in one of the compartments of the chamber and the other compartment was left blank, the percentage of eggs laid in the compartment containing the susceptible IS 18363 volatiles was much higher (71 ± 5.1%) than the compartment with IS 2146 volatiles (219.0 ± 9.1%) under no-choice situation. After extracting with n-hexane, the residue of the dry leaves elicited much less egg laying than the unextracted dry leaves. On the other hand, the hexane extract of the susceptible cultivar elicited 68.0 ± 7.8% oviposition as compared to only 54.8 ± 10.9% by the resistant. Thus, the lower content of n-hexane in IS 2146 contributes to the insect's non-preference for the cultivar.

Larval orientation. As mentioned before *(ICIPE Annual Report, 1983)*, the neonate larvae emerging on the oviposited plant are arrested in large numbers on both the cultivars. The role of the chemical stimuli from the plants in larval attraction and arrest was examined during 1984. Larvae were released on a disc of wet filter paper impregnated with desired amounts of n-hexane extract or acetone extract of the n-hexane-extracted leaves of the two cultivars. The percentage of larvae remaining on the filter paper discs after 2 h would be relative to the degree of attraction arrest by the extracts. The untreated wet filter paper arrested only 46.7 ± 15.2% larvae whereas the percentage of larvae arrested was 63 ± 6 by the hexane extract of IS 18363, 68 ± 9 by the hexane extract of IS 2146, 85 ± 4 by the acetone extract of IS 18363 and 80 ± 6 by the acetone extract of IS 2146. Thus, the acetone extracts of both the cultivars were equally more effective than their n-hexane extracts.

Larval feeding. The neonate larvae settling in the whorls of the sorghum plants have been reported to feed on IS 18363 more than on IS 2146. On developing to the third instar, the larvae may feed on the foliage or bore into the stem making tunnels. Feeding by the third instar larvae on the foliage was therefore compared for the two cultivars. The area of the leaves consumed in 48 h was higher (4.3 ± 0.4 cm^2) on IS 18363 than that on the resistant IS 2146 (3.4 ± 0.6 cm^2). Thus, like the first instar larvae, those in the third instar also showed a feeding non-preference for IS 2146 leaves. The plant characters responsible for this are under investigation.

Basis of cowpea resistance to the pod borer, *M. testulalis*

S.H.O. Okech, K.V. Saxena

Three cowpea cultivars have been taken up for this study: susceptible Vita 1, and resistant Vita 5 and TVu 946. The approach and methodology described above are being followed for this insect as well. The results obtained thus far are summarized below.

Oviposition. On the resistant cowpea cultivar TVu 946, oviposition is less than on the susceptible Vita 1 because TVu 946 contact characters, particularly chemical stimuli, are less effective in eliciting the response. Thus, non-preference for TVu 946, caused by inadequate chemical stimulants, is involved in its resistance, particularly in a choice situation.

Larval orientation. Attraction and arrest of neonate larvae and their settlement on the resistant TVu 946 is lower than on susceptible Vita 1. This difference is due to the lower chemical stimulant content of the resistant cultivar rather than any repellent factors.

Larval feeding. On the flowers, larvae feed as much on Vita 1 and TVu 946, though the latter's flower juice is less phagostimulatory than the other. Feeding on the leaves, though quite low on Vita 1, is still less for TVu 946. The same applies to the stems and pods of this cultivar and this is involved in the cultivar's resistance in the absence of flowers.

Larval food utilization/nutrition. Food utilization and nutrition on stems and pods of TVu 946 is less than on Vita 1. Thus, antibiosis is partly involved in the resistance of TVu 946 when larvae feed on stems and pods.

Larval development. On the flowers, larval establishment is equally high for Vita 1 and TVu 946. On pods, when flowers are not available, development is lower on TVu 946 than on Vita 1. On stems, in the absence of pods and flowers, development is lower on TVu 946 than on Vita 1. The development on the leaves when flowers and pods are not available, is very low on Vita 1 and even lower on TVu 946. Differences in larval development on leaves, stems and pods, are caused by those in both feeding and food utilization/nutrition.

Oviposition by the sorghum shootfly on certain cultivars in relation to their relative resistance or susceptibility

G.C. Umathan, K.V. Seshu Reddy

Shootfly oviposition and infestation on eight sorghum cultivars (IS Numbers 2122, 2123, 2205, 2291, 4660, 5092, 5480, and 18551), reported to be resistant to the shootfly, and two susceptible cultivars (Serena and CSH 1) were studied under field conditions (natural infestation). The eight resistant cultivars and CSH 1 were also tested for preference for oviposition in a two-choice situation in the screen-house. The eight resistant cultivars received considerably less shootfly eggs and showed fewer dead-hearts than the susceptible cultivars. The results of the two earlier experiments and field studies indicate that non-preference for oviposition is an important mechanism for resistance to shootfly in the eight test cultivars. There was also evidence of a certain degree of antibiosis in IS 2291.

Genetics of plant resistance to insects

R.V. Pathak

Sorghum resistance to stem borers. Genetic analysis of sorghum resistance to stem borers (mainly *C. partellus*) was performed in crosses involving susceptible, resistant and tolerant cultivars. The results revealed that resistance to stem borers is polygenic in inheritance. The locally adapted cultivar, Serena, showed a high degree of tolerance to stem borer infestation, particularly to stem tunnelling, and gave the highest grain yield. All resistant cultivars were poor in grain yields. Genetic analysis revealed that resistance to leaf damage and stem tunnelling appear to follow different genetic patterns. Resistance to leaf damage was governed by both additive and non-additive genes while resistance to stem tunnelling was governed predominantly by additive genes.

Further experiments were carried out to study the genetic variations of resistance and tolerance. The analysis involved parents, F_1 and F_2 of three different crosses. The parents involved in the study had a wide range of expression for plant height, leaf damage, stem tunnelling, stem lodging and grain yields. Estimates of degree of dominance in F_1 and inbreeding depression in F_2 suggested that susceptibility was dominant over resistance in susceptible/resistant and susceptible/tolerant crosses, while resistance was dominant over tolerance in the tolerant/resistant cross.

The variation pattern of leaf damage and stem tunnelling in F_1 and F_2 progenies suggested that they are different and independently inherited parameters. Thus, both resistance and tolerance mechanisms are operating in sorghum resistance to stem borers.

Estimates of broad sense heritability, genetic coefficient of variation and expected genetic advance for stem tunnelling are presented in table 10. The heritability estimates were rather low in all crosses. The estimates of genetic coefficient of variation considered in conjunction with heritability estimates would indicate that large grain yield from the selection result from the cross Serena/IS 2146. The estimates of expected genetic advance also indicated that about 55% reduction in stem tunnelling should result from selection of the top 5% (the least tunnelled) of plants among the segregates of the cross Serena/IS 2146.

Table 10. Estimates of heritability, genetic coefficient of variation and expected genetic advance for percent stem tunnelling in sorghum

Cross	Heritability (broad sense)	% genetic coefficient of variation	Expected genetic advance (% of mean)
Swarna/IS 2146	0.25	19.45	19.00
Swarna/Serena	0.23	19.43	19.32
Serena/IS 2146	0.47	38.97	54.98

IS 2146 - Resistant, Swarna - Susceptible, Serena - Tolerant.

Based on the above genetic analysis, exploitation of resistance/tolerance mechanism is suggested through recurrent selection (population improvement) to develop stem borer-resistant cultivars.

Cowpea resistance to M. testulalis. Cowpea resistance to the pod borer, *M. testulalis* was studied in the cross between resistant parent TVu 946 and susceptible parent Emma 60 (ICV 5). Resistance to *Maruca* was measured on two parameters: percentage pod damage and percentage seed damage. The variation pattern in the F_2 suggested that resistance to *Maruca* is polygenically inherited. However, a comparison of F_1 from mid-parent value indicated that susceptibility was partially dominant over resistance. The seed size (100 - seed weight) appears to be somewhat associated with resistance or susceptibility of the seed. The resistant parent, TVu 946, had less 100-seed weight, (7.2 g), as compared to the susceptible parent ICV 5 (12.6 g). The F_1 progenies were found to be as susceptible as the parent ICV 5 with a 100-seed weight of 11.9 g. Percentage seed damage appears to be a more reliable measure of damage by *M. testulalis* than percentage pod damage.

Cowpea resistance to aphids. In addition to known sources of resistance to aphids, two new ones, ICV 11 and ICV 12 have been developed through induced mutations. These mutants are promising for improved grain yields as well. The resistance ability of these mutants have been confirmed through screening by artificial infestation in the screen-house. Preliminary studies in crosses between

resistant and susceptible cultivars suggested that resistance is completely dominant over susceptibility in F_1 and appears to be governed by a single gene pair.

BIOLOGICAL CONTROL SECTION

The long-term objective of the Biological Control Section is to study and develop biological control as a component of an integrated pest management (IPM) programme that suits subsistence farming systems in tropical Africa. The cereal stem borers, *C. partellus* and *B. fusca*, which are major pests of maize and sorghum; and the pod borer, *M. testulalis*, a major pest of cowpea, have been chosen as models in an effort to improve the production of these important food staples in Africa.

Field and laboratory studies were continued over the period to identify natural mortality agents (insect pathogens and parasitoids) associated with field populations of the target pests under mono and intercrop systems at Mbita Point Field Station. An extensive survey was also carried out throughout the Lake Basin (Kenya side) towards the end of the long rainy season (July-August, 1984) to record the distribution pattern of parasitoids and pathogens of cereal stem borers in this region.

Parasitoid studies on cereal stem borers in monocrop and intercrop systems

G.W. Otieno

Work on parasitoid incidence in relation to crop phenology and population densities of *C. partellus* on sorghum and maize monocrops was concluded. Systematic studies on the effect of intercropping on pest population densities in relation to incidence and activity of natural enemies (parasitoids) were conducted during the long rainy season of 1984 at the station and on a subsistence farm at Gingo, near the station. A randomized block design with 5 cropping combinations replicated 3 times was used. The following five combinations were selected on the basis of previous work on intercropping under the Bionomics and Applied Ecology Section of the programme: sorghum/cowpea, maize/sorghum, sorghum pure stand, cowpea pure stand, and pure stands of the local varieties of sorghum (Serena), maize (Katumani) and cowpea (Ex-Luanda). Weekly insect counts were made by examining and dissecting samples of 4 plants each of maize, sorghum and cowpea per plot. Field-collected cadavers were examined for mortality agents in our Insect Pathology Unit.

Results from sorghum and maize monocrops confirmed the predominance of the pupal parasitoids, *Dentichasmias busseolae* and *Pediobius furvus*, in field populations of *C. partellus* and *B. fusca* at the Station. The population build-up of the stem borer larvae on sorghum and maize started one week after plant emergence (WAE) and that of pupae 4 WAE. The larval and pupal populations on sorghum rose to a peak 12 WAE. On maize, larval population showed two peaks at fourth and nineth WAE, whereas the pupal population reached a peak during the sixth and ninth WAE.

On both maize and sorghum, infestation of pupae by parasites was recorded from the seventh or eighth WAE, reaching a maximum at harvest. A new addition to last year's list is an egg parasitoid of *B. fusca* (? *Telenomus* sp., Scelionidae) recorded from a subsistence farm at Gingo. The parasitoid *Apanteles sesamiae* (Braconidae) was found to be the commonest and most widely distributed larval parasitoid of *C. partellus* and *B. fusca* in the Lake Basin region towards the end of the long rainy season (July-August, 1984).

The first set of results from intercropping experiments at the Station (long rains, 1984) showed larval population densities of *C. partellus* on sorghum (cumulative data throughout the season) to be highest in pure stands of sorghum (mean of 0.31 larvae per plant), intermediate in maize/sorghum (0.22 per plant) and lowest in sorghum/cowpea combinations (0.18 per plant). However, corresponding estimate of parasitoid numbers were too low to account for the observed differences.

Insect disease dynamics within the intercropping system

W.A. Otieno

Utilization of insect disease-causing microorganisms such as bacteria, fungi, nematodes and viruses, is now widely recognized as an important component of integrated pest management throughout the world. However, most of the information available on the applications of insect pathogens against agricultural crop pests is based largely on monocrop systems. The resource-poor subsistence farmer in the tropics, especially in Africa, often practices intercropping. In order for him to benefit from the emerging new technologies on biocontrol agents, it becomes necessary to understand the basics of insect disease dynamics within an intercropping system as a prelude to field applications.

Field mortality of the cereal stem borer, C. partellus, *in different intercropping patterns.* A regular weekly sampling regime to recover *C. partellus* was maintained throughout the growing periods of sorghum and maize plants. After 13 weeks of sampling the 13 samples collected, larvae and pupae, were separated into live and dead ones.

Results indicate (Table 11) that a significantly higher disease incidence (8.57%) occurs on *C. partellus* in the sorghum/cowpea and sorghum/cowpea/maize combinations (8.64%) than in sorghum/maize combination (4.93%).

Distribution of pathogenic microorganisms among dead, field-collected C. partellus. Laboratory diagnosis

Table 11. Field mortality of the spotted stalk borer *C. partellus*, in different intercropping patterns at Mbita Point Field Station (Long rains, 1984)

Intercrops	Total collected	Diseased	% mortality
Maize-sorghum	304	15	4.93
Maize-cowpea	49	3	6.1
Sorghum-cowpea	140	12	8.57
Sorghum-cowpea-maize	185	16	8.64
TOTAL	678	46	6.78

of field-collected dead *Chilo* indicates that the bacteria are the most important mortality agents (36.76%), while the viruses are the least important (4.41%). See table 12.

Table 12. Incidence of pathogenic microorganisms in field collected *C. partellus* at Mbita Point Field Station (Long rains, 1984)

Pathogens	No. dead (n = 68)	% mortality
Bacteria	25	36.76
Fungus	20	29.41
Nematode	13	19.12
Protozoa	7	10.29
Virus	3	4.41

Infection and mortality of crop borers on sorghum and cowpea

M.O. Okeno

Plots of pure sorghum and cowpea crops were planted, hand-weeded, and fertilizer applied to them according to standard agronomic practice. Sorghum stems were sampled continuously from planting to post-harvest, dissected and live larvae and larval cadavers recovered. Diagnosis for causes of mortality was carried out by examining the haemolymph and portions of fat-body tissue in water-mounts under dark-field and phase-contrast microscopy.

In the preliminary bioassays conducted to test for microorganisms with potential for biocontrol, laboratory-reared third-instar *C. partellus* larvae were fed on diet treated with a semi-purified inoculum prepared from field-collected larval cadavers. Mortality was recorded every 48 h.

The major stem borer was *C. partellus*, but the other borers, *B. fusca*, *E. saccharina* and *S. calamistis* were also found. Mortality build-up was slow initially and, although it started about 4 weeks post-plant emergence (PPE), it reached its peak (14.7%) at late plant maturity (9 weeks PPE). Thereafter, larval mortality declined to less than 3% at harvest (14 weeks PPE). Similarly, in the legume pod borer *M. testulalis*, larval mortality due to microorganisms was low, the highest level recorded being 12% in late growing season. In laboratory-maintained cultures of *M. testulalis*, the protozoa *Nosema* sp. was an important mortality factor.

Bacteria were recorded in the highest proportion of the larval cadavers of the cereal stem borers (77.8%), but fungi (19.2%), protozoans (7.9%) and nematodes (2.9%) were also observed. In laboratory-maintained cultures of *M. testulalis*, the protozoa *Nosema* was an important mortality factor.

Preliminary bioassays and subsequent screen-house trials showed that the protozoan recovered from *M. testulalis*, (Nosema sp.) is highly pathogenic to *C. partellus*, and therefore has a high potential for use in the control of the stem borer.

INSECT MASS REARING TECHNOLOGY SECTION

R.S. Ochieng

This section is divided into two sub-sections: Research and Development (R & D) and Support Services. The R & D sub-section is the research wing whose function is to formulate and test insect diets for mass production. Its other function is to initiate and develop new techniques for mass production of insects. The support services sub-section caters for the demand of all other programmes and units at ICIPE.

During 1984, our efforts were mainly directed towards the production of *C. partellus*. Improved rearing techniques were required to meet the increasing demand for the insects. It was, therefore, necessary to study some factors that are believed to affect the production of *Chilo* on mass scale.

One factor which was thought to have profound effect on *Chilo* production was the relative humidity on the eggs. Studies of various relative humidities experienced throughout the entire embryonic period, or during part of it, and their effect on egg hatch and development times were undertaken. Such knowledge is vital in establishing optimum relative humidity conditions during embryonic development, and in applying an appropriate egg collection and egg incubation strategy.

The eggs of *C. partellus* used in these experiments were obtained from the laboratory colony kept on artificial diets for over one year (about 10 generations). The experiments were done in sandwich boxes with light fitting lids kept at ambient laboratory temperatures. Eggs were collected on wax paper. In the morning, the eggs were counted under the microscope and then placed on filter papers. In each humidity level tested, 200 eggs were set up. Loaded filter papers were immediately placed into the sandwich boxes with appropriate test humidities established over different concentrations of sulphuric acid, introduced into the container at least one day before use. Care was taken not to leave the sandwich boxes open long during the various experimental manipulations, in order to avoid disturbance to the humidity equilibrium. Hatchability, checked after 5 days after oviposition, was used as a criterion for the effect of humidity on the eggs.

The effect of varying constant incubation environment on egg mortality throughout the egg's developmental period is shown in table 13. At all the relative humidities tested, the embryonic development proceeded normally. At values below 80% RH many eggs which were developed fully to black-head stage did not hatch. Many black

Table 13. Percent hatchability of *C. partellus* eggs at different humidities

Incubation environment (RH %)	Number of eggs placed	Unhatched eggs with black head (embryo)	% egg hatchability (range)
100	405	85	77.28
90	402	99	73.83
80	400	120	68.25
70	401	189	47.13
60	400	256	36.0
50	359	203	19.68
40	400	251	10.5
30	398	229	2.5
20	395	269	0.0
10	402	248	4.97
0	430	427	0.00

heads at medium humidities had their bodies emerged half way through the egg. Eggs appeared desiccated at the lowest relative humidity values tested. A large number of developed larvae appeared shrivelled, and about 30% eggs did not have any embryonic development and had extensive desiccation. At 10% RH, 95% of the eggs did not hatch, although 62% of them contained well-developed embryos, indicating the presence of a water loss prevention mechanism. At 100% RH, the hatchability was above 77%.

In experiments exposing the eggs to various relative humidities for different periods of time then transferring them to 100% RH for completion of development at room temperature, it was shown that prolonged exposure to non-saturated humidities does not result in significant reduction in hatchability. However, there was a marked reduction in egg hatchability at 40% RH (Table 14). At 90% RH even the exposure for 72 h did not significantly reduce egg hatchability. The eggs only became sensitive at later stages of development.

Table 14. Percent hatchability of *C. partellus* eggs exposed for different periods of time to various RH before transfering to 100% RH for completion of development at room temperature (100 eggs/treatment)

Exposure duration (RH)	% hatchability under different RH			
	40	60	70	90
6	87	77	78	70
12	83	95	85	80
18	82	87	64	79
24	74	66	94	93
36	83	83	93	96
48	64	65	63	75
72	43	73	78	64

The studies show that *C. partellus* eggs are not very sensitive to low relative humidity. Egg development proceeds to hatching only at atmospheres close to saturation point. Prolonged exposure to low relative humidity affects egg hatch but does not adversely affect embryonic development. The data suggests that the embryonic development is not affected by low relative humidity.

In *C. partellus* at 10% RH, 62% of the eggs did not hatch although developed embryos could be seen through the chorion. Under these conditions, either the embryos had not completed their development or they were too weak to break through the chorion. A large number of embryos broke the chorion at 70% RH but desiccated half way trying to wriggle out.

It has thus been shown that *C. partellus* eggs possess a mechanism which protects them against desiccation. This allows the development of the embryo but, the development appears to weaken this mechanism which probably results in the disturbance of water balance due to the loss of water molecules through the permeable chorion of the egg.

Results obtained in these studies have assisted in improving phases of the mass-rearing system regarding egg collection and incubation. Egg masses are placed in tightly closing sandwich boxes in which the relative humidity is kept as close to saturation as possible by spraying distilled water onto the filter paper on which they are placed.

INSECT MASS-REARING TECHNOLOGY SECTION (IMRT) NAIROBI BRANCH

The main objective of IMRT Nairobi Branch is to mass-produce high quality target insects, competitive with the wild type, for experimental use by various research programmes.

During 1984 the Nairobi branch mass-reared and supplied target insects to programmes and units as shown on the chart. The unit also maintained laboratory animals for blood sucking arthropods and also supplied rabbits, rats, mice and hamsters for experimental purposes.

Primary users of insect and mammals reared by the unit

Programmes and units	Livestock Ticks	Medical Vectors	Tsetse	Chemistry & Bioassay	Histology and Fine	Sensory Physiology	Crop Pests	Other Institutions
Tsetse (*G. morsitans*, *G. pallidipes*)			X	X	X	X	X	X
Stem borers (*C. partellus*, *E. saccharina*)				X		X		X
Wax moth (*Galleria mellonella*)							X	
House fly (*Musca domestica*)		X						
Mosquito (*A. aegypti*)				X				X
Cotton stainer (*Dysdercus fasciatus*)				X				X
Armyworm (*Spodoptera exempta*)				X		X		X
Rabbits	X	X	X	X	X	X	X	X
Rats		X	X				X	X
Mice	X	X	X					
Hamsters		X						

Livestock Ticks

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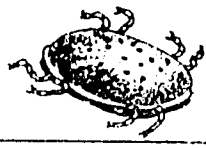

Livestock Ticks Research Programme

As a result of recent developments concerning the availability of vaccines and drugs for the control of tick-borne diseases, and particularly East Coast Fever (ECF), and the availability of biological methods of controlling the ticks that transmit these diseases, it is considered probable that new technologies will shortly become available for the integrated control of ticks and tick-borne diseases of livestock. Further, it is considered that these integrated control technologies will result in a biologically stable situation with regard to disease, and that they will be environmentally acceptable and dependent on the absolute minimal use of acaricides.

The traditional method for controlling ticks and the diseases they transmit is the close-interval application of acaricides to livestock in dips or sprays. To control ECF, twice-weekly applications are necessary. This procedure has many disadvantages: the high cost of installing, maintaining and staffing dips and spray races; the high cost of acaricides; the development of resistance to acaricides by the ticks, and high levels of acaricide residues in beef and dairy produce. But perhaps the greatest disadvantage of using acaricides to control ticks is the fact that an inherently unstable situation results where regularly dipped cattle are completely susceptible to disease and naive to tick infestation. If for any reason acaricide application fails, catastrophies can occur.

As a result of work carried out by the UNDP/FAO Regional Project (Research on tick-borne diseases and tick control, RAF/67/077) from 1967 to 1977 at Muguga in Kenya, and since then continued at the Veterinary Research Department of the Kenya Agricultural Research Institute (KARI) and at ILRAD, Nairobi, curative drugs and an experimental vaccine against East Coast Fever have been produced. Successful field trials with the experimental vaccine were carried out in Kenya and Tanzania during the 1970s.

At a recent conference on theileriosis at ILRAD in Nairobi, attended by delegates from many countries in Africa and elsewhere, concern was expressed regarding the present situation of relying on acaricides to control ticks and great support was given for the development of biological methods to control ticks. These would be used in conjunction with curative drugs and vaccines against the tick-borne diseases. It was considered that such an integrated approach would create a stable situation which would obviate most of the disadvantages associated with the use of acaricides.

What are the prospects? As stated above, curative drugs and vaccines are now, or shortly will be, available for the control of the diseases of cattle transmitted by ticks. In addition, it has been known for many years that cattle can be made resistant to ticks, and the technique is now being used in Australia to control Boophilus microplus, *the vector of babesiosis.*

BIOLOGICAL CONTROL OF TICKS

M.P. Cummings

As a result of the considerations mentioned in the introduction, ICIPE set out to investigate the possibility of producing biological methods for controlling ticks. Two different approaches have been used to induce resistance in host animals and are referred to as Type 1 resistance and Type 2 resistance.

Type 1 resistance. It has been widely reported during the last 30 years that resistance to many species of ticks can be induced in many laboratory animals following tick infestation. Type 1 resistance in cattle was reported in the early part of this century in cattle in Queensland, Australia, which developed resistance to *B. microplus* following infestation. However, it was only within the last 10 years that this finding has been applied in the field, necessitated by the widespread development of resistance to acaricides by *B. microplus* and made possible by the availability of curative drugs and a vaccine for the control of babesiosis, the only disease transmitted by *B. microplus*.

At ICIPE it has been found that cattle become resistant to *Rhipicephalus appendiculatus*, the vector of ECF, when approximately 500 adult ticks are allowed to feed on them. Resistance also develops when cattle are exposed to ticks in paddocks. Resistance has been produced in *Bos taurus* as well as in *B. indicus* cattle, and is long lasting – at least 2 years.

Resistance appears to be stimulated in cattle in response to antigens inoculated in the saliva of the feeding tick. When ticks feed on a resistant animal, they secrete antigens in the saliva. These antigens stimulate an immediate type hypersensitivity reaction, and marked swelling occurs at the site within 20 min of attachment. This reaction interferes with the ability of the tick to feed properly. The effect is most marked against larvae, less against nymphs and least against adult ticks. In highly resistant cattle, very few larvae, less than 25% of nymphs, and less than 50% of adult ticks are able to complete feeding, and the engorged weight is also reduced. The smaller ticks which are produced after moulting have a reduced survival potential when exposed to temperature and humidity stress and the females produce smaller egg batches of reduced viability.

Two experiments which demonstrate that resistant cattle can control field populations of *R. appendiculatus* have been carried out.

- Individual tick-naive cattle were exposed singly in each of five paddocks infested with ticks. Initially, the tick populations in each paddock increased and reached a peak after 9 months, when hundreds of adult ticks were seen on the cattle. Thereafter, the ticks decreased in number and after 2 years had almost disappeared. On removal from the paddocks the cattle were found to be highly resistant.
- In the second experiment, a pair of resistant cattle were exposed in a paddock infested with larval ticks, and pairs of tick-naive cattle were exposed successively in a similar paddock. Thousands of adult ticks were produced in the tick-naive cattle paddock and very few adults in the tick-resistant cattle paddock.

Another important finding is that tick-resistant cattle, when infected with ECF, greatly affect the infection rates in larvae or nymphs which feed on them, resulting in reduced transmission of the disease. It is probable that resistance to ticks in cattle will enhance the efficacy of vaccination against ECF by reducing challenge.

Type 2 resistance. This approach to produce resistance in host animals originated at ICIPE, and depends on the observation that mammalian gamma globulins ingested by ticks pass unchanged from the blood meal into the ticks' haemolymph. It was found that when homogenates of fed female ticks were inoculated into rabbits or cattle, antibodies were produced against antigens from the tissues of the tick. When ticks are fed on the immunized host animals, the antibodies ingested in the blood meal react with the target antigens, interfere with their normal function and a high tick mortality results. The reduced numbers of female ticks which do engorge either do not lay eggs, or produce eggs which have a markedly reduced viability. At least 10 target antigens which are involved in type 2 resistance have so far been identified, one of which has been obtained in a purified form. Purification of the remaining antigens is in progress, and the possibility that hormones and enzymes might be used as target antigens is being investigated.

Largely because of the possibility of patenting results, practically nothing has been published on type 2 resistance. We do know, however, that colleagues in universities and research institutes in the United States, Canada and Australia are achieving positive results using different species of ticks, and that at least one commercial company in UK is involved. There appears little doubt therefore, that type 2 resistance will shortly become available to enhance type 1 resistance for the control of ticks and indeed, that type 2 resistance in host animals might be produced against other blood sucking parasites.

THE USE OF IRRADIATED *RHIPICEPHALUS APPENDICULATUS* TICKS TO INDUCE RESISTANCE TO TICK INFESTATION

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

During this period, we continued our investigations on the use of irradiated *R. appendiculatus* ticks to induce resistance. We have had difficulty in obtaining regular supplies of suitable rabbits for experiment and the drought conditions in central Kenya have caused problems in maintaining cattle for experiment.

Resistance engendered by ticks treated with three levels of radiation.

Earlier work had shown that doses of 1.2 and 2.4 krad of gamma radiation prevented the ticks from breeding, but did not interfere unduly with feeding.

Three pairs of rabbits were used in this experiment and 20 male and 20 female *R. appendiculatus* ticks were fed on the left ear of each, confined in a cloth bag. The ticks had received 0, 1.2 and 2.4 krad and two rabbits were used for each radiation dose. Two more rabbits served as

Table 15. Feeding performance on rabbits by two successive batches of irradiated *R. appendiculatus* adults, followed by a test feed with untreated nymphs

Host No	Dose on ticks (krad)	1st feed (females) Left ear only mean ± SD	2nd feed (females) Left ear mean ± SD	2nd feed (females) Right ear mean ± SD	3rd feed (nymphs) Left ear mean	3rd feed (nymphs) Right ear mean
1	0.0	322 ± 78	26 ± 59	26 ± 20	2.7	2.3
2	0.0	483 ± 65	132 ± 92	234 ± 65		
3	1.2	392 ± 64	141 ± 108	183 ± 67		
4	1.2	432 ± 65	94 ± 67	132 ± 63	3.6	3.4
5	2.4	263 ± 119	21 ± 10	113 ± 73	3.6	3.5
6	2.4	312 ± 62	22 ± 18	100 ± 46	3.6	3.8
7	Control				8.1	7.1
8	Control				7.9	8.4

3rd feed, 100 nymphs on each ear

Rabbits no. 2 and 3 died before the 3rd feed

controls. The feeds were exactly repeated 34 days later, except that the ticks were divided equally between both ears of the hosts. After a further 28 days, a test feed was done on each ear of every rabbit, including the controls, using 100 untreated nymphs. Engorged females were weighed individually, but the engorged nymphs only in bulk.

The results are given in table 15. During the second feed, the mean engorged weights were reduced, often severely. In every case the effect was greater in females from the left ear, which had been fed on already, than in those from the right ear which had not. The mean reductions in weight of 78% respectively were significantly different ($P < 0.05$), confirming that the resistance shown by the hosts contained both a systemic component (in the blood) and a local effect in the ear tissues. In the final assessment it was apparent that all the surviving rabbits that had had ticks feeding on them were markedly resistant compared to the controls, but the ear-to-ear difference in engorged weight was no longer significant. The data were too few for a final comparison of the effects of differing doses of radiation on the ticks' ability to induce resistance.

Possible complications of male tick sterility in inducing resistance in the host.

Earlier, we had shown that female ticks treated with 1.2 krad did not differ consistently in mean engorged weight if either sex alone, or both sexes together, were irradiated. They were only a little lighter in weight than controls in which both sexes were untreated. We repeated the experiment using ticks which had received 2.4 krad, and also wished to see if their ability to induce resistance in the host might be affected. We needed also to see if sterility in the male interfered with the ability of the female to feed after mating, or later to convert the blood meal into eggs, since these processes were under examination in other experiments.

Only two rabbits were available. Irradiated and non-irradiated ticks were fed on them as shown in table 16. All female ticks were weighed individually before use in order to select those of good size (2.0-2.9 mg) and again after engorgement. A sub-sample of 5 was taken at random from each combination of those completing engorgement on days 8 and 9 of the feed. Their production of eggs was weighed and an estimate of the percentage hatch of larvae from each egg batch was made. A single test feed using 100 nymphs was started on each host 28 days after the application of the adults to feed. The engorged nymphs were weighed in bulk. The results were compared with those from the same control hosts as in the previous experiment.

The mean weight of the engorged females when both sexes were irradiated was significantly lower ($P < 0.001$) than if both sexes were untreated. Irradiated females laid no eggs over a 31-day period after engorgement, whereas all non-irradiated females started oviposition in 5 to 7 days. However, as expected, the eggs laid by females mated with irradiated males were almost completely sterile. It was also apparent that the host rabbit for the irradiated females was as highly resistant to subsequent tick infestation as the one receiving the non-irradiated females.

An experiment is now in progress on rabbits in which induction of resistance to infestation by irradiated and non-irradiated ticks is being investigated at 3 levels of infestation.

Table 16. Feeding performance on rabbits by adult *R. appendiculatus* irradiated with 2.4 krad, followed by a test feed with 100 untreated nymphs.

Host	Radiation dose (krad) ♂♂	Radiation dose (krad) ♀♀	Engorged females mg mean ± SD	Engorged females % conversion[1]	Nymphs % hatch	Nymphs mg mean
1 left ear	2.4	2.4	243 ± 95	0.0		
1 right ear	0.0	2.4	282 ± 90	0.0		2.9
2 left ear	0.0	0.0	378 ± 52	54.1	95	2.7
2 right ear	2.4	0.0	342 ± 104	55.3	1	
7 left ear } control						8.1
7 right ear } control						7.1
8 left ear } control						7.9
8 right } control						8.4

1-Percentage of the engorged weight converted into eggs

Induced resistance to *R. appendiculatus* infestation in cattle in the laboratory

We have already shown that on tick-naive rabbits, adult *R. appendiculatus* irradiated with 2.4 krad of gamma radiation, though completely sterile, feed to about 2/3 of the weight of untreated ticks. The duration of the feed is also extended, which might be advantageous since we are dealing with antigens injected into the host through the tick saliva during feeding (type 1 resistance). In collaboration with colleagues at the Kenya Agricultural Research Institute, (KARI) Muguga, we also showed that in cattle resistant to *R. appendiculatus*, there is interference with the transmission of East Coast Fever. The objective of the present work is to render cattle type 1-resistant for use in further ECF transmission studies, and for use in field experiments to control ticks by means of resistant cattle.

Two year-old Boran cattle were bought from a ranch in Laikipia District where it is normally too dry for *R. appendiculatus* and where *Theileria parva* (causative organism of ECF) is also absent. A random sample of 5 animals was tested and found to be serologically negative for *T. parva*. These were placed in stalls and used for the following experiment using adult ticks irradiated with 2.4 krad.

Day 0, 1st test feed
Day 14, feed 500 irradiated adults on each ear
Day 34, 2nd test feed
Day 55, 3rd test feed
Day 75, feed 250 irradiated adults on each ear
Day 114, 4th test feed

The results summarized in table 17 show that some degree of resistance was engendered. The mean engorged weight decreased significantly ($P < 0.05$). The cattle results are also compared with the pooled control rabbit results. When individual cattle were examined for the first and fourth tests, it appeared that one animal had failed to develop resistance. There was an increase in the mean engorged weight of the test nymphs on this animal, whereas on the remaining four animals a mean reduction of 32% in engorged nymphal weight was registered.

The mean duration of the test feeds varied from 6.8 days in the second feed to 11.7 days in the third feed. Environmental temperature is known to affect the duration of the nymphal feed, but not to the extent shown here. Mean duration to first engorged nymphal detachment was also calculated for each feed and was found to be highly correlated ($r = +0.95$) with the mean duration of the feed, indicating that some controlling factor was at work early in the feed.

Table 17. Development of Type 1 resistance to ticks in cattle.

	1st feed (day 0)			4th feed (day 114)		
	days	%fed	mg	days	%fed	mg
Five cattle	8.8±0.5	90.2±3.1	8.9±0.4	7.9±1.9	81.3±9.0	6.7±1.5
	(5)	(5)	(5)	(5)	(3)	(5)
Two rabbit controls	4.7±0.1	85.0±1.4	9.4±0.4	5.3±0.5	95.0±1.4	9.8±0.5
	(2)	(2)	(2)	(2)	(2)	(2)
Cattle-fed NN as % of controls[1]	166	99	96	149	89	73
Min °C	9.8				11.6	
Max °C	23.2				24.8	

Notes
1=Pooled data from 7 control rabbits only (one died during 3rd feed)
Mean results (±SD) for engorged ticks in test feeds with 100 nymphs (NN), sample size in brackets
Feeds of irradiated adults (equal numbers of each sex) were started on day 14
(1,000 ticks per host) and day 75 (500 ticks per host). Mean environmental temperatures are given for the first 5 days of each feed

FIELD TEST TO ASSESS CATTLE HOST-RESISTANCE TO *R. APPENDICULATUS*

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

A field test is essential for studies on natural or artificial host-resistance to ticks.

With single-host ticks like *B. microplus*, this test is carried out by applying a known number of larvae on the animals and subsequently counting the number of females between 4.5 and 8 mm in length from day 18 to 22. The application of 100 nymphs (NN) of *R. appendiculatus* on one ear of cattle or rabbit has been previously used in the laboratory to assess resistance. In the course of an on-going experiment, cattle, believed to be susceptible to *R. appendiculatus*, were brought to Intona Ranch and the 100 NN test performed on them to evaluate their resistance status.

Fifteen cattle, up to then regularly dipped in quintiophos (Baedip, Bayer E.A. Ltd.), were washed with detergent on 3 successive days and their ears thoroughly cleaned and clipped. Earbags, made of nylon monofil of 236 μm pore size, were applied to the ears with the use of chloroprene glue. Nymphs from the tick colony at Muguga were transported to the field by placing the tubes on wet sand in a polysterene box. The results of the test and a comparison between right and left ears are presented in table 18. It can be seen that no significant differences were observed between the ears, and the animals appeared to be *R. appendiculatus*-susceptible.

A few earbags were lost and, although immediately replaced, this may explain the relatively low percentage of engorged nymphs recovered. The weather was hot and the ticks were drying within the earbags. The bags were therefore opened twice a day (1000 and 1500 hours) instead of a single collection at 0900 hours as in the laboratory.

Cattle stayed on pasture all the time, except during heavy rains when they were put under cover. They did not

Table 18. Results of 100 NN tests carried out on cattle in the field; comparison through a paired sample 't' test of results on right and left ear

	Right ear Mean ± S.E.	Left ear Mean ± S.E.	
Percentage engorged	66.5 ± 4.0	66.0 ± 4.2	0.2 NS
Mean weight (mg)	8.0 ± 0.2	7.8 ± 0.2	1.2 NS
Mean No. of days	4.1 ± 0.1	4.0 ± 0.1	0.6 NS

Critical value of t with 14 df is 1.8 (P<0.1)

show discomfort and immediately after earbag and tick application they grazed normally.

This test has been shown as a reliable and useful way of assessing resistance to 3-host ticks in the field. It can be used until a serological test is developed.

EFFECT OF TICK INFESTATION ON CATTLE

Effect on cattle

Most of the work to assess the effect of tick infestation on cattle has been carried out in Australia for *B. microplus* with little or no work done on this problem in East Africa.

A study of the effect of artificial infestation with the Brown Ear tick *R. appendiculatus* on cattle was followed by a field trial to assess the effect of tick challenge on Boran (*Bos indicus*) cattle in an ECF endemic area of Kenya.

Artificial infestations with R. appendiculatus. Three similar groups of 10 cattle each, 5 to 14 months of age, were used. For 24 weeks, one group was infested with 200 male and 200 female *Theileria*-free *R. appendiculatus* (400-tick group) and another group received 20 males and 20 females every week (40-tick group). The third tick-free group acted as a control (0-tick group). The animals were weighed and bled once a week and the ticks counted on days 2 and 5 after tick application.

Although the two tick-infested groups showed lower weight gains than the controls at the end of the infestation, the difference was not significant. This may have been due to high variability within treatment groups. More detailed analyses demonstrated that during weeks 1 to 12, the percent weight gain was significantly lower in tick-infested groups than in the controls (Table 19).

Haemoglobin concentration (Hb) and packed cell volume (PCV) values were generally higher in control animals, but no marked trends were observed in either red blood cell (RBC) or white blood cell (WBC) counts.

The percentage of ticks feeding on day 2 did not differ significantly between the 2 groups of cattle. However, by

Table 19. Results of 3-factor analyses of variance on the percentage weight gain of three groups of cattle over four consecutive six-week periods

	Mean percentage weight gain		
	Controls	40-tick	400-tick
Weeks 1 - 6	27.6a	23.7b	22.4b
Weeks 7 - 12	17.1	15.1	15.7
Weeks 13 - 18	10.7	13.2	10.2
Weeks 19 - 24	7.8	8.3	10.8

Numbers followed by different letters, significantly different

day 5 a significantly higher percentage of the females of the 40-tick group remained attached than of the 400-tick group, $F(1,36) = 5.6$ ($P<0.05$), with a higher overall percentage of ticks feeding during weeks 1 to 6 than in any other period (Tables 20 and 21). The animals were not resistant to *R. appendiculatus* before the experiment started but had developed resistance by the time the work was completed (Table 22). This was the first attempt to assess the effect of adult *R. appendiculatus* infestation on liveweight gain of cattle under known levels of tick infestation.

Table 20. Mean numbers and percentages of *R. appendiculatus* females feeding on the 40-tick group of cattle on days 2 and 5 after infestation

	Day 2		Day 5	
Period	Number feeding	% over total applied	Number feeding	% over total applied
Weeks 1 - 6	17.0	85	11.0	55
Weeks 7 - 12	14.8	75	8.8	44
Weeks 13 - 18	16.5	83	8.2	41
Weeks 19 - 24	16.8	85	8.8	44

Table 21. Mean numbers and percentages of *R. appendiculatus* females feeding on the 400-tick group of cattle on days 2 and 5 after infestation

	Day 2		Day 5	
Period	Number feeding	% over total applied	Number feeding	% over total applied
Weeks 1 - 6	160.3	80	58.7	29
Weeks 7 - 12	165.5	83	52.6	26
Weeks 13 - 18	162.2	81	44.5	22
Weeks 19 - 24	169.3	85	49.8	25

The combination of factors such as a good feeding regime and the development of host resistance to ticks (in which grooming played an important part) may explain why cattle suffered from tick effects at the beginning of the trial and later recovered.

Table 22. Results of one-way analyses of variance of the results of 100 nymphal tests performed with *R. appendiculatus* on three experimental groups of cattle before and after tick infestation

	Percentage engorged				Mean engorged weight			
	0-tick	40-tick	400-tick	F(2,27)	0-tick	40-tick	400-tick	(F(2,27)
Before	75.7	93.1	91.6	2.6 NS	92.1	97.2	91.9	1.7 NS
After	98.6[a]	67.5[b]	51.5	4.7*	70.5[a]	53.9[b]	56.2[b]	12.2***

Notes. Numbers followed by different letters are significantly different
***P<0.05 ***P<0.001**
Results expressed as percentage of results obtained on control rabbits

Suppressed immune responses in tick-infested cattle. Lymphocyte reactivity in the cattle described above, was tested by assaying transformation responses to concanavalin A (Con A). All animals were tested on five occasions throughout the six-month period of the trial. Eleven incidents of low responsiveness to Con A were identified, as shown in table 23. No lowered responses were detected after ten weeks.

Table 23. Lymphocyte proliferation responses to Concanavalin A

Cattle group	Weeks					
	1½	6	10	14	19	24
Controls						
40 tick	444[a]	397				
	541	506	506			
	559	559	559			
400 tick	463	463	541			

***Reduced responses: 5000 counts per minute, combined with a stimulation ratio of < 15 on an initial and repeated test within 1 week. Generally lymphocytes gave mean counts of about 54,000 C.P.M. and S/R of 60**

[a]Identification numbers of individual cattle

To assess the effect of infestation on the induction of a primary immune response to antigen, the cattle were inoculated with duck red blood cells (DRBC). One third of each group was infected at monthly intervals after infestation, thus assessing status at 4, 8 and 12 weeks. Delayed type hypersensitivity (DTH) to DRBC was assessed after inoculation, and haemagglutination titres were measured on a weekly basis. Reduced DTH responses among the tick-infested group, as compared to the control group, were seen in those animals inoculated at 8 weeks, but not at 4 or 12 weeks. Haemagglutination antibody titres were higher in the control animals inoculated with DRBC at 4 and 8 weeks, but not at 12 weeks. To assess the effect of tick infestation in the development of immune response, all cattle were vaccinated with S 19 *Brucella* vaccine, one week before tick infestation. Half the animals in each group were revaccinated at 2 months, the remainder at 4 months. DTH brucellergen was assayed 30 days after the second inoculation. *Brucella* agglutination titres were assayed weekly. Cattle inoculated at 2 months showed differences in DTH responses between the infested and control groups. Infested groups had lowered responses. All animals inoculated at 4 months responded in a similar manner.

Maximum *Brucella* agglutination titres did not differ between groups before revaccination, though titres were generally maintained longer in the control group. After revaccination at 2 months, the maximum mean titres of the control group were twice those of the infested groups, and again responses were more prolonged in the control group.

It appears then, that sporadic and temporary immunosuppression occurs during *R. appendiculatus* infestation and that this happens quite early during tick challenge.

Field trial to assess the effect of tick infestation on cattle immunized against theileriosis. The study on the effect of tick infestation on cattle immunized against theileriosis was carried out at Intona Ranch in the Transmara Division of Narok District in Kenya. Twenty Boran heifers were immunized against theileriosis by infection with local strains of *Theileria parva parva, T. parva lawrencei* and *T. mutans*, followed by treatment with parvaquone (Clexon, Wellcome Kenya Ltd.) on day 12 after infection.

Acaricidal treatment using toxaphene (Coopertox, Wellcome Kenya Ltd.) was administered to one group of 12 cattle every week, leaving the remaining group of 8 untreated. All cattle were weighed and bled every week and half-body adult tick counts recorded. Table 24 shows the tick species found on the cattle, in order of abundance.

Table 24. Estimated mean weekly total adult tick loads, derived from counts of both ears plus half-body counts x 2

Tick species	Undipped	Dipped
R. appendiculatus	45.9	13.9
Amblyomma spp	35.8	1.8
B. decoloratus	6.4	0.2
R. evertsi	8.4	2.2
TOTAL	97.5	18.1

By the end of the trial, dipped cattle had gained significantly more weight than the undipped group t = 2.45, P<0.05 (Table 25), equivalent to 0.55 kg per animal per week. There was a significant positive correlation between mean adult counts and difference in weight gain between the dipped and undipped cattle (Fig. 1).

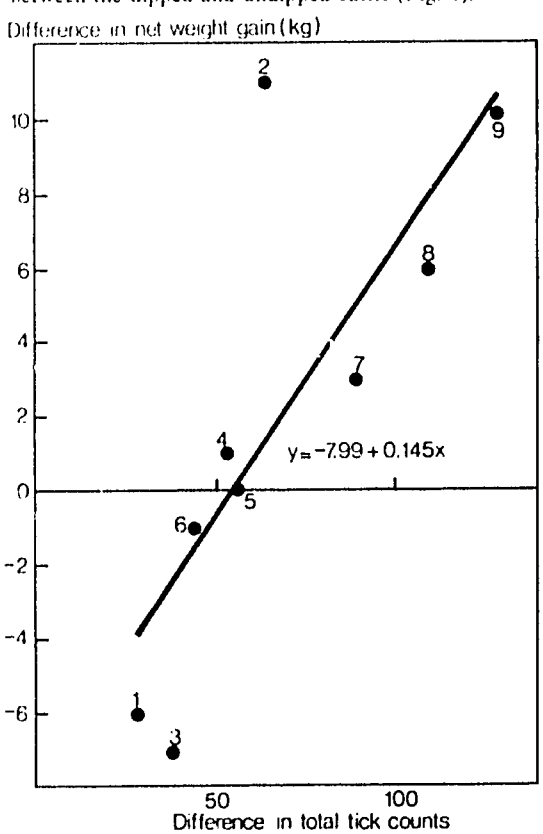

Figure 1. Regression of difference in weight gain on difference in total tick count per 3-week period. Nine periods are considered. F(1,7) = 10.3; P > 0.05)

Generally, lower values of Hb, PCV, RBC, and WBC counts were recorded in the undipped cattle but none of these was significant.

Table 25. Changes in mean body weight of undipped and dipped cattle

Period	Undipped	Dipped
	Mean (kg) ± S.E.	Mean (kg) ± S.E.
Week 0	270.6 ± 4.4	268.5 ± 3.4
Week 10	297.5 ± 5.2	306.4 ± 6.0
Week 20	305.6 ± 5.8	307.5 ± 5.4
Week 30	327.7 ± 4.8	342.2 ± 6.[illegible]
Gain (weeks) 0–30*	57.1 ± 4.8	73.7 ± 4.2

*Difference between undipped and dipped cattle significantly different t = 2.47, P < 0.05

As a result of recent improvements in immunization techniques against theileriosis, it was possible to assess the direct effects of tick infestation on the productivity of cattle in the field.

The trial showed that for the area concerned, and under the tick challenge described, dipping had a beneficial effect on weight gain estimated at 78 g per animal per day. Although we cannot extrapolate these results to other areas, they suggest that, in the absence of tick-borne diseases, the economic threshold for tick control in East Africa could be improved by a more rational use of acaricides.

RESISTANCE INDUCED BY LABORATORY AND FIELD-DERIVED *R. APPENDICULATUS*

J.B. Chaka

More work was carried out during the period under review to investigate the development of host resistance induced by laboratory and field-derived *R. appendiculatus* strains, separately. Four infestations of 500 larvae of each strain were applied successively on each of 5 rabbits. The two strains were then compared on the basis of the rate of development and level of host resistance attained. A test involving infestation with 100 larvae and 50 nymphs of one strain on one ear of each rabbit and a similar lot of the other strain on the other ear was carried out after the fourth infestation. The same procedure was used for nymph infestations.

Two observations were made of larval infestations (Table 26). The first one was that while the field-derived

Table 26. Mean weight and precentage (±SE) of larvae engorging on rabbits previously successively infested with 500 larvae of Narok strain and laboratory strain.

Infestation	% fed		Mean weight (mg)	
	Lab strain	Narok strain	Lab strain	Narok strain
First	90.2 ± 1.4	86.8 ± 3.1	0.52 ± 0.01	0.56 ± 0.02
Second	67.8 ± 6.5	78.0 ± 9.7	0.45 ± 0.02	0.54 ± 0.02
Third	42.0 ± 6.4	58.6 ± 6.0	0.40 ± 0.02	0.48 ± 0.02
Fourth	33.8 ± 3.5	60.5 ± 9.1	0.40 ± 0.2	0.46 ± 0.02

Narok larvae fed at a lower percentage on susceptible hosts (first infestation) they showed a lower rate of development of host resistance with respect to percentage fed, such that at the fourth infestation, twice as many Narok larvae fed as the laboratory larvae. The second observation was that the mean engorged weight of the Narok larvae on susceptible hosts was significantly higher (P<0.01) than that of the laboratory larvae and remained so through successive infestations. The nymph infestations gave similar results (Table 27).

Table 27. Mean weight and percentage (± S.E.) of nymphs engorging when rabbits were successively infested with 50 nymphs of two strains

Infestation	% fed		Mean weight (mg)	
	Lab strain	Narok strain	Lab strain	Narok strain
First	95.6 ± 2.0	89.7 ± 4.5	9.08 ± 0.22	9.82 ± 0.25
Second	81.0 ± 3.0	88.5 ± 2.2	4.06 ± 0.37	6.14 ± 0.51
Third	75.5 ± 9.6	84.0 ± 4.7	3.62 ± 0.30	4.60 ± 0.41
Fourth	73.0 ± 3.4	88.0 ± 2.9	3.84 ± 0.38	4.62 ± 0.46

When all the rabbits were tested for resistance against both strains, it was noted that while the laboratory larvae fed as well on rabbits previously infested with larvae of the same strain as on those infested with Narok larvae (Table 28), the Narok larvae fed to significantly heavier weights (P< 0.05) on rabbits previously infested with laboratory larvae than on those infested with their own strains. The percentage of larvae feeding in this test were also slightly higher. Test Narok nymphs behaved similarly as opposed to laboratory nymphs. Test with nymphs gave similar results but to a lesser extent (Table 29).

Table 28. Mean weight and percentage (± S.E.) of test larvae and nymphs engorging on rabbits after 4 infestations with 500 larvae of two strains

Test ticks	Infested with Lab strain larvae		Infested with Narok strain larvae	
	% fed	wt (mg)	% fed	wt (mg)
Lab larvae	34.0 ± 11.0	0.40 ± 0.03	18.5 ± 1.5	0.40 ± 0.01
Narok larvae	76.7 ± 7.4	0.53 ± 0.01	40.5 ± 18.5	0.42 ± 0.05
Lab nymphs	90.7 ± 1.8	5.5 ± 0.2	90.0 ± 0.0	6.2 ± 0.0
Narok nymphs	78.7 ± 8.5	7.2 ± 0.5	84.0 ± 12.0	5.9 ± 0.1

Table 29. Mean weight and percentage (± S.E.) of test larvae and nymphs engorging on rabbits after 4 infestations with 50 nymphs of two strains

	Rabbits infested with Lab strain nymphs		Rabbits infested with Narok strain nymphs	
	% fed	wt (mg)	% fed	wt (mg)
Lab larvae	46.0 ± 6.4	0.34 ± 0.04	59.8 ± 7.3	0.38 ± 0.01
Narok larvae	55.5 ± 2.5	0.42 ± 0.04	71.0 ± 5.0	0.42 ± 0.02
Lab nymphs	86.0 ± 4.0	4.99 ± 0.42	94.0 ± 2.0	4.64 ± 0.35

After the test nymphs had moulted, the scutal lengths of the emerging adults were measured. It was noted that for both strains the male scutal lengths were significantly more variable (P<0.01) when the nymphs had fed on rabbits previously infested with larvae or nymphs of a different strain. The scutal lengths of the males from Narok nymphs were even more variable (P<0.001) than laboratory males when the nymphs had fed on rabbits infested with laboratory larvae or nymphs. The data for the female ticks were not so clearly defined, possibly because female tick size is much less affected by host-resistance.

On the basis of the results obtained so far, it is to be noted that field-derived Narok strain was superior to the laboratory strain in terms of survival on hosts made resistant to both strains, much more so at a medium level

of host resistance. The increased variability of tick size when nymphs fed on hosts previously infested with another strain is an indication of inherent differences in the antigens injected by the different tick strains during feeding.

DEVELOPMENT AND SURVIVAL OF *R. APPENDICULATUS* UNDER DIFFERENT FIELD CONDITIONS

D.K. Punwa

As indicated in last year's report, studies have been carried out on the development and survival of the tick *R. appendiculatus* at the Transmara Division of Narok District and at Mbita Point Field Station of South Nyanza District, Kenya.

The Transmara area has a medium altitude (1600 m) with very high rainfall, while Mbita Point Field Station has a lake shore altitude (1230 m) but low rainfall. The species, however, is found under both these conditions.

Engorged ticks of all stages (larvae, nymphs and females) were exposed under field conditions and allowed to complete development under these conditions which are carefully monitored.

Table 30. Development periods of tick *R. appendiculatus* at the Transmara Division, Narok District, during 1984

Season	Pre-oviposition	Egg hatch	Larva moult	Nymphal amount
February	4 days	2 weeks	No sample	3 week
March	5 days	2 weeks	2 weeks	3 weeks
April	5 days	sample damaged	2 weeks	3 weeks
May				
June	6 days		3 weeks	3 weeks

While the study has just been started at Mbita Point Field Station, the work was started at the Transmara in February 1984. The results are shown on tables 30 and 31.

Table 31 Survival of *Rhipicephalus appendiculatus* at the Tansmara Division, Narok

	March	April	May	June	July
Adults	Unmoulted	100%	98%	98%	90%
Nymphs	Unmoulted	95%	47%	24%	20%

ACQUIRED RESISTANCE IN RABBITS IMMUNIZED WITH CRUDE MIDGUT HOMOGENATE AND FRACTION I PROTEIN OF MIDGUT

C.K.L. Mango, M.P. Cunningham, C.A. Agunyo

Groups of rabbits were inoculated with either crude midgut homogenate or Fraction I protein of midgut fractionated through Sephadex G-100. They were regularly boosted and test-bled and tested for precipitating antibodies using Ouchterlony method. When a strong precipitin reaction was observed, after 5 months and 4 months, rabbits immunized with crude midgut and Fraction I protein were challenged respectively with 35 female and 35 male ticks on one ear and 100 nymphs and 200 larvae on the other ear.

Observations were made on engorged weights of adult females, nymphs and larvae, mortality, weight of egg masses produced by females and finally the percent hatchability of the eggs.

One month after first challenge, a second challenge was done, the rabbits having been boosted once between the challenges. A third challenge was done two months after the second one, two boosters having been administered and similar observations made.

Crude midgut homogenate

Engorged weights. The mean fed weight of adult females during the first challenge was 294.7 ± 8.1 and dropped to 84.1 ± 13.6 and decreased further to 47.4 ± 9 mg during the second and third challenge respectively. On the other hand, the mean fed weight of female ticks fed on control rabbits were 401 ± 5.2 and 385.5 ± 12.9 mg during the first and third challenge respectively. Unfortunately there were no controls during the second challenge.

The mean engorged nymphal weights of ticks fed on immunized rabbits dropped from 8.8 ± 0.1 to 4.5 ± 0.2 and 4.0 ± 0.4 mg per nymph during the first, second and third challenge respectively compared to those fed on control rabbits of 9.9 ± 0.4 and 9.7 ± 0.2 mg/nymph during the first and third challenge respectively.

The mean larval fed weights also decreased from 0.58 ± 0.01, to 0.34 ± 0.03 mg during the three challenges, compared to 0.62 ± 0.08 and 0.54 ± 0.01 mg for control ticks during the first and third challenge.

Egg production. The highest means were achieved during the first challenge (145.8 ± 7.3 mg per tick) followed by a sharp drop to 28.0 ± 5.7 mg/tick during the second and lowest in the third challenge of 15.4 ± 3.3 mg/tick. On the other hand, the control ticks had mean weights of 212.1 ± 5.4 mg and 182.2 ± 11.6 mg/tick during the first and third challenges respectively.

Hatchability. The percentage hatchability of eggs resulting from feeding on immunized rabbits was 68.2% ± 2.8 and 51.4% ± 7.2 as compared to 80.0% ± 2.0 for ticks fed on control rabbits, during the first challenge. Unfortunately, we did not have controls during the second challenge.

Mortality. The mean percent mortality among adult ticks fed on immunized rabbits during the three challenges are 18.2% ± 6.3, 34.2% ± 5.3 and 28.6% ± 6.3 respectively, whereas the ticks fed on control rabbits the percent means are 5.7% and 2.9% during the first and third challenges respectively.

Among nymphal ticks, mortality was low with percent mean mortalities of 10.8% ± 2.7, 6% ± 0.6 and 18.6% ± 5.1 during the first, second and third challenges respectively.

The larval ticks show a progressive increase of mortality percent of 20.7% ± 3.1, during the first, 34.7% ± 5.5, during the second and 44.0% ± 5.1, during the third challenge. The mortality among the control larvae was 13.4% ± 6.5 during the first and 6.6% ± 0.6 during the third challenge.

These results show that rabbits immunized with crude midgut homogenate acquire resistance which is manifested in reduction of fed-weight in adult females

and their egg output during the first challenge. During subsequent challenges (2 and 3), there is a great reduction in fed weights of adult females, nymphs and larvae. Among the female ticks, the reduced fed-weight result in reduced egg output as well as hatchability. There is also increased mortality among ticks fed on immunized rabbits compared to those fed on controls.

Results of Fraction I protein of midgut

Engorged weights. On first challenge, the ticks fed on immunized rabbits took large blood meals of mean weights 370.7 mg ± 34.7. However, on second challenge the mean fed weight fell drastically to 64.4 mg ± 11.2 per female tick. The nymphal ticks did not show reduced mean fed weight on the first challenge (8.6 mg ± 0.3), on second challenge, the nymphal mean fed weight dropped considerably to nearly half (4.8 mg ± 0.2).

The larval mean weight similarly was normal on first challenge, 0.47 mg ± 0.1, and dropped to 0.39 mg ± 0.01 during the second challenge.

Egg production. On the first challenge, egg production was low 135.4 mg ± 15.7 per tick, on second challenge it was very much reduced to 27.3 mg ± 5.8 per female, compared to 172.1 mg ± 16.3 per control female.

Hatchability. The percent hatchability of eggs of ticks fed on immunized rabbits during the first challenge was 48.6% ± 10.5 compared to 75.1% among control ticks.

Mortality. The mean percent mortality among the adult female fed on immunized rabbits during the first and second challenges are 40.4% and 49.6% compared to 7.1% for the control ticks. Among the nymphal ticks there does not seem to be a pattern. The means were 61.5% and 14.9% as compared to 1.5% and zero in controls during the first and second challenges respectively. Within the larval ticks, the mean mortality rate recorded is 68.9% and 40.0% compared to 29.7% and 9.4% in controls during the first and second challenge.

The results show that Fraction I protein of tick midgut when used to immunize rabbits results in resistance which causes adverse effects to ticks fed on the rabbits. The resulting effects are reduction in blood intake which in turn results in reduced reproduction and egg hatchability. Also, all stages of ticks feeding on immunized rabbits experience higher mortality compared to the control ticks. Fraction I protein shows improved adverse effects compared to crude midgut homogenate. Therefore it is to be purified further and used for immunization.

IMMUNOGENICITY AND PARTIAL CHARACTERIZATION OF AN EMBRYONIC TICK CELL LINE FROM *R. APPENDICULATUS*

M. Nyindo, E.S. Dhadialla, L. Awuor

In the previous annual report it was stated that an embryonic cell line from *R. appendiculatus* was established from eggs, 21 days post-oviposition. However, this cell line did not grow fast enough to provide sufficient antigen to be used for experimental purposes. Another cell line has recently been established from eggs, 18 days post-oviposition, which has a high cell turn over (doubling time interval from 48 to 72 h). This cell line, like the previous one, consists of 2 cell types: fibroblast-like and epithelial-like.

A soluble antigen was prepared from the cell line. It was injected into rabbits after emulsifying in Freund's complete adjuvant. One booster injection of the antigen in Freund's incomplete adjuvant was administered 30 days later. A second booster was given in the incomplete adjuvant 3 weeks later. The rabbit was bled 14 days later for immune serum. A double immunodiffusion test was set up using the immune serum against the following antigens from *R. appendiculatus* as shown on figure 2: Vitellin (a), soluble antigen from the cell line (b), protein extract from adult fed female ticks (c), egg extract (d), midgut (e), saline (f) was used as control. As shown in figure 2 the immune serum against the cell culture soluble antigen reacted against all antigens of *R. appendiculatus* tested. No explanation for the reaction between whole tick homogenate and the cell culture soluble antigen can be given at present.

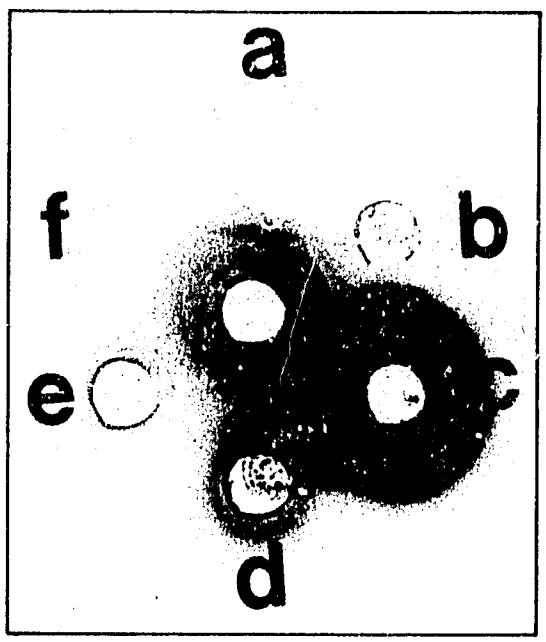

Figure 2. Results of double immunodiffusion test between the antiserum against cell culture antigen and different antigens.

The IgG fraction of the immune serum was conjugated with fluorescein isothiocyanate and the conjugate was applied onto the tick cells grown on coverslips. The results of the direct immunofluorescent staining confirmed the presence of 2 cell types as revealed by light microscopy. The epithelial-like cells stained more intensely than the fibroblast-like cells.

The existence of the 2 cell types was further confirmed by the transmission electron microscopy.

Medical Vectors

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Medical Vectors Research Programme

During the year 1984, Medical Vectors Research Programme continued to focus its activities on the epidemiology of leishmaniasis. The major areas of activities were as follows:

- *In Baringo where the existence of both visceral and cutaneous leishmaniasis has been established.*
- *In Mount Elgon area, studies of* Phlebotomus pedifer *and* Phlebotomus aculeatus *were carried out. These two species of phlebotomine sandflies are undistinguishable in the female forms through ordinary morphological characters.*
- *In Machakos and Kitui leishmaniasis foci, investigations were continued on breeding sites and sandfly taxonomy.*
- *In the laboratory, the host-parasite relationships, parasite identification through isoenzyme techniques and vector studies through biochemical and isoenzyme techniques were carried out.*
- *A PhD student concluded his investigations on the ecology of* Anopheles *mosquitoes and is in the process of writing up his thesis.*
- *The programme was involved in collaborative investigation with the CBRU on a natural plant extract which showed some potential in the control of leishmaniasis.*

In these areas of investigations, the programme made some significant achievements which are highlighted in this report.

EPIDEMIOLOGICAL INVESTIGATIONS OF LEISHMANIASES

M.J. Muiruri, C. Kamau, F.J. Kyai, D.M. Omogo, J. Mwandandu, J. Ndambuki

Breeding sites of phlebotomine sandflies

The breeding sites of phlebotomine sandflies were intensively investigated. Termite hills and animal burrows were carefully excavated and soil samples taken at specific depths of these niches. These were incubated in the laboratory in containers with an area of approximately 660 cm². The samples were vigorously agitated and searched to ensure that there were no adult flies. These samples were then maintained at specific humidity and temperature. They were covered first with fine mesh cloth netting then with black polythene sheeting to maintain darkness. Moisture was maintained by sprinkling water on the soil. Containers were examined mornings and evenings for adult flies.

Table 32 summarizes the number of species that emerged from soil samples taken from 4 animal burrows. Ten species of sandflies were isolated *(Phlebotomus martini, Sergentomyia antennatus, S. bedfordi, S. ingrami, S. africanus, S. clydei, S. adleri, S. affinis, S. schwetzi, S. graingeri.* Sex ratio (184 females and 266 males) is also shown in this table. The percent prevalence of the various species shows *S. antennatus* and *S. ingrami* with the highest prevalence, 47.33% and 35.56% respectively. *S. adleri, S. schwetzi* and *S. graingeri* were the least prevalent, 0.002.

Table 33 gives the summary of various species of sandflies that emerged from soil samples obtained from two termite hills. Nine species were recorded having emerged from these soil samples. There were 57 females and 62 males. The most prevalent species that emerged were *S. antennatus* (50.42%) and *S. ingrami* (24.37%). The least prevalent species was *S. affinis* (0.01%).

Table 32. Species, number, sex and percent emergence of sandflies from four animal burrows. Marigat, March-May 1984

Species	Total	Female	Male	Percent composition
S. antennatus	213	98	115	47.33
S. bedfordi	27	16	11	6.00
S. ingrami	160	62	98	35.56
S. africanus	7	3	4	1.56
S. clydei	32	2	30	7.11
S. adleri	1	1	0	0.002
S. affinis	3	2	1	0.01
S. schwetzi	1	0	1	0.002
S. grauogeri	1	0	1	0.002
P. martini	5	0	5	1.11
Total	450	184	166	98.69
Unidentified	6			

Table 33. Species, number, sex and percent prevalence of sandflies emerging from termite hills soil samples

Species	Total	Female	Male	Percent composition
S. antennatus	60	31	29	50.42
S. bedfordi	8	5	3	6.72
S. ingrami	29	13	16	24.37
S. africanus	3	1	2	0.03
S. clydei	9	2	7	7.56
S. adleri	3	3	0	0.03
S. affinis	1	1	0	0.01
S. schwetzi	2	0	2	0.02
P. martini	4	1	3	0.03
Total	119	57	62	89.19
Unidentified	1			

Host prevalence studies

Experiments on host preference using various animal baits have previously been carried out in Kenya by ICIPE scientists. The general conclusion by these workers was that *P. martini* and *S. garnhami* prefer reptilian hosts. These studies were, however, conducted in the laboratory using sandflies which had been captured the night before the experiment.

A study designed to observe natural behaviour of sandfly species in their natural resting sites, the animal burrows in Marigat, was designed to afford phlebotomine sandflies equal accessibility to various hosts for 6 months, January-June 1984, in order to quantify host selection and determine host preference.

A preliminary survey of feeding habits of various species was conducted in Marigat, using sandflies trapped from termite hill ventillation shafts. The engorged female flies were washed in detergent saline and the heads severed and preserved for identification. The rest of the body was placed in a gellatine capsule and sent to Imperial College, Silwood Park, UK, for blood meal identification.

A dog, a goat, chickens, lizards, rats and a mongoose were used in each trap. These were placed inside a cage and covered by a clear polythene sheet coated with castor oil to trap sandflies which were attracted to the animal bait. The open end of the cage was placed facing the animal burrows so that flies could be attracted to the host and possibly enter to feed and be trapped in the process of either getting inside or leaving after feeding. A control trap without a bait was set up in the same way.

The traps were set up in the evening at 1830 hours and flies which had been trapped over the night were collected in the mornings at 0700 hours. The flies were then taken to the field laboratory where they were washed in detergent saline and mounted on glass slides and identified into species.

The flies which were freshly fed on the bait were recorded after identification into species to determine the number of flies that actually fed on the animal bait. Table 34 summarizes results of blood meal analysis of flies trapped from termite hills ventillation shafts. Three *P. martini* fed on bovid hosts, 1 on mammal, 3 on dog and 3 on reptile. In general the *Sergentomyia* species fed mainly on reptilian hosts the only exception being where 1 *S. antennatus* fed on a dog and 1 *S. schwetzi* fed on bovid and mammal baits.

Various hosts, including mammalian, avian, and reptilian, revealed different attraction to different species of sandflies. Ten species of sandflies were investigated during these experiments and included the already incriminated vector of visceral leishmaniasis, *P. martini*, the vector of *Leishmania major*, *P. duboscqi*, and other groups belonging to the *Sergentomyia* genus.

Sandfly colony

It has been possible to study the biology of several species of sandflies and to colonize several species including *Sergentomyia* viz *S. ingrami*, *S. schwetzi*, *S. antennatus*, *S. clydei*, and *S. bedfordi*. Our sandfly colony reared various species of phlebotomine sandflies. Because of lack of proper environmental chambers, it was difficult to colonize the more delicate species like *P. martini*, *P. pedifer*, *P. elgonensis and S. garnhami*, but we have recently purchased environmental chambers and have vigorously embarked on colonization of these. Dr. T. Gemetchu who joined the Programme in October 1984 is in charge of a programme to rear and colonize the difficult species. During this period 1240 sandflies were issued to scientists for experiments within the programme.

Table 34. Blood meal and analysis of sandflies trapped using suction tube from termite hill ventilation shafts in Marigat, Baringo District

Species	Bovid	Mammal	Dog	Reptile	Negative	Total
P. martini	3	1	3	3	7	17
S. antennatus			1	50	20	71
S. bedfordi				2	2	4
S. schwetzi	1	1		32	27	61
S. africanus				15	7	22
Total	4	2	4	102	63	175

ARTIFICIAL INFECTION OF SANDFLIES WITH *LEISHMANIA DONOVANI*

J.B. Kadda, M.P. Nyamori, R. Musyoki

Two years ago when pilot work on the laboratory colonization of Kenyan sandflies was undertaken, plans were made to carry out experimental transmission of leishmania to be able to pinpoint vector species.

In our current studies, the susceptibility of laboratory-reared sandflies to *L. donovani* was investigated. Sandflies were fed on infected hamsters.

Feeding through a membrane

Sandflies were fed on defibrinated hamster blood containing culture forms of *L. donovani* through a membrane of a 1 to 2-day old chick. In the absence of a microenvironment chamber, the flies were maintained in an oven at 22-26°C with relative humidity fluctuating between 40 and 90%, and offered an unlimited supply of apple juice. They were dissected and microscopically examined for presence of parasites on various days, after the infective feed, ranging from day 2 to day 10.

Feeding on a hamster

Sandflies were fed on hamsters which had been infected with amastigotes and or primary culture forms of *L. donovani*, 3 to 4 months earlier. The flies were maintained in an oven and dissected for microscopical examination in the same way as the flies which were fed through a membrane. A breakdown of sandflies species fed and microscopically examined for the presence of parasites is given in table 35. The hamsters on which sandflies fed are still under observation and hence conclusions cannot be drawn as to whether or not the fed flies were able to infect parasites.

Table 35. Artificial infestation of sandflies with *Leishmania donovani*

		Sandflies investigated		Dissections	
		Total	Fed	Fed	Dissected
S. schwetzi	MF	53	47	28	65
	AF	96	48	23	35
S. antennatus					
	MF	2	2	0	0
	AF	13	13	0	0
S. ingrami	MF	227	141	77	71
	AF	16	11	5	4
S. garnham	MF	8	8	0	4
S. garnham	MF	8	8	0	0
P. elgonensis	MF	1	1	0	0
P. martini	MF	1	0	0	

MF - Fed through a membrane
AF - Fed on hamster
Only 1 *S. ingrami* was found parasitaemic

Of the 71 *S. ingrami* which were fed through a membrane, promastigotes were found in one sandfly on day 2 post-infection. In the various species, the flies which were without demonstrable parasitaemia had bacterial infection in the guts. The lack of parasite growth in the sandfly guts which contained bacteria reaffirms the antileishmanial effect of bacteria in the sandfly gut.

Investigations are continuing in order to compile data based on large numbers of different species of laboratory-reared sandflies to be able to confirm the susceptibility of Kenyan sandflies to *L. donovani*.

BIOCHEMICAL CHARACTERIZATION OF LEISHMANIAL ISOLATES FROM HUMANS AND WILD ANIMALS

B.M. Okot-Kotber, R. Ndurumu

In our report last year (1983), we highlighted the importance of the identification of *Leishmania* isolates from wild animals and sandflies, in epidemiological investigations. During that period basic facilities in terms of laboratory and *in vitro* culture methodology were still being established to enable us to carry out identification studies. Thanks to those efforts, we have been able to move ahead with the work whose results are being reported here.

A selected number of isolates from man and wild animals and reference strains representing the major groups of *Leishmania* spp. were mass-cultured *in vitro* following the procedure reported in last year's annual report. The following strains were used in the study:

Reference materials

L. major	- LRC L119
L. major	- LRC L137
L. donovani	- MAN/IN/80/DD8
L. aethiopica	- MAN/ET/83/Belehu/76/83
L. adleri	- LRC L121
L. agamae	- LRC L123

Unidentified isolates

ICIPE 126	from human spleen
ICIPE 140	from a lizard liver
ICIPE 180	from a genet cat spleen
ICIPE 224	from elephant shrew spleen
ICIPE 151	from a mongoose spleen
ICIPE 172	from a mongoose liver

The mass-cultured strains were processed for biochemical studies by sonicating in the cold in an equal volume of phosphate-buffered saline with 1% triton x-100 (PBS-pH 7.2). One half was treated with phenylmethylsulphonyl-fluoride - a proteinase inhibitor- to protect proteins from breaking down during processing. This fraction was used for general protein profile analysis. The other half was not treated and was used for isoenzyme studies following sonication and centrifugation. The supernatants were stored in liquid nitrogen, in the form of 10 µl beads, until required.

The following glycolytic isoenzymes were used for the identification of strains:

Alcohol dehydrogenase (EC 1.1.1.2)
Glucose-6 phosphate dehydrogenase (EC 1.1.1.49)
Glucosephosphate isomerase (EC 5.3.1.9)
Hexokinase (EC 2.7.1.1)
Isocitrate dehydrogenase (EC 1.1.1.42)
Malate dehydrogenase (EC 1.1.1.37)

Malic enzyme (EC 1.1.1.40)
Mannose-phosphate isomerase (EC 5.3.1.8)
Phosphoglucomutase (EC 1.7.5.1)
6-Phosphogluconate dehydrogenase (EC 1.1.1.44)

The extracts were subjected to isoelectrofocusing in either agarose or polyacrylamide. All these 10 selected isoenzyme systems consistently differentiated between strains. Figure 3 shows a typical isoelectrofocusing pattern of isoenzymes of promastigote extracts from the following isolates: ICIPE 126, 140, 180 and 224, and reference materials, DD8-*L. donovani*, L 119 and L 137 *L. major* and 76/83, *L. aethiopica* and L 457, a lizard material from Kenya of unknown identity. The enzyme is

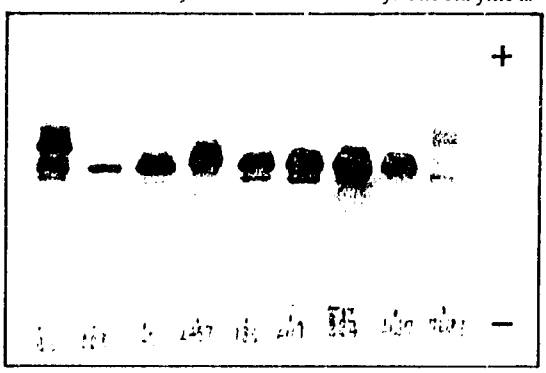

Figure 3. Typical isoelectrofocusing patterns of isoenzymes of leishmanial promastigote extracts from various isolates. Isolates: ICIPE - 126, 140, 180 and 224. Reference materials: DD8 - *L. donovani*, L 119 and L 137, *L. Major* and 76/83, *L. aethiopica* and L 457, a lizard material from Kenya of unknown identity. Enzyme: glucose - 6 - phosphate dehydrogenase. Running conditions: 1% agarose, 1500 V, Current maximum - 150 mA and power constant—15 W.

glucose-6-phosphate dehydrogenase. Running conditions are 1% agarose, 1500V, current maximum 150mA and power constant 15W. In all 10 isoenzyme systems, isolates from a lizard, genet cat and elephant shrew have the same patterns as those of LRC-L 119 (*L. major*, reference material from a Kenyan wild animal) and LRC-L 137 (*L. major* reference material from a cutaneous leishmaniasis patient in Israel). However, the strain, ICIPE 126, isolated from a kalaazar patient, did not have any similarities in the 10 isoenzymes with the *L. donovani* reference material from India. Similar results were obtained when the extracts were subjected to general protein analysis. Isoelectrofocusing of these extracts gave about 40 protein bands (Fig. 4). This is another very promising tool for strain identification and we are now working on it to improve on the quality.

The extracts from ICIPE 151 and ICIPE 172 isolates from mongooses were tested on one isoenzyme system (isocitrate dehydrogenase) and the patterns were found to be different from the leishmanial strains included in this test. These results show that genet cats and elephants shrews may be potential reservoirs of cutaneous leishmaniasis in Kenya and that the Kenyan strain of the kalaazar causative agent, ICIPE 126, is not similar to the Indian strain MAN IN 80/DD8. The lizard strain, ICIPE 140, raises very interesting speculations since it has the same isoenzyme profiles as *L. major*. More studies are underway to ascertain this apparent similarity.

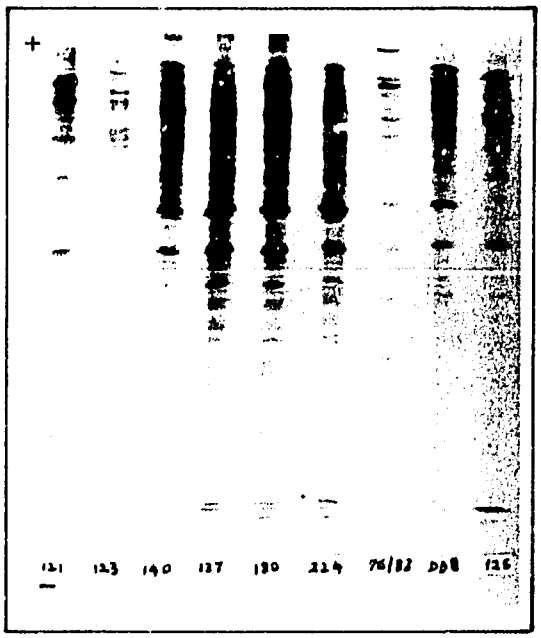

Figure 4. Isoelectrofocusing of general proteins of the same materials as in figure 3.

TAXONOMIC STUDIES BASED ON PHLEBOTOMINE SANDFLIES (DIPTERA: PHLEBOTOMIDAE) OF KENYA

F. M. Rogo, M. Okulo

Research continued during this year on the taxonomy of phlebotomine sandflies in leishmaniasis endemic areas of Kenya.

Forty one species of sandflies, belonging to two subgenera, *Phlebotomus* (10 species and one subspecies) and *Sergentomyia* (30 species) exist. Although morphological characteristics have been used to identify most of these sandflies, this criterion cannot be used to distinguish certain female vectors of cutaneous and visceral leishmaniasis from closely related species (cryptic forms). *P. pedifer* and *P. elgonensis*, *P. martini*, *P. vansomeranae* and *P. celiae* respectively, are not easily distinguishable using these characteristics. Isoenzyme analysis and micro-morphological techniques were used to try and differentiate between these cryptic forms. For the other sandfly species, morphological characteristics were used to distinguish them, but a taxonomic review of Kenyan sandflies had to be done because of the absence of complete taxonomic descriptions of species in this region and the absence of up-to-date identification keys. This therefore necessitated complete measurements of Kenyan sandflies, which have been used for a redescription and construction of an identification key for Kenyan sandflies.

Isoenzyme analysis of cryptic forms

This involved specific detection of enzymes following electrophoresis on 10% starch gel supporting matrix. The thorax and abdomen of single flies were homogenized with a glass rod in 5 μl deionized water in a

microtitre plate. The homogenates were applied to the gel using anchor threads and electrophoresed at a constant voltage (400V). Staining procedure followed recipes of Harris and Hopkinson.

In the *P. pedifer* group, 14 enzymes have been examined for individual sandflies. Of these 10 were detected with varying degrees of success and four of these have been found to be diagnostic enzymes for this group. The enzymic differences occurring in this group may be useful in differentiating *P. pedifer* from *P. aculeatus* during epidemiological studies in cutaneous leishmaniasis endemic areas of Kenya where these species occur sympatrically.

In the *P. martini* group, members of the group, especially *P. vansomeraniae*, and *P. celiae* are very scarce in the wild. The availability of these species was made even more difficult by the fact that all gravid females obtained from the wild and reared in the laboratory produced only *P. martini* (sensu stricto) males and females. Because of the rarity of the two species, it was difficult to compare them using several enzymes. Therefore, 3 enzymes which were found to be diagnostic for the *P. pedifer* group were used to try and characterize the few available specimens of this group. Only one enzyme is able to separate *P. vansomeraniae* alone from *P. martini* and *P. celiae* which are indistinguishable using the same enzyme. The other two enzymes show too much inconsistency to be diagnostic.

Micro-morphological examination of sandfly eggs using the scanning electron microscope

Eggs obtained from gravid females of the vectors and closely related species were mounted on metal stubs and allowed to rest in chambers with 5% gluteraldehyde fumes, and then double-coated with 100-200A° carbon and Au-Pd alloy in a vaccum evaporator, the chorion sculpture of the eggs were then examined and photographed using the scanning electron microscope, this was done to try and use the sculpturing pattern on the eggs to diagnose the vectors from closely related species.

The most common patterns observed were polygons and ridges in both the *P. pedifer* and *P. martini* groups. However, a very high degree of intraspecific variation was observed within the chorion sculpture of eggs laid by different females of the same species, indicating that the chorion structure is not a reliable diagnostic method for distinguishing between these cryptic species.

SCREENING NATURAL PLANT PRODUCTS AGAINST LEISHMANIA PARASITES

B.N. Odero

Leishmaniasis, the infectious disease caused by protozoan parasites of the genus *Leishmania*, is transmitted by female phlebotomine sandflies. The diseases exist in the Old and the New World in visceral and cutaneous forms and as mucocuteneous in South America.

The original leishmanial chemotherapeutics were based on pentavalent antimonials and the aromatic diamidines of 1930s. These are still the drugs of choice. Many chemical compounds have been tested against leishmania parasites since then, but none proved useful.

In our laboratory, several natural products from Kenyan plants, have been screened against promastigotes of *L. donovani in vitro* during the period under review. One antileishmanial component was identified. Further work to study its effect on the amastigotes (tissue forms) of *L. donovani* and *L. tropica in vitro* and *in vivo*, is in progress. Additional investigations expected to be conducted on the new compound include: phamacodynamics, toxicology and chemistry.

ESTIMATION OF THE SURVIVAL RATES OF FIELD POPULATIONS OF MALARIA VECTORS IN KENYA

C.M. Mutero

Field studies on the survival rates of *Anopheles funestus* and three members of the *A. gambiae* complex were concluded at the end of May, 1984. A final and detailed report on the findings will be available at the end of 1984.

Preliminary analysis of the results have nevertheless shown interesting species and seasonal differences in the survival and hence vectorial capacity of genetically defined populations of the *A. gambiae* complex. Previous methods of estimating survival rates of field mosquito populations were elaborated in the course of the present studies and investigations were also conducted on the occurrence of pre-gravid development in the different mosquito species during different seasons.

A complete analysis of the field data is expected to reveal hitherto unknown ecological and genetic heterogeneties, particularly with a members of the *A. gambiae* complex in Kenya. Such information combined with previous findings will undoubtedly make a positive contribution towards a better understanding of the local vector systems, and especially in relation to planning of appropriate strategies for their control.

Tsetse

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Tsetse Research Programme

In spite of many years of research and attempts at control measures, relatively little impact has been made in the control of trypanosomiasis. Indeed, tsetse and trypanosomiasis are still a problem in many areas of sub-Saharan Africa. For instance, in Kenya, it is estimated that out of 570 000 km² land area, nearly 138 000 km² is tsetse-infested. Although much valuable information has been accumulated on tsetse, there are still major gaps in the knowledge of the ecology and behaviour of this important vector of trypanosomiasis. In an attempt to fill some of these gaps, the attention of ICIPE Tsetse Research Programme has been directed towards examining new methods of tsetse control and also improving on existing strategies. The specific objectives are to develop environmentally safe tsetse control methods through studies of tsetse behaviour, vectorial capacity of tsetse populations, and through studies on the factors involved in the natural regulation of tsetse populations.

NKURUMAN TSETSE AND TRYPANOSOMIASIS PROJECT

R.D. Dransfield, M.F.B. Chaudhury, J.K. Gichuki, S.R. Tarimo

The objectives of this multidisciplinary team project are; first, to develop a dynamic epidemiological model of the trypanosomiasis challenge to cattle in the Nkuruman area, of which a major component will be a tsetse population model, secondly, to test the model in the field, and lastly to use the model to develop an integrated control package for trypanosomiasis in the area.

The area under study at Nkuruman comprises about 30 km² between the Ewaso Ngiro River and the escarpment, with vegetation ranging from open plains near the river to thickets and woodland near the base of the escarpment. The area is normally heavily utilized by Maasai for grazing their cattle, although with the 1984 drought the larger herds were moved to the top of the escarpment.

Phase I of the project, which involved monthly sampling of the tsetse for population data and trypanosome infection rates, over an 8.5 km transect from the river to the escarpment, has been successfully completed and the data are being analyzed by computer to provide the basis for a population model. Phase II which is now underway involves sampling on two additional transects, as well as work on pupal ecology, quantification of the level of predation and parasitism and dispersal studies.

In addition, cattle in the area are being monitored for trypanosome infection rates and a small herd of cows has been purchased for experimental purposes. Work has recently started on testing locally available odour sources (e.g. cow, sheep and goat urine) as attractants for use in control strategies in Phase III of the project. Cooperation with the local Maasai people has been excellent.

VECTOR POPULATION DYNAMICS

R.D. Dransfield, M.F.B. Chaudhury

Population sampling

Since the biconical trap is being used as the principal method of sampling adult tsetse, work is continuing on some of the problems associated with using traps to monitor population change. One of these is the 'site effect' whereby catch size is greatly dependent on the exact location of the trap. The first approach to this was to use a chemical attractant on the basis that if flies are attracted from a greater distance, inter-site variability may be reduced. In addition, chemical attractants found

to be effective in Zimbabwe could be tested on Kenyan *Glossina pallidipes* populations. For population sampling, a relatively cheap chemical is required for which the response to different dose rates is known. A series of experiments were therefore carried out to assess the efficacy of various different ketones (acetone, methylethyl ketone (MEK) and methyl vinyl ketone (MVK) and 1-octen-3-ol. This was done in collaboration with Dr. Saini, of the Sensory Physiology Research Unit, who has carried out behavioural bioassays using the same chemicals on *G. morsitans*.

Field trials were carried out by placing the chemicals in jars on the ground shaded by rocks at 30 cm from the base of biconical traps. Different known dose rates of the ketones were obtained by varying the diameter of the opening of the jars. Three dose rates with a control were tested for each of the three ketones using repeated Latin square design. High dose rates of each chemical were then compared simultaneously with a control. The effects of 1-octen-3-ol (constant dose rate) with and without acetone and with and without MEK were then assessed.

The results of these experiments using analysis of variance of the log transformed data are shown in table 36. Catches of *G. pallidipes* were increased by the use of acetone and MEK but not markedly by MVK. In the latter case the chemical underwent a colour change when exposed to field conditions, suggesting chemical change and possibly explaining its lack of activity. The experiment to compare high doses of the three ketones gave similar indices of increase confirming previous results, although the treatment effect was not significant owing to high variability. The high dose of acetone was most effective giving a mean 2.3-fold increase. In the first experiment with 1-octen-3-ol, this chemical was not found to be attractive, but the sample had been previously used elsewhere and was discoloured by contact with the rubber septum. When a fresh sample was used it was found to nearly double the catch, and together with a low dose of MEK the catch was nearly tripled.

The effects of the odour attractants used on age distribution and sex ratio of the samples were also assessed. No consistent differences in the age distribution were noted, but both acetone, (χ^2_{MH} = 7.46, P<0.01), and 1-octen-3-ol, (χ^2_{MH} = 9.42, P<0.01), were found to be more effective for males than females. For the high dose of acetone over three trials, the mean index of increase for males was 2.16 compared to 1.77 for females.

As a result of these experiments, the high dose of acetone was chosen for future experiments on the site effect because it produces a relatively consistent effect on catch size of *G. pallidipes*. It appears to be stable under field conditions and it is comparatively cheap and readily available. Thus in June 1984, the high dose of acetone was used on 41 traps on the regular transect on alternate days over a 6-day period. These data are at present being analysed to assess whether inter-trap variability has indeed been reduced, and whether the apparent pattern of distribution differs when an odour attractant is used.

The second approach to reducing inter-site variability was to try to understand more fully the factors causing it.

Table 36. The effect of various ketone and 1-octen-3-01 on the catch size of *G. pallidipes* with biconical traps at Nkuruman.

Dose rate (mg/h)	Detransformed mean catch (10^{-2}) 6h	Index of increase	F-ratio
Acetone			
Control	0	10.2	5.9*
Low dose	139	18.1	1.8
Medium dose	463	18.2	1.8
High dose	2391	27.9	2.7
MEK			
Control	0	5.9	
Low dose	77	13.4	2.7	4.3*
Medium dose	190	10.3	1.7
High dose	1546	9.8	1.7
MVK			
Control	0	30.6	-
Low dose	64	25.2	0.8	5.4*
Medium dose	145	29.6	1.0
High dose	703	37.7	1.2
3 Ketones			
Control	0	44.9	--
Acetone	2216	81.9	1.8	2.9NS
MEK	1694	69.4	1.5
MVK	936	48.0	1.1
Acetone + 1-octen-3-ol			
Control	0	26.3	—
Acetone	2216	62.2	2.4	12.5***
Octenol	50	30.0	1.1
Acetone Octenol	2216 50	53.7	2.1
MEK + 1-octen-3-ol			
Control	0	47.0	--
MEK	85	73.9	1.6	10.3***
Octenol	50	88.0	1.9
MEK Octenol	85 50	125.6	2.7

*P < 0.5*** P < 0.0001, ns non-significant

+ Octenol sample discoloured

These are thought to be visibility around the trap, light intensity (i.e. degree of shading) and proximity of hosts. The latter two were examined by emptying 4 traps at 15-minute intervals throughout the day and taking light intensity readings at the traps at 30-minute intervals, together with continuous readings of net solar radiation, temperature and humidity. Herds of cattle and goats moved along the track between the 4 traps at intervals and the times were recorded as well as any game animals seen. Preliminary analysis suggests that hosts passing near the traps cause only a transitory increase in catch size primarily when they pass within the range of visual attraction of the trap (15-20 m). These samples are still being taken for one day each month on the regular sampling trips and are also being used for fat-haematin analysis to assess changes in the nutritional state of the population.

Overall mortality and recruitment rates

R.D. Drummond, M.F. Chaudhury

The prolonged drought at Nkuruman in 1984 has had a pronounced effect on the distribution of *G. pallidipes* compared to 1983 when flies were largely restricted to the riverine thickets, woodland and valley areas, although a very low density population continues to persist along the Ewaso Ngiro River. Apparent tsetse densities within the former habitats have fluctuated greatly ranging from over 150 flies per trap per day in the riverine thickets in February and September to less than 60 per trap per day in several other months. The percentage of teneral flies has also varied widely, supporting the previous supposition that both survival and recruitment rates vary from month to month thus invalidating conventional techniques for estimating survival rates from the age distribution. Mark-release-recapture studies and estimates of the recruitment rate from the pupal studies now underway should enable the recruitment and survival rates to be estimated as well as the level of immigration and emigration.

Effect of salivary gland hypertrophy on survival rate

I.K. Gooder, A.P. Jones, R.D. Drummond

It has been suggested that *G. pallidipes* with virus-associated salivary gland hypertrophy may be in a stressed condition and that this may result in decreased longevity. This suggestion is based on observations that the degree of hypertrophy increases with age of the fly and that the incidence of infected flies has been observed to be inversely correlated with population density in one study area at the Kenya Coast. We have reasoned that if longevity is reduced in flies with hypertrophied salivary glands, the percentage infected should be lower in the older age groups.

Table 37 shows the frequency of hypertrophied salivary glands in various age groups of *G. pallidipes* dissected at Nkuruman. The overall frequency of enlarged glands is 1.2% in the 10 231 flies dissected. There is a remarkable homogeneity between the various wing fray categories of both male and female flies, with no evidence of any

Table 37. Frequency of hypertrophied salivary glands in *G. pallidipes* at Nkuruman, July 1983—June 1984.

		Wing fray category					
		1	2	3	4	5 6	Total
Males		1.33	1.30	1.40	1.4	0	1.3
	n	1123	2130	861	227	9	4360
Females	%	1.2	0.8	1.3	1.4	3.5	1.04
	n	1223	2915	1260	416	57	5871
Total	%	1.3	1.0	1.2	1.4	3.0	1.2
	n	2346	5055	2121	643	63	10231

decrease in the incidence of hypertrophied glands with age; if anything there is apparent increase in wing fray categories 5 and 6. These data suggest that the virus has no effect on the longevity of flies.

Reproductive biology of *G. pallidipes*

M.F. Chaudhury, R.D. Drummond, I.K. Gooder

Females caught in the traps were examined to obtain information on insemination, fertilization, egg and larval developmental rates and reproductive abnormalities including abortion rate. While the data are being processed through the computer for detailed analysis, several preliminary observations are reported here.

In *G. pallidipes*, unlike other tsetse species studied, mating and insemination occur several days after emergence of the female. Under laboratory conditions mating and insemination generally occur when the female is 7 to 9 days old (usually mated with a 10 to 14 day-old male). At Nkuruman, females of physiological age group *Oa* (age 1 to 5 days) and a substantial number of the age group *Ob* (age 6 to 10 days) have not been inseminated. Many of the uninseminated *Ob* females possessed a developing follicle as long as 1.4 mm. Normally these females should have been inseminated by this stage of follicular development. The percentage of uninseminated *Ob* females was relatively higher in the hotter months (about 12% in December 1983 and January 1984). Peculiar cases of unfertilized eggs in the uterus of inseminated females have been detected, but they were rare comprising less than 1% of the females examined. We have not observed ovulation in uninseminated females, a case commonly observed in laboratory colonies resulting in non-production of progeny.

As regards oocyte and larval development, a mature ovulated egg of *G. pallidipes* is usually 1.6 mm long or longer. We have observed however that the eggs of the first pregnancy cycle do not attain the usual size but are normally small (sometimes as small as 1.40 mm). This could be a contributory factor to the relatively high rate of abortion in the first pregnancy cycle which has been observed both at Nkuruman and Lambwe Valley.

Observations on the oocyte maturation in the ovary and the duration of the various stages in the uterus are yielding interesting information. Measurements of the largest developing follicle and the corresponding uterine content indicate that female flies are caught in the traps at three main stages of the pregnancy cycle: immediately following ovulation, immediately after the egg is hatched and at second instar larval stage. This may indicate the timing of blood meals during a pregnancy cycle.

Examination of *in utero* larval stages indicates that there are considerable variations in the rate of larval development among females caught. A large number showed pronounced retardation in larval development particularly in drier months. Many of these retarded larvae probably do not produce viable pupae. This may be a significant contributory factor to pupal mortality and thus the overall recruitment rate.

Larviposition and pupal biology

M.F. Chaudhury, R.D. Dransfield

Natural larviposition sites have now been detected in the vicinity of the three transects and regular sampling is underway from marked sites. Large numbers of live pupae as well as many empty puparia have been collected. In addition some artificial larviposition shelters have been constructed and these are proving effective. Individual pupae are weighed and kept in vials for emergence of adults or to detect mortality due to parasitism or other factors. Adults of the bombylid *Exhyalanthrax* spp. have emerged from some of these pupae.

TRYPANOSOME VECTOR INTERACTIONS AND DISEASE EPIDEMIOLOGY

Vector infection rate

S.R. Tarimo, T.K. Golder

Data collected so far indicate that *G. pallidipes* is the major vector contributing to trypanosomiasis at Nkuruman. Three trypanosome species have been observed, *Trypanosoma vivax*, *T. congolense* group and *T. brucei*. Of the 10 281 tsetse dissected between July 1983 and July 1984, 3.5% had a *T. vivax* infection, 0.8% *T. congolense* and 0.03% had a *T. brucei* infection. The highest infection rates were recorded for *T. vivax* in January 1984, and for *T. congolense* in July and October 1983 and March 1984 (Table 38). Infections of *T. brucei* and *T. congolense* group have been separately inoculated into individual mice, and stabilates made and stored for further experiments such as finding out whether the *T. brucei* are of the human or animal strain(s) and whether the *T. congolense* group contains the related species *T. simiae*. The possibility of a tsetse harbouring more than one parasite, e.g. *T. congolense* and *T. brucei*, is also being looked into as this has been suggested by observations in the Lambwe Valley.

Infection rate and age of tsetse

S.R. Tarimo, T.K. Golder, R.D. Dransfield

Table 39 shows the relationship between age and infection rate. The trypanosome infection rate shows a sharp increase from ovarian age 1 through 5 but then a drop in age groups 6 to 7. This drop in infection rate in the old tsetse (which has also been observed at the Kenya Coast and at Lambwe Valley) is interesting in that it suggests that either infection is having an adverse effect on longevity of the fly, or the old tsetse are losing their infection.

Table 39. Trypanosome infection rates in ovarian age group Ob-2 in *G. pallidipes* at Nkruman, July 1983 - June 1984.

		Ovarian age						
		Ob	1	2	3	4	5	6-7
Infection rates %		0.7	0.8	2.9	3.8	6.9	9.7	7.5
	n	240	654	456	342	490	487	255

It should be noted however that when wing fray is used as an index of age, no decline in infection rate in the older flies is apparent. This paradox should be clarified as more data on flies in the older age categories is gathered. Once the effects of climatic factors and feeding patterns have also been analysed, a mathematical function will be generated in an attempt to predict seasonal and spatial variations in the infection rate.

Infection rate and abortion rate

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

While there may be many different causes of abortion, in general they result from some form of physiological stress. We have recorded the frequency of abortions in *G. pallidipes* at Nkuruman in order to assess their contribution to reproductive loss and to determine if the stress of trypanosome infections increases the abortion frequency. The data on empty uteri are summarized in table 40 (this will slightly overestimate the frequency of

Table 40. Abortion frequency in unifected and trypanosome-infected *G. pallidipes* at Nkuruman, January—June 1984.

		Ovarian age						
		1	2	3	4	5	5/7	Total
Unifected								
Abortion rate %		6.5	6.8	4.9	4.4	5.2	4.6	5.6
	n	648	443	327	456	440	238	2552
Infected								
Abortion rate %		33.3	7.7	6.7	2.9	6.4	5.9	6.8
	n	6	13	15	34	47	17	132

Table 38. Monthly trypanosome infection rates of *G. pallidipes* at Nkuruman, July 1983—June 1984

			JY	A	S	O	N	D	JAN	F	M	A	M	JUN	Total
Male	*T. vivax*	%	2.9	3.7	2.0	3.5	4.6	3.2	13.6	3.9	4.6	3.4	3.9	2.9	4.1
	T. congolense	%	2.1	0.2	0.5	0.2	0.6	0.4	1.2	1.4	0.4	0.5	1.1	0.7	0.7
	T. brucei	%	0	0	0	0	0	0	0	0	0	0	0	0	0
		n	239	428	399	461	348	250	243	280	497	411	439	451	4428
Female	*T. vivax*	%	1.9	2.6	1.3	2.9	3.8	3.3	7.5	4.4	3.1	1.0	2.5	2.1	3.0
	T. congolense	%	1.6	0.5	0.4	1.6	1.4	0.5	0.8	1.3	1.5	0.2	0.8	0.0	0.9
	T. brucei	%	0.5	0	0	0	0	0	0	0	0.1	0	0	0	0.05
		n	369	381	520	555	499	366	387	619	519	410	854	374	5583

true abortions which is to be estimated using data on the length of the largest developing follicle) and show several interesting points. The relatively high abortion rate in Nkuruman compared to Lambwe Valley can be attributed to the harsher environmental conditions at Nkuruman. The highest abortion rates appear to occur in ovarian age categories 1 and 2. When abortion rates in infected and uninfected flies are compared, there is no significant difference in abortion frequency (X^2 ldf = 0.33, N.S.). The higher variability between age groups of infected flies is almost certainly due to the much smaller sample sizes. We may conclude therefore that trypanosome infection does not increase the abortion rate.

Tsetse feeding patterns

S.R. Jarvis, J.K. Golder

More than 1000 blood meals from *G. pallidipes* and *G. longipennis* have been collected on filter paper from Nkuruman. A total of 697 samples are currently being analysed by ELRAD. Preliminary results are summarized in table 41. Suids make up the highest percentage, and these are at present being tested to distinguish between warthog and bushpig. Other favoured hosts include buffalo, kongoni and cattle and, surprisingly, waterbuck and wildebeest. Both the latter species are relatively uncommon at Nkuruman, and are not normally regarded as favoured hosts.

Table 41. Feeding patterns of *G. pallidipes* at Nkuruman (n = 436)

	%
Suids (warthog and bushpig)	32.1
Water buck	11.2
Buffalo	10.3
Cattle	6.2
Wildebeest	5.7
Kongoni	5.5
Human	5.3
Bushbuck	3.7
Eland	3.2
Grant's gazelle	3.2
Giraffe	2.8
Goat	2.5
Elephant	1.8
Impala	1.6
Hyena	1.6
Sheep	0.9
Rabbits, Hare	0.9
Oryx	0.7
Felidae (lion and serval)	0.7
Avidae (birds)	0.0

N.B. Some blood meals were unidentified due to the advanced stage of digestion of the blood. A few rare species of animals have yet to be tested for (e.g. klipspringer, honey badger).

Infection rates in hosts

S.R. Jarvis, J.K. Golder

In order to develop an epidemiological model, trypanosome infection rates in livestock and wild hosts are required. Work has commenced in 1984 on monitoring infection rates in cattle.

A sample of cattle in a large herd of about 150 animals belonging to local Maasai are bled monthly and tested for trypanosome infection. Two capillaries and both thick and thin smears are taken, and both *T. vivax* and *T. congolense* infections have been detected. The packed cell volume (PCV) and girth of the animals are also recorded. This herd of cattle is moved from one area to another in search of grazing and because of the drought was moved to the top of the escarpment above the main study area in mid 1984. Sampling has however continued.

Another small herd of cattle (10 animals) belonging to a local Maasai has remained in or near the study area with ICIPE supplementing their feeding with hay. These animals have been given Isometamidium chloride (Samorin ®) for prophylaxis. The first breakthrough of infection occurred after 3 months. ICIPE has a further 3 cows which are kept with this herd. They are not given prophylaxis but are checked weekly for infection and treated with Berenil when necessary. These animals are at present used for experimental purposes but when the drought ends they will form the nucleus of a larger herd for which the 'Berenil index' will be estimated to give a measure of trypanosomiasis challenge.

Distribution and abundance of possible mechanical vectors

R.D. Dransfield, J.K. Golder

Sampling of tabanids, stomoxyds, hippoboscids and *Musca crassirostris* has continued using biconical traps and water traps. In addition, biconical traps have been set in local manyattas, and biting flies around the cattle have been collected by hand nets. The data indicate that *Haematobia minuta*, *M. crassirostris* and *Hippobosca* spp. are the main species feeding on cattle.

TSETSE TRAPPING STUDIES

M.L.A. Owaga

Field studies initiated in mid 1983 on screening a variety of natural products and chemicals as aids in attracting tsetse to traps continued. In the initial experiments, carbon dioxide and acetone were each found to be effective attractants in their own right, but gave even better results when dispensed together. Attention was, however, turned to natural products, namely waste products such as urine, faeces and body wash, from at least two wild hosts of tsetse, as possible effective olfactory attractants. It was shown that buffalo urine was the most promising of these products.

Since May 1984, experiments were initiated using buffalo urine under a variety of test conditions to elucidate its effectiveness as an olfactory attractant for *G. pallidipes*. The studies involved determining the position of the bait sample, in relation to the trap for maximum effectiveness; establishing at what volumes it starts to effect significant increase in the catch; determining how best to dispense it; comparing it with a similar sample from a closely related domestic species, cow; establishing the role of evaporative rate; and finding ways of

maximizing its potency e.g. by adding acetone which had proved effective in boosting the attractiveness of CO_2 to tsetse.

The results are summarized in table 42. Teneral flies of both sexes were excluded from the table and from the analysis. There was no significant difference in yields between the traps having the sample bait placed inside, at the foot of the trap, or at a distance from the trap. All of them gave higher yields than the unbaited trap.

However, there was a significant difference ($P<0.05$) between male and female *G. pallidipes* in the trap with the sample bait placed 40 cm away from the trap pole, it captured more males (Table 42). Another important observation was that the trap with the bait inside had a lot of tsetse flying within the upper cone and not entering the cage, (these were excluded from the counts). The two traps which had bait samples placed at a distance had only tsetse inside the collecting cages and hardly any flying about within or near the trap.

The age of the bait sample did not make any significant difference in its potency, however, the three-week-old and the five-month-old samples captured more tsetse and the difference might have been significant if there were more replicates.

Cattle urine performed poorly as a bait compared to buffalo urine, effecting only 1.8 times increase in the catch size whereas that of the buffalo gave 9.6 times increase for females and 8.2 times for males.

The rate of evaporation of the sample played an important part. The two bait samples which evaporated at the rate of 15-20 ml during the seven hours of operation gave statistically significant increase over those which evaporated at the rate of 1.5-2.5 ml in the same time.

From these and earlier observations made in the last annual report, it was concluded that buffalo urine is worth examining chemically so that the potent substances can be isolated and identified. It may be a very useful aid in tsetse sampling or control by removal trapping.

G. PALLIDIPES ECOLOGICAL STUDIES IN THE LAMBWE VALLEY

D.A. Turner

Research on *G. pallidipes* ecology in the Lambwe Valley was hampered considerably by the depletion of the fly population by spraying operations initiated in May 1984, when yet another attempt was made to interrupt

Table 42. Results of analysis of variance and Duncan's multiple test

Experiment	Sex	ANOVA: Degree of freedom	Mean square	F ratio	Duncan's test (* P 0.05 ** P 0.01)				
Age of sample						2	4	3	
1 control				**	1	*	*	*	
2 fresh sample	0	11	0.179	10.29	2				
3 3 wk sample					4				
4 5 month sample						3	4	1	
					2			*	
					3			*	
	0	11	0.179	5.58	4			*	
Buffalo and cattle urine						5	6	3	
1 control	0	11	0.15	13.84	1			*	
5 4th trap (unbaited)						5			*
3 buffalo sample						6		*	
6 cattle sample					6			*	
						5	6	3	3
					1			*	*
					5			*	*
	0	11	0.121	15.86**	6			*	*
Rate of evaporation	0	9	0.045	7.51		8	10	11	9
1 control					1			*	*
8 75 ml, small neck					10			*	*
9 75 ml, large neck					11			*	*
10 150 ml, small neck									
11 150 ml, large neck						4	2	11	9
					1			*	*
	o	9	0.07	4.19	4			*	*
					2				*
					5				
				**		13	15	12	14
Position of bait					1	*	*	*	*
1 control	0	14	0.112	17.82	13				
12 sample inside					15				
14 sample 40 cm away					12				
15 sample 75 cm away									
	o	14	0.126	9.2**		13	15	12	14
					1	*	*	*	*
					13				
					15				
					12				

trypanosomiasis transmission in the area. Investigations carried out during the period include:

- Surveys to determine the limits of *G. pallidipes* distribution in the environs of the Lambwe Valley, in the Gembe and Gwassi hills particularly, which flank the valley to the east. Low density (< 1 fly/trap day) populations were found to exist in all of the small, isolated patches of thicket at the tops of hillside gullies on all sides of the hills. This information was sought to gain some further understanding of why past attempts to eradicate *G. pallidipes* were unsuccessful, and also as essential data in the event that a proposal materializes for tsetse eradication in the Lambwe Valley by the sterile insect method.
- Determination of the nutritional status of *G. pallidipes* populations from fat haematin analyses. Samples were collected bimonthly from populations occupying thicket, woodland and conifer plantation vegetation types to assess whether there are correlations between nutritional status and seasonal factors and tsetse density. Samples are being analysed by the Tsetse Research Laboratory, Langford, Bristol. Results returned so far are still too few to attempt any interpretation of the data.
- The use of electric screens to investigate aspects of sampling bias in biconical trap captures, with particular reference to sex ratio, age composition and female pregnancy. Results so far indicate that in so far as age structure and pregnancy are concerned, trap captures provide a good representation of the active population and that males appear to be under-sampled in biconical traps. It was also hoped that electric screens would provide some data on arthropod predators of adult tsetse, possibly in pursuit of tsetse congregated around biconical traps. So far only in one instance was a known tsetse predator (an Asilid fly) caught.
- Investigations of pupal ecology, with particular reference to estimating mortalities from predation, parasitism and underdevelopment from visual examination and measurements of puparia. This work continues to be held back by the labour and time required to locate puparia in the Lambwe Valley, where they do not appear to have an aggregated distribution which normally facilitates searching. Attempts to construct artificial larviposition sites were not successful. It is hoped that the *G. pallidipes* colony established at Mbita Point Field Station will soon be in a position to supply puparial material for field studies.

G. PALLIDIPES POPULATION DYNAMICS IN RELATION TO THE EPIDEMIOLOGY OF AFRICAN TRYPANOSOMIASIS IN LAMBWE VALLEY WESTERN KENYA

L.H. Otieno, S.R. Tarono, S. Darji

These studies are aimed at collecting data for *G. pallidipes* population dynamics and their trypanosome infection rates so that trypanosomiasis risk in Ruma (inside the park) and at Riamakanga (an interphase between game animals and human activities) could be determined. The studies are also meant to provide baseline data for the envisaged control attempts using sterile insect technique. Studies initiated last year in two study areas were continued. *G. pallidipes* population was sampled using the biconical trap once every month. The number of flies caught per trap (six traps in each study site) was noted. A sample of the female flies caught was aged according to the method described by Saunders, and these plus an equal number of males from the same trap, were dissected and examined for the presence of trypanosomes.

BIOLOGICAL CONTROL STUDIES

Inducible immune proteins in the haemolymph of *Glossina morsitans morsitans*

G.P. Kaaya, L.H. Otieno, S. Darji

Humoral responses of *G. m. morsitans* to various species of bacteria and to *T. brucei* were investigated. Further to our earlier observations that vaccination of *G. m. morsitans* with sublethal doses of live *Escherichia coli* confers protection against subsequent lethal doses, we have now demonstrated remarkable increases in two haemolymph proteins in vaccinated *G. m. morsitans*.

Using SDS-Polyacrylamide gel electrophoresis and suitable molecular weight markers, we have demonstrated that the two immune proteins possess molecular weights of ca. 17 and 70 Kilodaltons and that their increase begins at approximately 18 h and 48 h respectively after vaccination. *E. coli*, *Enterobacter cloacae* and *Acinetobacter calcoaceticus* induced marked production of these proteins only when injected alive. Injection of heat-killed bacteria had no effect. Live *Micrococcus luteus*, *Bacillus subtilis* and *T.b. brucei* also failed to induce production of these proteins.

However, injection of live *T.b. brucei* into the haemocoels of *G.m. morsitans* was followed by a rapid elimination of the trypanosomes so that only approximately 1% of the injected numbers were present 48 h post-injection and they became progressively sluggish as they stayed in *Glossina* haemocoels. These observations strongly suggest the presence of anti-bacterial and anti-trypanosomal factors in tsetse haemolymph and further experiments to characterize them are in progress.

Potential of the tsetse virus in the biological control of *G. pallidipes*

M.O. Odondo

Laboratory-reared teneral *G. pallidipes* were inoculated with the tsetse virus by microinjection into the haemocoel, and feeding through micropipettes. The inoculated tsetse were reared on rabbits for 45 days and, during this time, feeding, flight and mating activities were recorded. The tsetse were dissected and the conditions of the salivary glands and gonads noted. F_1 pupae were allowed to emerge, dissected and the salivary glands examined for hypertrophy and the gonads for sterility.

There was no reduction in the activity of inoculated tsetse. Infection level was 23.5% in the treated adults

(Table 43). All infected males were sterile while females were fertile. There was no significant difference in the maternal age at larviposition, F_1 pupal weight, or incubation period of F_1 pupae between treated and untreated tsetse.

Table 43. Infection and sterility in *G. pallidipes* inoculated with the tsetse virus

	infection		sterility*
Treatment	00	+ + 04	
Inoculated	12.8	30.8	12.8
Control	3.1	4.0	3.1
Offspring	86.0	58.3	80.0

*males only

A high proportion of F_1 adults (65%) were infected with category 4 hypertrophy of salivary glands. All males with enlarged glands were sterile. The evidence obtained shows that the tsetse virus could be used as a sterility-imparting factor in the biological control of *G. pallidipes*.

Fly population densities have continued to be very high (110-574 flies trap day) in the Ruma thicket compared to (45-359 flies trap day) in Riamakanga thicket which is quite close to human settlements. In both study areas observations so far reveal no direct relationships between the fly density and trypanosome infection rates. In both areas, lowest fly densities were observed during the long rains in the month of April. The data from the two study areas are being analyzed by computer.

Mixed trypanosome infections

It has been observed from time to time during the above studies that some flies showing apparent *T. congolense* infections as revealed by fly dissection do harbour cryptic *T. brucei* infections. This was discovered when titurates of *G. pallidipes* proboscis infected with trypanosomes were inoculated into susceptible mice. The ensuing infection usually turned out to be mixed *T. brucei* and *T. congolense*. *T. congolense* normally appearing much later. The slow growth *T. congolense* parasitaemia showed clearly that the growth rates of the two trypanosome species differed. This is a point worth noting as it may well affect many experimental designs. It also underlines the need for a more accurate method of determining trypanosome species infection in the tsetse.

Preservation of live trypanosomes in tsetse tissues

In order to reduce the work load during field studies, it has become necessary to devise a method which can be used to identify trypanosome-infected flies after a period of cryopreservation.

A preliminary approach to this problem has involved attempts to cryopreserve trypanosome-infected flies in phosphate-buffered saline glucose (PSG) mixed with 10% glycerine and 7.5% Dimethyl sulfoxide (DMSO).

Two trials carried out so far indicate that trypanosomes in the tissues survived very well in samples cryopreserved at pH 8.0.

G. PALLIDIPES REARING AT MBITA POINT FIELD STATION

E.H. Otieno, R.N. Ochieng, M.I.B. Chaudhury

The use of sterile insect technique in the integrated approach to *G. pallidipes* control in the Lambwe Valley depends very much on the ability to rear this species in the laboratory. With this objective in mind, attempts to colonize *G. pallidipes* from Lambwe Valley at Mbita Point Field Station were started in March 1983 in a makeshift insectary. The initial attempts were very successful.

In order to facilitate further work, an improved insectary was therefore constructed based very largely on the earlier prototype. The only major improvements were a concrete floor and a concrete wall up to 1 m from the floor. The rest of the building was thatched with grass. Electrical lighting was also introduced to facilitate work at night. With this arrangement, the insectary maintained fairly uniform humidity and temperature. Flies introduced in this insectary are doing very well. Emergence rates have ranged from 85-95%. Pupal weight has averaged 37.0 mg with a range of 25-42 mg. Flies emerging from puparia weighing below 25 mg do not usually survive. No drastic mortality has been noticed. The colony size at the time of writing was 1510 mated females.

Chemistry and Bioassay

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Chemistry and Bioassay Research Unit

This year's report reflects a shift in the Unit's research activities towards the implementation of 1984-86 Strategic Plan. Reports on the purification and characterization of tick proteins and the Maruca *allomones from the cowpea plant underline greater emphasis which will be given to tick-antigen and chemical basis of plant resistance work respectively in the coming years. The installation of a VG 12-250 quadrupole gas chromatograph mass spectrometer means that our chemical work, particularly that involving volatiles (pheromones, plant odours, tsetse host odours etc.), can be pursued with greater vigour.*

Apart from the GC-mass spectrometer, a number of new equipment arrived during the year: a high-speed centrifuge and a low temperature freezer for biochemical work; a Packard Model 4530 liquid scintillation counter coupled with Phillips P2000 I microcomputer and a Birchover radioisotope camera for the ICIPE/IAEA Radioisotope Project. What remains now to fill up the gaps is an FT NMR equipment and a number of smaller items like a UV spectrophotometer, drop counter current instrument, a spinning band column, preparative liquid chromatography equipment etc. It is hoped that funds will be available to enable the unit to acquire these in the next two years.

A SYNOPSIS OF THE UNIT'S MAJOR ACCOMPLISHMENTS

P.G. McDowell, A. Hassanali

Protein biochemistry

A carboxyl proteinase from the gut of partly engorged female *R. appendiculatus* ticks has been partially purified and shown to be similar to cathepsin D of mammals. Further purification of the enzyme as well as immunological studies are in progress.

Vitellin, the major egg yolk protein from the eggs of *R. appendiculatus* has been purified (~95%) and shown to be a heme-containing glycolipoprotein. The purified vitellin reacted with anti-serum raised in rabbits against fed adult female whole tick homogenate, thus confirming that it is one of the 14 antigens present in the homogenate. Purification of other potential antigens is underway.

The affinity constant (Ka) of an ecdysone-binding protein fraction was estimated by isotope dilution technique and shown to be about 5×10^{5} at room temperature. Continued interest in the purification and characterization of the ecdysone-binding protein stems from the possibility that it may be used to produce antibodies in livestock which would sequester and inactivate ecdysone carrier protein in ticks.

Allelochemical research

Progress has been made in the fractionation and isolation of feeding deterrents from ethyl acetate extract of a *Maruca*-resistant variety of cowpea, TVu 946. A mixture of mildly active phytosterols isolated from the active fraction is currently being investigated spectrally. Isolation of the more active antifeedants is underway.

Two more projects were initiated during the year: isolation of sorghum shootfly oviposition stimulant from a susceptible sorghum variety and gas chromatographic examination of volatiles from susceptible and resistant varieties of sorghum seedling. However, it is too early to report on these this year.

Pheromone research

Pheromone research is now set to go ahead with the

installation of the new mass spectrometer. The emphasis will be on crop pest moths and projects on *Maruca* and *Chilo* have already commenced, although it is too early to report on these this year. Some outstanding questions on volatile pheromones in *R. appendiculatus* are being answered, and further to the report last year on the presence of 2,6-DCP in adult female *R. appendiculatus*, the same compound has been detected in the larval stage of the tick. Several other compounds have also been identified.

Anti-insect compounds from tropical plants

A new pterocarpan was isolated from *Tephrosia hildebrandtii* with antifeedant activity against *M. testulalis* larvae. This finding has raised the possibility that cowpea phytoalexins, which also have the pterocarpan skeleton, might constitute a basis for induced resistance in the plant against *M. testulalis*.

The antifeedant property of the leaf extract of *Calpurnea aurea* against the African armyworm has been shown to be due to the presence of a dehydro derivative of 0-(2-pyrolylcarbonyl)-virgiline previously isolated from species of *C. aurea* found growing in South Africa. Interestingly, 0-(2-pyrolylcarbonyl)-virgiline itself, shows no antifeedant activity against the armyworm.

The larvicidal effect of the hexane extract of *Plumbago zeylanica* against *Aedes aegypti* larvae has been shown to be due to plumbagin previously identified at ICIPE as the antimicrobial and insect antifeedant agent present in *Plumbago capensis*. Fractionation of a potent larvicidal fraction of an extract of *Spilanthes mauritiana* and identification of the purified components is underway.

Eight closely related limonoids obtained by isolation or partial synthesis have been screened for antifeedant activity against *Spodoptera exempta*, *E. saccharina* and *M. testulalis*. No simple structure-activity relationship has so far emerged. However, promising levels of activity have been demonstrated by deoxylimonin which has been selected for further derivatization to compounds with potential systemic antifeedant properties.

IAEA/ICIPE radioisotope project

This three year project started late in 1983 with the establishment of a small radioisotope standard laboratory and the ordering of a number of items of equipment. The purpose of the project is to establish facilities for employing nuclear techniques in insect entomological and biological research and training regional scientists in the use of such techniques. The project provides for 3 visiting experts for the three year period, each for 3 months. The first visit was completed between February and April 1984 and succeeded in establishing the physical facilities of the laboratory and providing some training to staff. The second expert visit due to take place early in 1985 will concentrate on further training of staff by application to their own research.

Lignocellulose project

Four fungi associated with the fungus-comb of *Macrotermes michaelseni* have now been completely identified. Mycotetes of *Termitomyces* which have shown high levels of cellulase activity was successfully cultured on potato dextrose agar.

Several lignin substructure model compounds were synthesized and used for assaying novel enzyme activities in the isolated organisms. Cellobiase oxidase, protocatechuate oxygenase, demethylase, an H_2O_2 depedent ligninase are some of the enzymes detected in various cultures. A start was made on the use of radiolabelled substrates to monitor the state of degradation of substructure and polymeric compounds.

DIGESTIVE ENZYMES OF THE BROWN EAR TICK, *RHIPICEPHALUS APPENDICULATUS* NEUMAN - SOME PROPERTIES OF A CARBOXYL PROTEINASE FROM THE TICK GUT

R.M.W. Vundla, G.V. Achieng'

The exploitation or manipulation of those special physiological and biochemical adaptations which enable ticks to digest and metabolize large volumes of blood may be important in tick control. The digestive enzymes constitute an essential component of the biochemistry of digestion in ticks, and an understanding of their nature and mode of action is therefore important.

A carboxyl proteinase from the gut of partly engorged (days 5 and 6 post-feeding) female *R. appendiculatus* ticks has been partially purified on sephadex G100. In this report, some chemical properties of the partially purified enzyme are presented.

Effect of substrate concentration on the enzyme activity. The partially purified enzyme was assayed in 0.25 M sodium formate - formic acid buffer pH 3.3, containing 0.2M sodium chloride, with a) haemoglobin, concentrations ranging from 0.1-2% and b) bovine serum albumin (BSA) concentrations of 0.1-2%. The enzyme hydrolyzed both haemoglobin and BSA. The Michaelis constant (K_m) values were 0.2% for haemoglobin and 0.53% for BSA (Figs. 5 and 6).

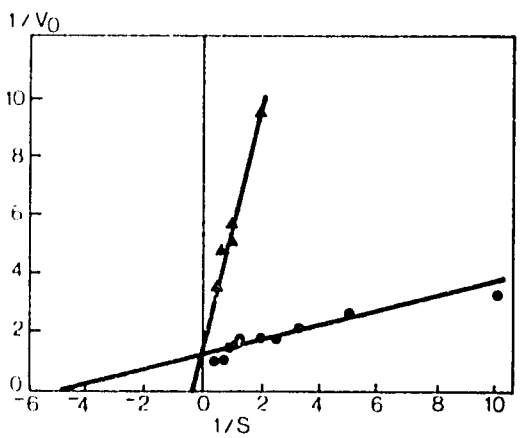


Figure 5. Lineweaver-Burk plot showing the effect of haemoglobin concentration on *R. appendiculatus* carboxyl proteinase activity (●—●) and competitive inhibition by 3 nM pepstatin (▲—▲)

Optimum pH. Phosphoric acid - formic acid-acetic acid buffers containing 0.2M NaCl and ranging in pH from 1.8 to 6.2 were used to assay the enzyme with haemoglobin

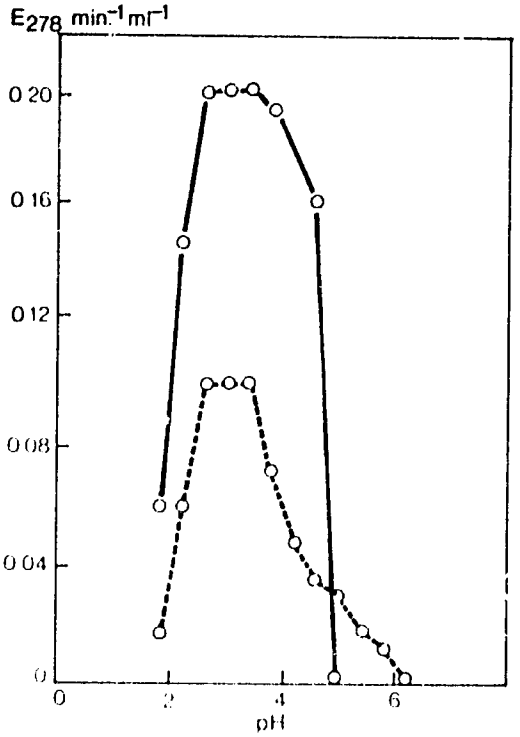


Figure 6. Effect of pH and *R. appendiculatus* gut carboxyl proteinase activity using BSA (O-O) and haemoglobin (O-O) as substrates.

and BSA. Optimum activity was obtained between pH 2.6-3.4 for both substrates (Fig. 7).

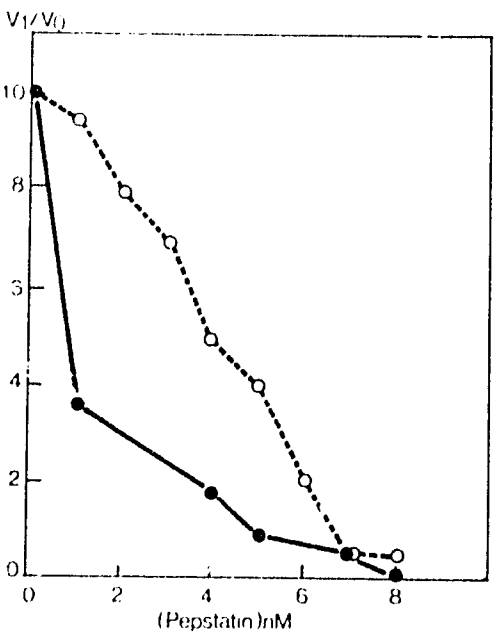


Figure 7. Effect of pepstatin on *R. appendiculatus* carboxyl proteinase activity. V_i - enzyme activity after pepstatin treatment, V_0^i - enzyme activity without pepstatin.

Effect of pepstatin. The proteinase was incubated for 15 min with increasing concentrations (1 - 8nm) of pepstatin and then assayed by the standard method. 4nm pepstatin reduced the proteinase activity to 50%. With 7nm pepstatin, the enzyme activity was down to only 5% (Fig. 8). Pepstatin inhibited the enzyme competitively.

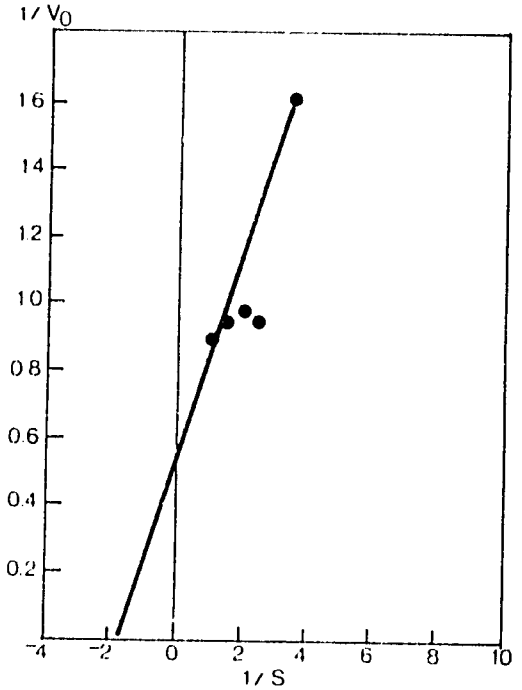


Figure 8. Lineweaver-Burk plot showing the effect of BSA concentration on *R. appendiculatus* gut carboxyl proteinase activity.

From the data reported here, it can be concluded that the enzyme is a carboxyl proteinase, very similar to cathepsin D of mammals. Work is in progress to purify and characterize the enzyme further.

PURIFICATION OF *R. APPENDICULATUS* VITELLIN

T.S. Dhadialla

Vitellin (Vn), the major egg yolk protein, from eggs oviposited by *R. appendiculatus* female has been purified by gel permeation and anion-exchange chromatographic steps. Upon electrophoresis on gradient (3-15%) polyacrylamide slab gels, Vn stained as a single band with a molecular weight of about 485 000 (Fig. 9). On the same gel, haemolymph samples from post-fed adult males and females and crude egg extract were also separated electrophoretically. Protein bands (presumptive vitellogenin) (Vg) with similar electrophoretic mobility were present only in haemolymph samples from females and not males. Staining tests for carbohydrates and lipids indicated that both Vn and presumptive Vg are glycolipoproteins. Vitellin also absorbed strongly at 400 nm indicating the presence of heme moiety on the Vn molecule.

Under dissociating conditions, the purified Vn electrophoresed into 8 protein-stainable bands (P1 to P8; Fig. 10) with molecular weights ranging from 43 000 for P8 to 160 000 for P1. Purified Vn was found unstable to heating at 72°C for 5 minutes.

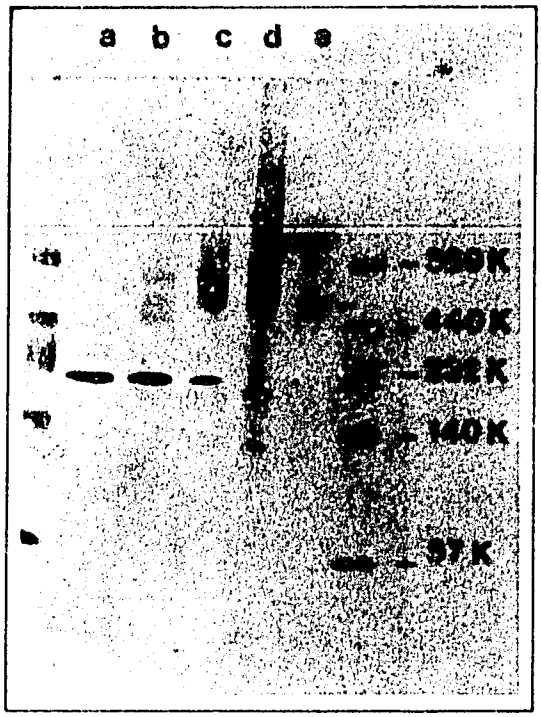


Figure 9. Slab gel electrophoresis of haemolymph and crude egg extract.
a—haemolymph of male, 10 days post-feeding
b—haemolymph of female, 10 days post-feeding
c—haemolymph of female, 10 days post-feeding when females had been irradiated with 2.4 krad gamma radiation as adults prior to attachment on host.
d—crude egg extract
e—purified vitellin
outer lanes—high molecular weight markers.

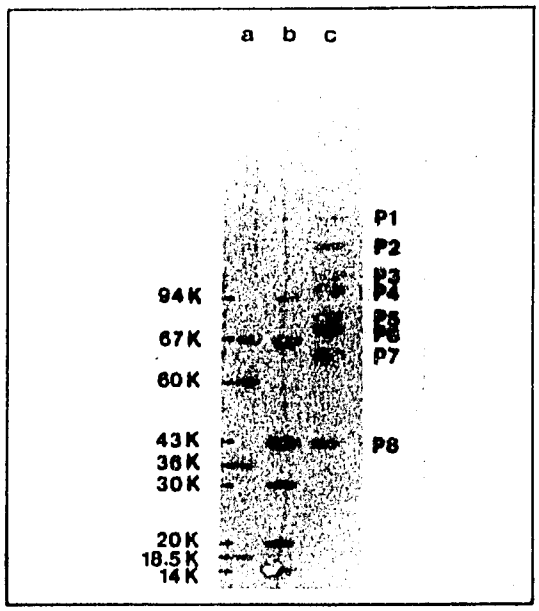


Figure 10. SDS-gel electrophoresis of purified vitellin.
a—high molecular weight markers
b—low molecular weight markers
c—purified vitellin

In an Ouchterlony immunodiffusion test, the purified Vn reacted with anti-serum raised in rabbits against fed adult female whole tick homogenate (R-500) giving a single precipitin line. Crude egg extract, on the same Ouchterlony, resulted in 3 precipitin lines one of which had complete identity with Vn. These results reflect the homogeneity of the purified Vn.

ECDYSTEROID CARRIER PROTEIN (ECP) FROM THE EGGS OF THE BROWN EAR TICK

D. Whitehead, T. Kezdi

The affinity of the low molecular weight proteins fractionated from the eggs of the brown ear tick (*ICIPE Eight Annual Report*, 1980, p. 84), with pI varying from 8.0 to 8.8, for ecdysteroids have been variously estimated (Table 44). ^{3}H-ecdysone injected into engorged *R. appendiculatus* is carried into the eggs where naturally occurring ecdysteroids have been found. At pH 7.7 40.5% of the label in the eggs adhered to DEAE cellulose but this could not be eluted using unlabeled 20-hydroxyecdysone (20-OHE) unless 0.75M NaCl was added.

From the isotope dilution data shown in figure 11 we have now estimated the affinity constant of (Ka), B'_{1-7} protein fraction to be about 5×10^{7} M using low specific activity ^{3}H-20-OHE at room temperature. We expect the Ka to be lower under optimal conditions. However, it compares well with the affinity of Calliphorin from blowfly larvae for ecdysteroids. The binding protein from the haemolymph of *Locusta migratoria* has a Ka = $1.03 \pm 0.05 \times 10^{7}$ M.

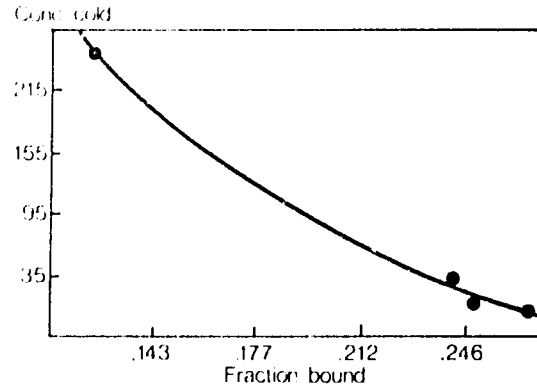


Figure 11. Proportion of ^{3}H - 20 - OHE bound to B'_{1-7} proteins following isotope dilution, plotted using the simplex method of computing data.

Although purification of ECP is not complete, from the evidence we have, we deduce the pI to be 8.2 - 8.3 and the molecular weight to be 33 300 approximately. This could be visualized using iso-electric focusing and partially characterized SD-PAGE. The possibility, that the tick embryos can also derive the steroid hormone required for development, from the conjugated form we have found to be present after injection of ^{3}H-ecdysone into gravid females, will be borne in mind. A carrier protein for the conjugated ecdysteroids might also exist if

Table 44. Assessment of the binding potential for ecdysteroids by various tick egg protein fractions

Methods used	Ligand	Protein fraction					
		B_{1-7}	B_4	B_6	BB	A	CD
RIA	^{3}H-20-OHE*	4.7		4.5	14.1	4.0	4.6
RIA	23, 24-$^3H_4$-E**	5.1	3.8	10.2	5.7	4.8	12.2
LH60 Sephadex	$^3H_4$-E	13.7					
	^{3}H-Ponasterone A***	23.5		22.4			
LH20 Sephadex	^{3}H-20-OHE	34.4					
G25 Sephadex	^{3}H-20-ONE	26.6					
with added	2.1 nmol	24.9					
20-OHE	8.2 nmol	24.4					
	62.5 nmol	12.3					
	*5 ci nmol	**50 ci nmol	-1		***170 ci nmol^{-1}		

it is not one and the same thing as the ECP we have studied.

CONTROL OF PUPARIATION AND SCLEROTIZATION OF THE POLYNEUSTIC LOBE CUTICLE OF *G. M. MORSITANS*

D. Whitehead

The mode of reproduction and subsequent development of the tsetse larva offers certain distinct advantages to the student of insect endocrinology. For instance, the events between the onset of pupariation (i.e. hardening of the peritreme around the spiracles) and pupation takes 10 to 12 h in blowflies whereas in tsetse they take 5 to 6 days. Hardening of the polyneustic lobes (analogous to the peritreme) takes place 2 days before larviposition. If the lobes were not sclerotized before birth, the air supply to the larva *in utero* could be cut off were the pregnant fly to feed during the last 2 days of her pregnancy. In 1981 it was suggested that, because ecdysteroids could not be detected using radioimmunoassay (RIA) at the time of lobe hardening, a non-steroid factor might be responsible for triggering sclerotization of the lobes. This interpretation is now questionable in the light of the following studies.

The rate of tyrosine uptake by the body was compared with uptake into the lobe cuticle *in vitro* just before sclerotization was due to start. The label entered twice as quickly into lobes when 20-hydroxyecdysone (20-OHE) was added to the tsetse ringer (Table 45) but ecdysone (E) the precursor of 20-OHE, did not stimulate uptake at the low titre used (deliberately to simulate the situation *in vivo*).

Table 45. Percent label taken up after incubating tsetse stage III larval cuticle *in vitro* with 50 n Ci of L-(U-^{14}C)-tyrosine* in 50 μl tsetse ringer for 1 h at 25 C and then for 18 h at 4 C

Treatment	Concentration of larva per μ	Lobe cuticle % label X ± SEM	Body cuticle % label X
Control		11.31 ± 0.54	10.2
With 20-OHE	2.38 nmol	19.45 ± 1.23	
With E	2.90 nmol	9.28	2.46
With larval blood		4.26	

*522m Ci mmol^{-1}

20-OHE stimulated metabolism of tyrosine in haemolymph taken from newly deposited larvae (Table 46) but the increase was mainly due to oxidation of DOPA. E stimulated incorporation of label into neutral (at pH 1.9) compounds X and Y which co-migrate with N-acetyltyramine and N-acetyldopamine. This is the first report that the sclerotizing agents can form *in vitro* in haemolymph rather than in the epidermis.

The role of ecdysteroids in programming the haemocytes and epidermis to synthesize the enzymes required to metabolize tyrosine thus:

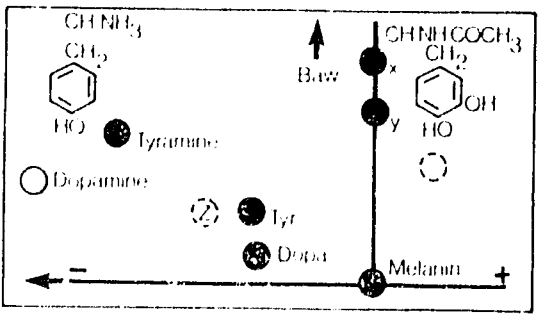


Table 46. Percent label metabolites derived from L-(U-^{14}C)-tyrosine in haemolyph from tsetse larvae at larviposition and pupariation

	Just deposited			Rounding-off	
	Nil	+ E^{-5}	+ 20OHE^{-4}	Nil	Constricted
Metabolites					
X & Y	0.50	1.62 (1.41)*	1.76	22.43	0.64
Tyramine	0.14	0.43	0.02	0.66	0.01
Dopa	5.57	1.03	7.13	5.52	4.38
Melanin	1.23	0.85	21.31	40.81	0.33
Unknown**		5.68			
Total	7.44	9.61	30.22	69.42	5.36

Notes.
Metabolites
Separated on paper by electrophoresis 0 pH 1.9 and chromatography in N-butanol/acetic/water: 4/1/1.
Haemolymp incubated 1H O R.T. —final volume 100(E)
Fmol, 83 Fmol (0.4 ng(E)$^{-1}$)
) Liberated hydrolysis (6n NCl) from the conjugate*
****) Unidentified compound(s) migrating on the positive side of tyrosine**

can easily be studied in tsetse where the (prepupation) events and pupation itself are widely separated in time. Use of the decarboxylase inhibitors MK 485 and Ro 4-4602, which inhibited pupariation for 18 to 24 h, showed that, because DOPA accumulated rather than tyrosine, tyramine was not the major precursor of the sclerotizing agent.

How 20-OHE activates tyrosine into lobe cuticle rather than the body cuticle deserves further study. The flux of

labeled metabolites, derived from ^{14}C-L-p-tyrosine injected into pregnant tsetse just before lobe hardening was due to begin, showed a dramatic activation of specific uptake of the precursors of the sclerotizing quinone (Table 47). That the process was mimicked *in vitro* when 20-OHE (but not its precursor, E) was added to the incubation ringer, in which excised white lobes were floating, shows that this stimulated uptake is specific for this hormone.

Table 47. Ration of label found in polypneustic lobes and body cuticle of stage III larvae *in vivo* after injection on day 7 of pregnancy of 0.1 Ci of L-($U^{14}C$)-tyrosine into the thorax of female *Glossina morsitans morsitans*

Elapsed time after injection	Status of the Larva *in utero*	Ratio of MG[1] in lobes / DPM sq.[1] integument
1h	"Barriers" open in lobe epidermis	11.5
2h	Lobes hardening	3.7
5h	Lobes hardened	1.8
24h	Preparing for larviposition	0.9
48h	Larviposition	0.9
49h	Head retraction	0.6
50h	Rounding off	0.6
51h	Sclerotizing puparium	0.1

CHEMICAL BASIS OF TVu 946 COWPEA STEM RESISTANCE TO *M. TESTULALIS* (GEYER)

D. A. Okonkwo, A. Hassanali, P. L. W. Nyange

We have previously reported the effects of ethylacetate extracts of stems of TVu 946 and Vita I cowpea cultivars on the feeding response of *M. testulalis* larvae (*ICIPE Eleventh Annual Report, 1983*). It was found that extracts of TVu 946 showed consistently greater feeding inhibition than those of Vita I in all tests. During the year under review, further work has been undertaken on extracts of these cowpea cultivars, with the objective of defining the roles of allelochemical factors in the extracts, isolating and identifying the allelochemicals involved and developing chromatographic techniques for quantifying these compounds in crude extracts from cowpea plants.

The work focussed on fractionation, high performance liquid chromatography (HPLC) analysis and bioassay. Fractionation was done by column chromatography and gave three major fractions based on polarity. Bioassay results of these fractions (Table 48) suggest that the active compounds are present largely in the middle fraction. Continued observations on larvae fed on discs treated with lower doses of these fractions showed no evidence of toxication indicating that the primary role of these allelochemicals is that of feeding inhibition. A mixture of phytosterols with moderate antifeedant activity has been isolated from one of the sub-fractions. Purification of the more active components is underway.

HPLC analyses of TVu 946 and Vita I extracts were carried out on a LC model 502051 (Varian aerograph), and the eluted compounds were monitored by a UV detector operated at 240 nm. Successful separation of the constituents of the extracts was achieved using MCH-5

Table 48. Antifeedant effects of extracts of stems of TVu 946 and Vita 1 on the feeding behaviour of *M. testulalis*

Extract weight	Average weight of control disc consumed (g)	Average weight of treated disc consumed (g)	*Percent feeding deterrency
TVu 946	2.64	0.44	82.6
Vita 1	2.79	2.73	2.15
Least polar fraction chromatographically isolated from TVu 946	3.76	4.07	NS
Medium polar fraction chromatographically isolated from TVu 946	3.90	1.00	74.4
Most polar fraction chromatographically isolated from TVu 946	3.99	3.17	20.6
Crystaline phytosterols isolated from the medium polar fraction	3.48	1.77	49.1

Dose—100 μg/disc for all extracts.
Percent feeding deterrency calculated as
$$100 - \frac{(\text{Average WT} + \text{Average WT})_{WC}}{\text{Average WT}}$$ **where WT = weight treated disc consumed; WC = Weight control disc consumed**
NS No significant feeding deterrency.

reverse phase column (Varian), and an elution programme involving 30-70% acetonitrile in water as the mobile phase.

The resulting chromatograms, (Figs. 11 and 12) demonstrate that almost a complete profile of compounds present in the organic extracts may be obtained by using a reverse phase column and a binary solvent system involving water and acetonitrite. This possibility opens up prospects for using the method to analyse crude extracts from floral parts, terminal shoots and pods of cowpea cultivars, and suggests a potential for

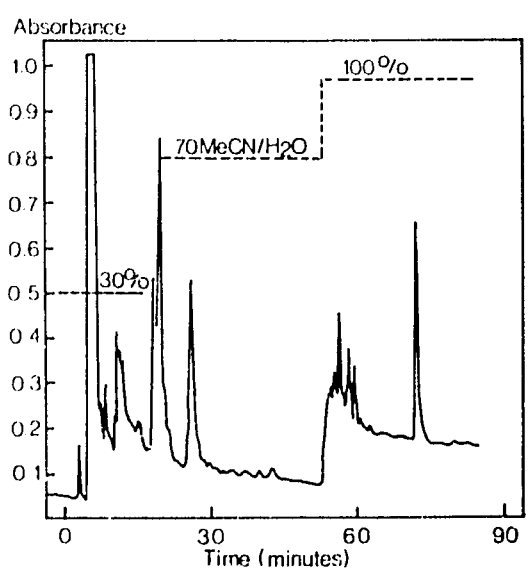


Figure 12. HPLC profile at EtOAC extracts of TVu 946 stems. The profile was obtained by using MC-5 reverse phase column (Varian) and a binary solvent elution programme involving 30 to 70% acetonitrile in water. UV detector at 240 nm; pump pressure max 350 psi; temperature, 21° C; flow rate, 1 ml/min; chart speed, 0.25/cm/min.

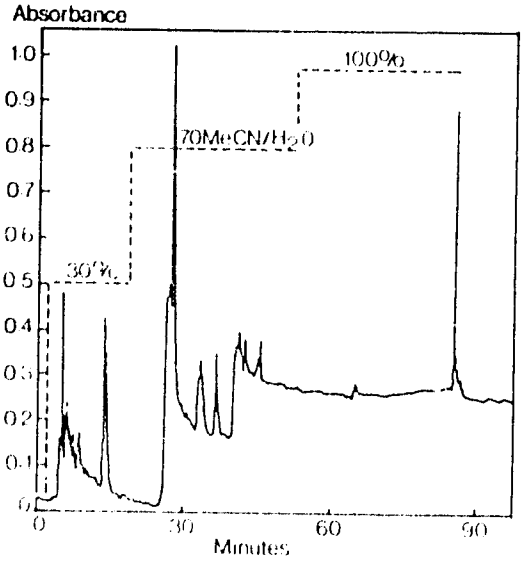


Figure 13. HPLC profile of EtOAC extracts of Vita 1 stems. Profile obtained using Mc-5 reverse phase column (Varian) and a binary solvent elution programme involving 30 to 70% acetonitrile in water. UV detector at 240 nm; pump pressure max 350 psi; temperature, 21 °C, flow rate 1 ml/min; chart speed 0.25 cm/min.

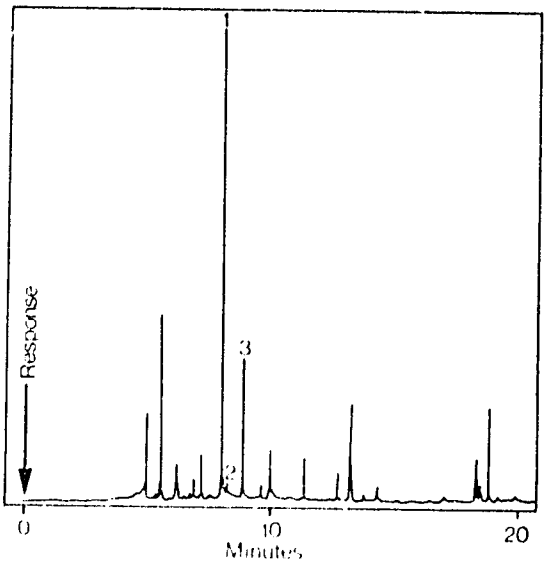


Figure 14. Capillary gas chromatograph of *R. appendiculatus* larvae extract using electron capture detection
Column: 50m x 0.22 mm, I.d. CP sil 5 fused silica
Injection mode : splitless
Analysis: 180 °C isothermal

the method in cowpea resistance improvement breeding programmes. The marked qualitative and quantitative differences between the two extracts is interesting. However, the full implication of this must await the identification of all the allelochemicals in the mixtures.

2, 6-DICHLOROPHENOL IN THE LARVAL STAGE OF *R. APPENDICULATUS*

P.G. Mabusela

In last year's report, evidence was presented for the presence of 2, 6-Dichlorophenol (2, 6-CDP) in adult stages of *R. appendiculatus*, predominantly in unfed females, which is released during feeding. Electrophysiological recordings on adult male olfactory cells have previously shown that extracts from larval *R. appendiculatus* also stimulate male olfactory cells with strong responses similar to those produced by adult female extracts and authentic 2, 6-DCP. An extract of the larval stage of *R. appendiculatus* has been examined by capillary gas chromatography [with electron capture detection (ECD)] and capillary gas chromatography-mass spectrometry (GC-MS). The presence of 2, 6-DCP in larval extracts of *R. appendiculatus* has now been confirmed.

Figure 14 shows a capillary gas chromatogram (ECD) of a larval extract. Component 1 (figure 14) has the retention time of 2, 6-DCP as demonstrated by comparison and co-injection with an authentic sample of the compound. Capillary GC-MS was performed on a 12.5 m silicone phase column with the spectrometer operated in the electron impact mode at 70 eV electron energy. Component 1 gave a spectrum closely resembling that of authentic 2,6-DCP as is shown in figure 15. Component 2 (Fig. 14) has also been identified from its

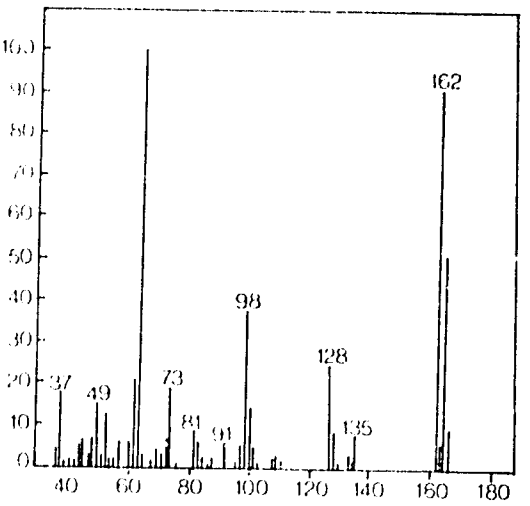


Figure 15. Mass spectrum of component 1, 2,6-Dichlorophenol

mass spectrum, shown in figure 16. Component 2 gave the spectrum of benzothiazole (mol wt. 135). A third component (but not that which is labelled 3 in figure 14) gave the spectrum of 2-undecanone shown in figure 17 (mol wt. 170). The assignment of component 2 as benzothiazole was confirmed by co-injection studies with the authentic compound. Component 3 had a retention time approximately 10 seconds longer than 2-undecanone. The ECD is not sensitive to 2-undecanone at low concentrations, however, flame ionization detection shows the 2-undecanone component clearly. Only very small peaks were detected by the FID at the retention time of component 3, indicating that the ECD is

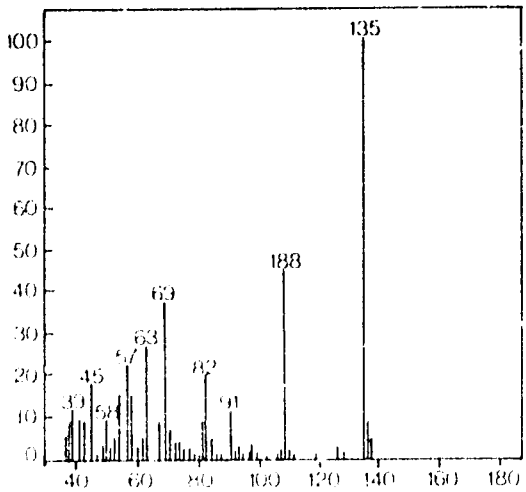


Figure 16. Mass spectrum of component 2 Miazole

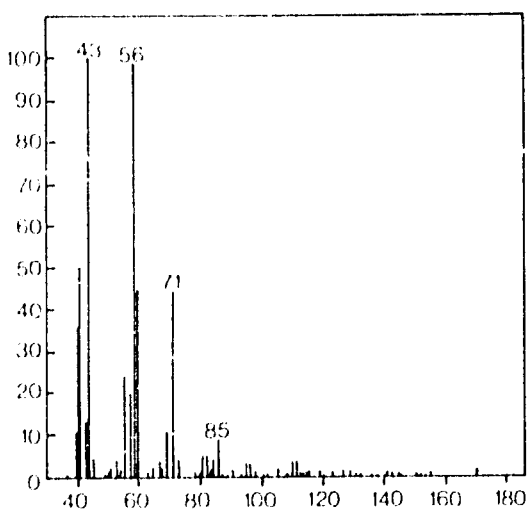


Figure 17. Mass spectrum of 2-undecanone

very sensitive to component 3. The structure of this and other components of the extract are still under investigation.

Thus both the larval and adult stages of *R. appendiculatus* possess 2,6-DCP. It appears likely that synthesis of the compound begins in the larval stage and continues through the nymphal stage (although this stage has not yet been examined) to the adult tick. Electrophysiological assays will be carried out on the other compounds identified (benzothiazole and 2-undecanone) to determine if they have any effects on male olfactory cells.

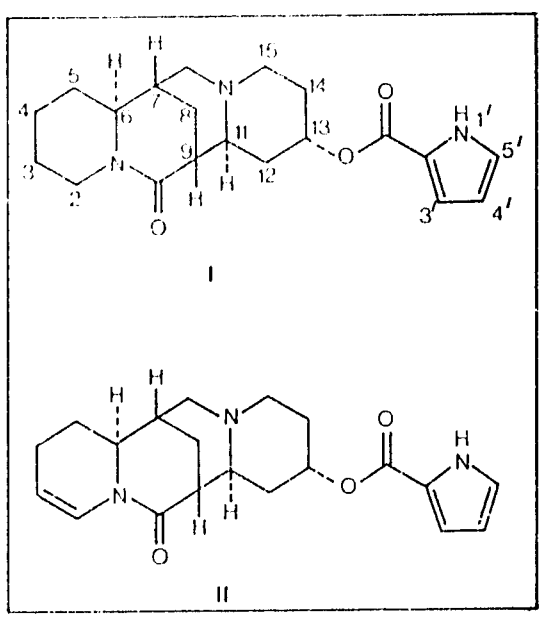
A clearer view of the volatile chemicals present in *R. appendiculatus* is beginning to emerge, although their overall role in behaviour still requires elucidation.

FEEDING DETERRENTS FROM *CALPURNEA AUREA* (PAPILLIONACEAE)

A. Chapya, C. Galeffi, G.B. Marini-Bettolo

In the course of our screening for naturally occurring anti-insect compounds from plants, we found that the crude methanol extract of fresh leaves from *Calpaurnea aurea* possesses a strong antifeedant activity against the African armyworm *Spodoptera exempta* (Wlk.)

The crude methanol extract was dispersed into water and the aqueous suspension was extracted with hexane, ether and ethyl acetate. The ether extract showed activity at 250 ppm and was fractionated using silica gel column chromatography (0.5% CH_3OH-$CHCl_3$) followed by counter current chromatography using a Craig-Post apparatus (200 stages, 10:10 ml, upper and lower phase) to give two major components, one of which showed antifeedant activity at 125 ppm. The non-active component was obtained as colourless crystals m.p. 265 C (CH_3OH), $[\alpha]_D^{20}$ -21 (C = .027 $CHCl_3$) and had a molecular formula $C_{19}H_{26}O_3N_2$ (by CI-MS 358 MH^+, and elemental analysis).



On the basis of physical and spectroscopic data, this compound was identified as 0-(2-pyrrolylcarbonyl)-virgiline I previously isolated from the South African *C. aurea* and *Beadea membranaceae* (Rubiaceae). Compound II is the dehydro derivative of I. Although the structures of I and II are very similar, the magnitude of their antifeeding activity is dramatically different. The dehydro-derivative I has no activity even at a concentration of 500 ppm.

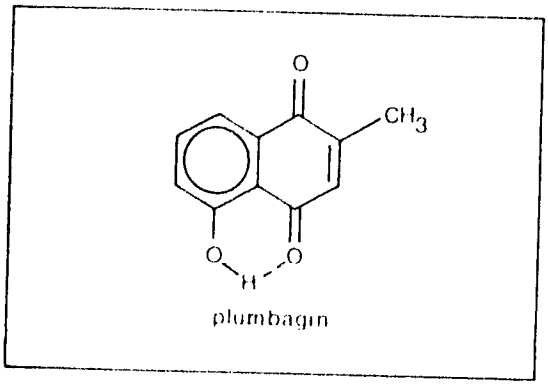
MOSQUITO LARVICIDE FROM *PLUMBAGO ZEYLANICA*

A. Chapya

We previously identified plumbagin (5-hydroxy-2-methyl-1, 4-naphoquinone), I, as the antimicrobial and

insect antifeedant agent from an ornamental shrub, *Plumbago capensis* (Plumbagaceae) found growing as a hedge near the University of Nairobi. The strong larvicidal activity of extracts of the roots of *P. zeylanica* found growing wild in Nzaui, Machakos area, led us to examine the constituents of this species.

The hexane extract of the crude methanol extract of the roots was chromatographed on silica gel using hexane with increasing amounts of ethyl acetate as the eluent. The active component was found to be plumbagin, which showed 100% mortality against third-instar larvae of *Aedes aegypti* after 24 h exposure at 0.01 μg/ml concentration and was identified by comparison with an authentic sample.



The wide occurrence of naphthoquinones in tropical plants as well as the relative high yields of the extract (plumbagin was obtained in 0.005% overall yield) suggest a potential for suitable naphthoquinone derivatives in mosquito control programme. We are currently carrying out a structure-activity study on available naphthoquinones as mosquito larvicides in order to identify the more active analogues.

A NOVEL 6a HYDROXY PRETOCARPAN ANTIFEEDANT FROM *TEPHROSIA HILDEBRANDTII*

B. Lwande, L. Hassanali, P.W. Njoroge, E.E.O. Jondiko

A new 6a- hydroxylated pterocarpan was isolated from the healthy roots of the herbaceous plant *Tephrosia hildebrandtii* of the family Leguminosae. It was named hildecarpin and assigned the structure and absolute spectroscopic data, optical rotation and chemical transformation. Hildecarpin showed antifeedant activity against the legume pod borer *M. testulalis* (Table 49) and antifungal activity against *Cladosporium cucumerinum*.

Hildecarpin bears a close structural resemblance to phytoalexins of the cowpea plant. Since *de novo* synthesis of the phytoalexins may be stimulated by artificially infecting the cowpea plant with non-pathogenic microbes, this may provide a method for inducing some measure of resistance in the plant against both *M. testulalis* and pathogenic microbes.

Table 49. The feeding inhibitory activity of hildecarpin against larvae of *M. testulalis*

Dose ug/disc	Batch No	Deterrence %	
100	1	83.4	Average
	2	92.9	85
	3	81.0	
40	4	51.8	Average
	5	52.0	52

For each batch, 20 larvae of the test insect were used. Two larvae were allowed to feed on a pair of treated disc and control disc. The result of each batch is based on a summationof 10 such feeding assays.

Deterrence defined as (1-WT) × 100, where WT = weight of treated disc consumed and WC = weight of control disc consumed.

ISOLATION AND PURIFICATION OF MOSQUITO LARVICIDES FOUND IN AERIAL PARTS OF *SPILANTHES MAURITIANA*

E.E.O. Jondiko

The methanol extract of wet stem and leaves of *A. mauritiana* collected from Kisii District was found to elicit 100% mortality at 0.003 g/ml after 7 h of application against third-instar larvae of *A. aegypti*. When the aqueous methanol extract was partitioned into chloroform, the resultant organic extract was found to be ten times more active than the whole methanol extract, while the aqueous phase showed no larvicidal activity. Further chromatographic purification of the chloroform extract

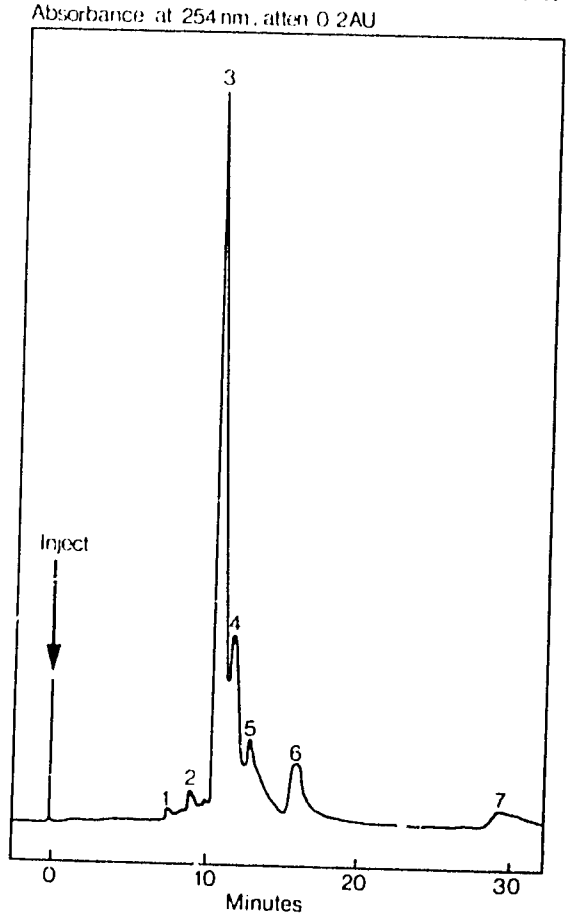


Figure 18. HPLC profile for fractions 5 and 6.

using silica gel and 30% ethyl acetate in hexane as solvent gave twelve fractions eliciting larvicidal activity. The most active fractions were subjected to high pressure liquid chromatographic purification, using MCH10 column (250 mm x 8 mm) and acetonitrile as the solvent. Figure 18 shows the profile obtained. The larvicidal activity of each fraction is given in table 50. Fractions 1, 2 and 3 have so far been purified to HPLC homogeneity.

Table 50. Mosquito larvicidal activity of HPLC fractions obtained from column fractions 5 and 6.

Fractions*	1	2	3	4	5	6	7
Time of observation (hr.)	24	17	1.75	1.75	1.0	1.0	17
Mortality percent	70	100	100	100	100	100	50

***Dose for each fraction: 0.01μg/ml.**

Work is now in progress to obtain the necessary spectroscopic data (^{1}Hnmr, ^{13}C nmr, UV) for structural elucidation. The mass spectrometric data shows that fractions 1 and 2 have the same mass number 229 which probably fits the molecular formula $C_{15}H_{19}ON$ while fraction 3 has mass number 247 corresponding to the formula $C_{15}H_{21}ON$.

EVALUATION OF LIMONOIDS FOR ANTI-INSECT ACTIVITIES

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

The longer term goals of the limonoid project at ICIPE are to carry out detailed structure-activity studies on limonoids on a number of ICIPE target insects, to identify potentially useful naturally occurring limonoids or readily accessible derivatives, and to synthesize and evaluate simple analogues of active limonoids.

In the present report more complete data on antifeedant activities of 6 limonoids (I-VI) against *S. exempta, M. testulalis* and *E. saccharina* are presented (Table 51).

Table 51. Feeding deterrent activities of limonoids against *S. exempta, E. saccharina* and *M. testulalis*

Test compound	Dose (μg/disc)	Feeding inhibition (%) *S. exempta*	*E. saccharina*	*M. testulalis*
I	100	a	62	75
	10	a	42	58
II	100	a	83	39
	10	a	81	56
	1	a	66	a
III	100	a	55	67
	10	a	48	b
IV	100	48	94	82
	10	a	65	76
V	100	37	74	81
	10	a	76	69
	1	a	73	b
VI	100	a	b	b
	10	a	b	b

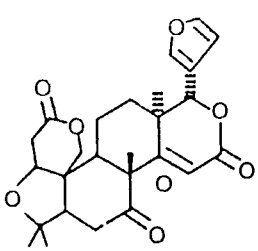
Inhibition calculated as

$$\left(1-\frac{WT}{WC}\right)\times 100,$$ **where WT = weight test disc eaten, WC, weight control control disc eaten.**

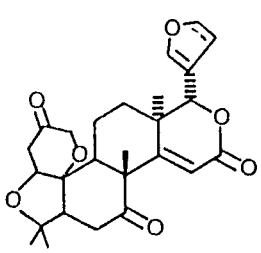
a **No significant activities**

b **Results not yet available**

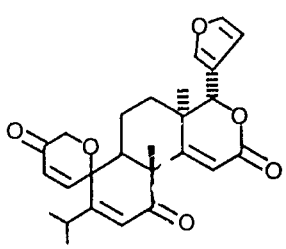
c **This is the first case we have observed where a depression of antifeedant activity is observed at the high dosage. Dose response data, we have so far, suggests a maximum activity at 20 μg/disc.**



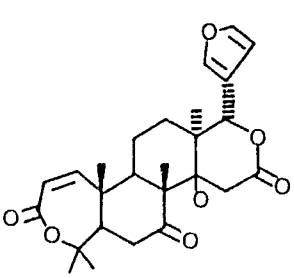
Limonin (I)



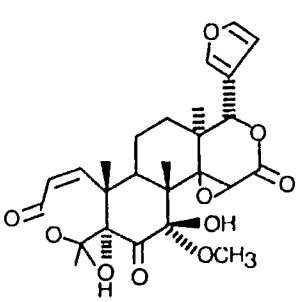
Deoxylimonin (II)



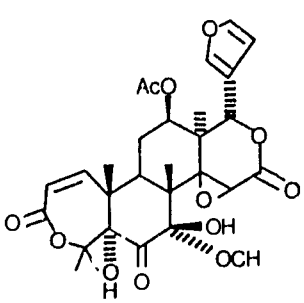
Citrolin (III)



Obacunone (IV)



Harrisonin (V)



Acetoxyharrisonin (VI)

From structure-activity relation point of view, this data is puzzling. If the moderate activities of obacunone (IV) and harrisonin (V) against the African armyworm are attributed to the presence of A-ring unsaturated lactone groups it is surprising to find that acetoxyharrisonin (VI) with an acetoxy group remote from the lactone ring is inactive even at concentrations higher than 100 μg/disc. On the other hand, the activities of the limonoids against *E. saccharina* and *M. testulalis* suggest that there may be several regions of interaction in the limonoid skeleton with the appropriate active site, or else there may be different receptors which interact with the different functional regions in the limonoid molecule. It is hoped that bioassays of other closely related limonoids and their simple analogues currently underway, as well as sensory physiological screening of the compounds would help throw some light on the structural units responsible for the activities of these compounds.

The C-7 keto group in these limonoids provides a convenient handle for manipulating the polarity of the molecules by appropriate derivatization and a number of such compounds derived from deoxylimonin and its 7-OH analogue are currently being investigated for systemic activity.

IAEA ICIPE RADIOISOTOPE PROJECT: NUCLEAR TECHNIQUES IN INSECT BIOCHEMISTRY AND ENTOMOLOGY

P.G. McDowell, A.R. McCaffery

The International Atomic Energy Agency (IAEA) has provided technical assistance to ICIPE to establish facilities at the Centre for radioisotope work. The aims of the project are three-fold. First, to equip the Centre with laboratory facilities suitable for the safe handling and measurement of radionuclides and to provide expert assistance to set up and establish proper functioning of the equipment and to institute safe working practices. Second, to provide expert assistance to train ICIPE staff in the use of radioisotopes through application of techniques to their own research work, thereby building up a base of experienced people. Third, to use this core of knowledge to provide training in radioisotope techniques in entomological research on a regional basis.

Much of the first objective has been achieved early this year during the visit of Dr. Alan R. McCaffery, the first of three visiting experts provided by IAEA. A small laboratory was modified to meet with class B specifications for a radioisotope laboratory and equipped with an isotope grade fume hood, decontamination sinks, work bench and storage facilities for radiochemicals. The major equipment for detection and assay installed was a Packard model 4530 liquid scintillation counter interfaced with a Philips P2000 T microcomputer for on- and off-line data manipulation. Other equipment installed were a manual GM counter (Tenelec, USA),autoradiographic equipment and high sensitivity film and a radioisotope camera (beta imager) and print projector (Birchover Instruments, UK) for non-destructive detection of radioactivity in thin layer chromatograms and gels.

A short course was also given to 12 members of staff covering such topics as properties of radionuclides, detection and assay of radioactivity, radionuclide handling, safety, protection and disposal, use of tracers, radiochromatography and radioimmunoassay. Instruction in the practical use of the available equipment was provided. ICIPE scientists were advised on ways of incorporating radioisotope techniques into their own work. To demonstrate the type of study which can be undertaken, a short research project was carried out to investigate the metabolism of a tritiated anti-allatal agent, precocene II, in *S. exempta*, a non-sensitive insect.

Metabolism of the pro-allatocidin precocene II in a lepidopteran

A.R. McCaffery

The pro-allatocidin precocene II is presumed to be metabolized by the corpora allata (CA) of sensitive insects such as *Orthoptera* to a reactive epoxide which bonds covalently to cellular macromolecules. This causes cell death and leads to the morphological and physiological symptoms of a cessation of juvenile hormone (JH) production by the CA : precocious metamorphosis and adult sterility.

The compound has been shown to have a singular lack of effect in Lepidoptera. This is particularly unfortunate since compounds of this type are clearly potential control agents and Lepidoptera represent one of the largest groups of pest insect species.

The recent commercial availability of tritiated precocene gives the opportunity to study in detail the fate of the compound in insects. The metabolism of precocene II in the lepidopteran, *S. exempta* was studied so as to indicate possible reasons for its inability to disrupt JH-controlled metamorphosis.

Unlabelled precocene II was topically applied in acetone to penultimate larval instar (V) *S. exempta* within 24 h of the previous moult showed that precocious metamorphosis did not occur in the treated insects. At high doses the compound was toxic with death following 2 to 5 days later. Doses of 50 μg produced 12.5% mortality by the time that the insects pupated, doses of 200 μg gave 50% mortality by the time the insects pupated and doses of 400 μg were rapidly fatal to all the test insects. Control insects topically treated with acetone alone showed no mortality whatsoever. Surviving test insects reproduced normally. Precocene II appears therefore to have no direct anti-allatal or anti-JH action in this insect. A series of authentic reference precocene metabolites was prepared (Dr. P.G. McDowell). These were assembled into a standard cocktail for use in the HPLC analysis of the products of tritiated precocene II metabolism. The mixture was prepared at frequent intervals and was composed of the following: cis + trans precocene II-3,4-diols, precocene II-4-ol, precocene II-3-ol and precocene II. The retention times of 6-(O)-desmethylprecocene II and 7-(O)-desmethylprecocene II were determined using limited amounts of authentic reference materials. Separation of the components was achieved using the Phase-Sep C-8 column with a gradient elution of 0-5 min, 25% acetonitrile in water and from 5 to 25 min a linear

gradient from 25% to 75% acetonitrile in water. UV detection was performed at 254 nm.

Doses of 1 μCi of precocene II in acetone with 50 μg of non-labelled carrier precocene were topically applied to fifth-instar *Spodoptera* within 24 h of the previous moult. The larvae were analyzed in various ways as follows.

After varying lengths of time the larvae were surface washed with acetonitrile, the washings reduced in bulk and a known portion analyzed by RPHPLC as above. (The radioactive content of a similar known portion was directly determined using liquid scintillation counting =LSC=. The eluent from the HPLC was collected in 0.5 ml fractions in scintillation vials containing scintillation cocktail, the samples counted by LSC and a radiochromatogram obtained. It has been assumed that co-chromatography of radiolabelled products with authentic reference standards gives a reasonably reliable, though not definitive, indication of their identity. After 2.5 h only 50% of the original applied dose of radioactive precocene was recovered by solvent washing. By 6 h, less than 5% of the original material remained on the surface of the insect. Up to 4 h after treatment at least 75% of the applied radioactivity remained as unchanged precocene II but by 6 h this was reduced to around 50%. The only other significant component isolated by radiochromatography appeared to be a non-polar material eluting by RPHPLC later than precocene II. This accounted for 10% of the radioactivity recovered at 4 h. By 6 h after treatment 7% of the recovered counts coeluted with the 3,4-diols.

The haemolymph was removed from test insects and ejected into ice-cold acetonitrile. The samples were thoroughly extracted then reduced in bulk and a known portion taken for RPHPLC analysis and LSC as above. The precipitate contained negligible radioactivity.

The titre of precocene II reached a peak of around 0.3mM at about 2 h after treatment. By 4 h this was below 0.1mM and decreasing rapidly. In all samples, precocene II accounted for less than 70% of the radioactivity recovered and apart from one sample was always below 50% of the recovered counts. Three other significant radiolabelled components were consistently found in haemolymph samples: precocene II-3,4-diols accounting for 30% of the haemolymph radioactivity 4 h after treatment, an unidentified polar material eluting before the diols and an unknown fraction eluting between precocene II-4-ol and 6-(O)-desmethylprecocene II and accounting for 14% of the radioactivity at 4 h.

The midguts of the treated insects were also examined. Samples were analyzed using the RPHPLC method described above. A variety of labelled components was always recovered: Polar materials 10-15%, precocene II-3,4-diols 10-15%, unknown (eluting between precocene II-4-ol and 6-(O)-desmethylprecocene II) 5-10%, precocene II-3-ol 10% and precocene II 20-60%.

These preliminary observations indicate that:

- Precocene II is without direct anti-CA action in *Spodoptera* and that high doses are toxic.
- Fifty percent of topically applied precocene disappears from the surface of the insect within 2.1/2 h. After 6 h less than 5% remains.
- A component less polar than precocene II and a material eluting with the diols both appear in increasing amounts on the cuticle surface after treatment.
- The maximum haemolymph titre of precocene II observed was 0.3mM at about 2 h after treatment. This is a titre which in sensitive insects would be effective at producing anti-CA actions. The titre falls rapidly thereafter.
- Significant quantities of the diols appear in both haemolymph and midgut samples as do fast eluting polar materials (conjugates?) and an unknown metabolite eluting between the 4-ol and 6-(O)-desmethyl metabolites.
- A material co-eluting with the 4-ol and 6-(O)- desmethyl metabolites.
- A material co-eluting with the 3-ol appears in the midgut.

It is concluded that whilst precocene II is without obvious action on the Ca, topical application of the compound results in haemolymph titres which would be effective in sensitive species. Nevertheless, the titre of authentic precocene decreases rapidly. Peripheral detoxication processes probably reduce the effective titre of the compound still further by metabolizing the compound to a variety of more polar components. These factors, together with possible unknown aspects of CA biochemistry probably render the compound ineffective.

IAEA SPECIAL PROJECT ON THE BIOLOGY BIOCHEMISTRY OF MICROORGANISMS FOR IMPROVED BIOMASS DEGRADATION

Biochemical section

H. Osore

Quantification of the rates of lignin degradation by microorganisms requires utilization of substrates of known structures. Accordingly, we embarked on the synthesis of several lignin substructure model compounds in 1984. Our attention was drawn mainly on models of the β-0-4 and β-1 type, which represent the major structural linkages in native lignin. Biphenyl linkage type models were also synthesized and of these dehydrodivanillin has been employed extensively in the search for novel enzyme activities.

The models were used to assay the following enzyme activities in cultures of *Fusarium semitectum, Trichoderma harzianum, Aspergillus niger, Aspergillus flavus, Actinomyces* spp. and *Termitomyces*:

- Cellobiose oxidase
- Polyphenol oxidase
- Protocatechuic acid oxidase (protocatechuate 3, 4 oxygenase)
- Cellobiose quinone oxidoreductase

An interesting finding was the detection of cellobiose oxidase in cultures of *F. semitectum* and *Actinomyces* spp. The extracellular enzyme utilizes molecular oxygen to oxidise cellodestrins to the corresponding aldonic acids. Degradation products were analyzed by GC and HPLC. Protocatechuate oxygenase was found in the fruiting bodies of *Termitomyces* and in the mycotetes.

The last quarter of 1984 saw the beginning of radioisotopic studies using ^{14}C-labelled model compounds and lignin polymers. This work was conducted during a study fellowship from IAEA to Dr. Osore in the USA.

A serious obstacle to the study of lignin biodegradation has been the lack of sensitive assays for measuring lignin decomposition. However, with the advent of sensitive specific radioisotopic methods, rapid advances have been made. The assay is based on incubation of the organisms in tightly closed 125 ml Erlenmeyer flasks in the presence of a known amount of the ^{14}C-substrate, and flushing out the liberated $^{14}CO_2$ at suitable intervals, trapping it in a scintillation cocktail incorporating ethanolamine, and eventually counting the vials in a liquid scintillation spectrophotometer. From the counts obtained, the percentage of the original ^{14}C liberated as $^{14}CO_2$ can be calculated.

Organisms selected for these studies were: *Trichoderma harzianum*, *Fusarium semitectum* and two species of *Actinomyces*. The white-rot fungus *Phanerochaete chrysosporium* BKM 1967 was used as reference organism. The fungi were tested against ^{14}C-ferulic acid (representative of monomeric unit in lignin biopolymer) and ^{14}C-(OCH$_3$) polyguaiacol.

Fusarium, *Actinomyces* A$_2$ and *Actinomyces* MAI oxidized ^{14}C ferulic acid to varying degrees. Figure 19 shows that *Fusarium* cultures oxidized ^{14}C ferulic acid by up to 63% within 6 days.

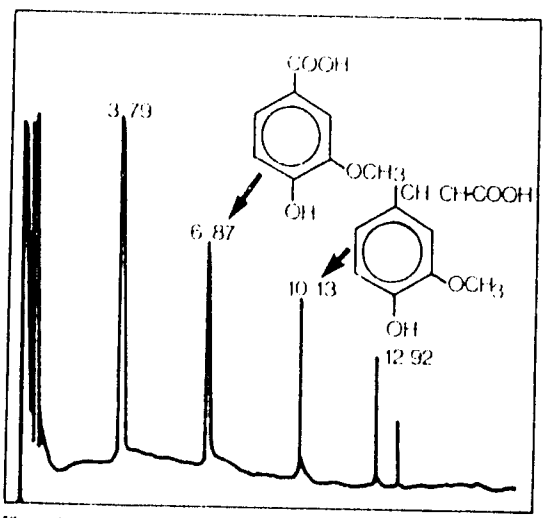


Figure 19. Oxidation of ^{14}C - [2] - ferulic acid to $^{14}CO_2$ by cultures of *Fusarium semitectum*, *Actinomyces* A2A and *Actinomyces* MA1

The dehydrogenation polymerisate (^{14}C-DHP) was degraded more slowly than ^{14}C ferulate. *Actinomyces MAI* released only 0.5% of the original ^{14}C as $^{14}CO_2$ after 20 days incubation. Likewise, *Fusarium* showed a slow release of $^{14}CO_2$, reaching a recovery of only 0.22% after 14 days incubation (Table 52).

Table 52. Oxidation of 14C (ring) - DHP by cultures of *Fusarium semitechtum* (total DPM = 4.79 × 10⁴)

Incubation time (days)	% ^{14}C Recovered as $^{14}CO_2$
1	0.010
4	0.066
7	0.096
9	0.130
10	0.144
12	0.157
14	0.220

The results obtained with ^{14}C-(Ring)-DHP clearly indicate that ring-labelled polymeric compounds are relatively harder to decompose microbially than side-chain monomeric compounds. Results obtained with ^{14}C-(Ring) vanillin and vanilic acid have shown that the degradability of such compounds falls intermediate between those of side-chain labelled monomeric compounds and ring-labelled polymeric compounds.

A number of substrates were used to assay for ligninolytic enzyme activities. A H_2O_2 - dependent ligninase that converts veratryl alcohol to veratraldehyde was detected in cultures of *Fusarium* and *Trichoderma*. Veratryl alcohol is converted by the H_2O_2 dependent ligninase to veratraldehyde which has a λmax value of 310 nM, making it easy to follow the reaction spectrophotometrically at this wavelength.

2-Methoxy-3-phenyl benzoic acid was used to assay for demethylase activity. This compound was demethylated by cultures of *Fusarium* to 2-hydroxy-3-phenylbenzoic acid, which has a λmax of 310 nM, whereas the parent compound absorbs maximally at 290 nM. In ligninase assays employing dehydrodivanillin, changes in the UV visible absorption spectra of the compound were monitored between 400 and 200 nM. Significant changes were only observed after 10-20 min incubation at 20° in the cuvette.

Capillary gas chromatography was used in conjunction with the above studies to elucidate degradation pathways for ^{14}C-ferulic acid. Figure 20 is an illustration of the metabolic products obtained after incubation for 15 days with cultures of *F. semitectum*.

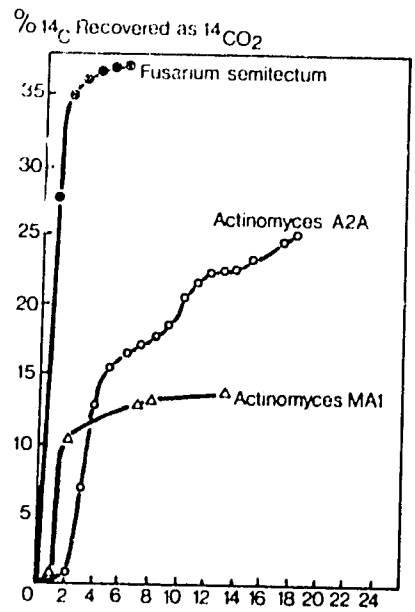


Figure 20. Capillary G.C. trace of metabolic products from ^{14}C- ferulate by *Trichoderma harzianum*

Microbiology section

M. Okech

Since our last report, complete identification of the four fungi associated with the fungus combs of *Macrotermes*

michaelseni has been done. They are now as follows: *F. semitectum*, *T. harzianum*, *A. niger* and *A. flavus*. The work on protozoans and anaerobes was shelved following the decision to narrow the work plan that was originally proposed.

In earlier reports, it was indicated that attempts to grow *Termitomyces* spp. from the mycotetes, spores and tissue cultures was unsuccessful and alternative methods were being explored. Since then, *Termitomyces* mycotetes have been successfully grown on potato dextrose agar. The mycotetes were collected from the fungus comb of *M. michaelseni* and surface sterilized as follows:

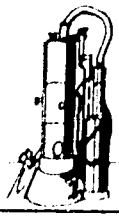
Washed in 0.5% sodium hypochlorite for 2 minutes, then washed in sterile distilled water for 5 minutes, then rinsed in 0.85% sterile saline and inoculated on to potato dextrose agar.

Within a week mycelia started to germinate from the mycotetes and in about three to four weeks mycotetes appeared on the mycelia.

The ability for the *Termitomyces* mycotete to produce cellulase was demonstrated according to standard methods. A positive result was obtained within 11 days. Their ability to produce 'ligninase' is currently being tested.

Histology and Fine Structure

Hyperglycemic activity in the corpus cardiacum-corpus allatum aorta complex of *G. morsitans* 65
Effect of precocene II on the milk gland of F1 generation *G. morsitans* (Westwood) 68
Ultrastructure of cultured embryonic tick cells 71
Sensory organs of *C. partellus* 72
Trichomes on maize leaf surfaces 72
Effect of ligation on spermiogenesis in *G. morsitans* 72
Function of the choriothete in tsetse 72



Histology and Fine Structure Research Unit

In 1984 work in the Histology and Fine Structure Research Unit focused on ultrastructural studies of neuroendocrine organs, reproductive organs, salivary glands of tsetse and sensory organs of crop borers. The objective was to provide various core programmes with the structural information needed in understanding and interpreting cell functions. A few examples of the activities have been selected to illustrate research achievements and at the same time indicate new horizons which have been opened.

- *The study of the corpus cardiacum - corpus allatum - aorta (CC-CA-A) complex of* Glossina morsitans, *a specialized organ which produces neurosecretions and juvenile hormones have shown that the complex in* G. morsitans *contains a hyperglycaemic factor that induces a considerable increase in total haemolymph carbohydrate.*
- *Retardation of tsetse testes development following ligation was another significant observation. This structural analysis of testes development has also suggested a number of important factors which should be considered for study in future including hormonal involvement in tsetse fly spermatogenesis.*
- *Based on previous investigations on the precocene - induced sterility in* F_1 *generation of* G. morsitans, *the present report gives an ultrastructural account of the structural transformations undergone by milk glands from* F_1 *generation following treatment of the female parent.*
- *The essential functions of the tsetse uterus include temporary storage of spermatophores received during mating and ensuring egg development into a mature larva. In performing the function of egg development, the uterus is modified to remove the chorion from the newly hatched first-instar larva and the exuvium from the newly-moulted second-instar larva. The work reported here suggests that the protein-mucopolysaccharide product which is secreted by the choriothete of the uterus acts as a cement to bind to the chorion and the exuvium during their removal.*
- *A cellular study at the transmission electron microscope level was undertaken on cultured embryonic tick cells in order to elucidate any morphological differences between the observed epithelial and fibroblast type cells. This can be used to speculate on the functional basis of these cells in relation to the synthesis of the antigen for experimental immunization against tick infestation with* Rhipicephalus appendiculatus.
- *In conjunction with the electrophysiological techniques, the electron microscope has revealed types of sensilla found on larval and adult antennae of* Chilo partellus. *The most significant observation to emerge from this study is the role of sensilla styloconicum and basiconica in the biology of* C. partellus *larvae.*
- *The morphology of the trichomes on certain resistant and susceptible maize genotypes were studied by scanning electron microscopy in order to determine the influence of such plant characteristics on oviposition by* C. partellus.

HYPERGLYCAEMIC ACTIVITY IN THE CORPUS CARDIACUM-CORPUS ALLATUM-AORTA COMPLEX OF *G. MORSITANS*

I. R. S. Awin, R. B. Mwangi, S. I. Okoma

Ultrastructural studies have shown that the corpus cardiacum (CC) cells produce an intrinsic granular secretion which is transported via axon-like cell processes to the aortic wall for release. The neurosecretory material from the brain transported via the nervus corporis cardiacum (NCC) are also released in the aortic wall, which is apparently the main neurohaemal organ in the retrocerebral system of *Glossina*.

The objective of this study was to investigate whether the neurosecretions in the corpus cardiacum - corpus allatum-aorta (CC-CA-A) complex in *G. morsitans* have hyperglycaemic activity.

Extracts of the glandular lobes of the corpora cardiaca from *Locusta migratoria* and the CC-CA-A complex from *G. morsitans* were prepared by disruption using a 150 W ultrasonic tissue disintegrator, and diluted with saline such that 0.033 glands were contained in 10 μl of saline for injection in *L. migratoria* and *Periplaneta americana*. For material injected into *G. morsitans* the dilution was 0.003 glands contained in 1 μl of saline. *L. migratoria* and *P. americana* were injected between any two abdominal sterna, while *G. morsitans* were injected through the arthrodial membrane at the neck. The haemolymph was collected by puncturing the arthrodial membrane at the base of one of the hind legs in *L. migratoria* and *P. americana*, but by slitting the neck membrane in *G. morsitans*.

Total haemolymph carbohydrate (anthrone-positive material) was determined by spectrophotometric absorbent readings. To determine the effect of flight on total haemolymph carbohydrate concentrations flies were placed in 30 cm^3 mosquito netting cages and stimulated to fly by rotating the cages at the rate of 45 cycles per min in all directions up to a maximum of 1 h. Results shown in table 53 indicate that the CC-CA-A extract from *G. morsitans* evoked a 65% increase in total haemolymph carbohydrate concentration (THCC) when assayed on *P. americana*. The response to the CC extract from *L. migratoria* when similarly assayed was slightly less than that observed for the CC-CA-A extract from *G. morsitans*. There was a slight and insignificant change in the THCC in control *P. americana* treated with tsetse brain extract. Tsetse treated with their own species CC-CA-A extract showed an increase of over 100% in THCC compared to the controls. A significant change in THCC was also observed in those flies treated with *L. migratoria* extract. However, the relative increase in THCC in the experimental flies treated with tsetse CC-CA-A extract was much higher than in the experimental flies receiving locust CC extract. There was no significant change of THCC in the flies receiving brain extracts from either locusts or tsetse.

When a group of flies was agitated to fly in a cage, there was a slight decrease in the THCC after 30 min of flight as compared to the resting control flies. However, within 60 min of flight the THCC was observed to be higher than that obtained for resting control tsetse. The difference in THCC between flies flown for 30 min and those flown for 60 min was significant at $P < 0.01$ and represented a change of 123% in THCC in 30 min.

It is well known from literature that an increase in blood sugar (trehalose) concentration is a measure of hyperglycaemic activity in insects. The observations reported here show that the CC-CA-A complex of *G. morsitans* contains a hypergiycaemic factor that causes considerable increase in total haemolymph carbohydrate (hence trehalose) concentration, not only in *G. morsitans* but also in *P. americana*.

EFFECT OF PRECOCENE II ON THE MILK GLAND OF F_1 GENERATION OF *G. M. MORSITANS* (WESTWOOD)

I. R. S. Awin, M. I. B. Chaudhury

Treatment of female parents with precocene II has been shown to cause sterility in the F_1 generation of female *G. m. morsitans*. This sterility involves abnormalities in follicle development and retardation in oocyte development.

The present study was carried out to investigate the effect of precocene II on the ultrastructural morphology of milk glands of both the treated parents and their sterile and normal F_1 offsprings.

Mothers were treated with precocene II soon after emergence, and on days 2, 9, and soon after larviposition,

Table 53. Effect of corpus cardiacum extracts on total haemolymph carbohydrate concentrations in adult female *P. americana* and 4-day-old *G. morsitans*

Tissue extract and origin	Total haemolymp carbohydrate concentration $\mu g/\mu l$ mean SE during rest	after injection	% change in concent	N
P. americana				
G. morsitans CC-CA-A	23.2±2.3	38.3±4.0	65.1	12
L. migratoria CC extract	22.1±4.5	31.5±3.3	42.5	12
G. morsitans brain extracts	23.0±2.1	25.7±3.6	11.7	12
G. morsitans untreated	7.2±2.7*			
G. morsitans CC-CA-A		15.5±2.2	115.2*	10
L. migratoria CC extract		11.0±3.6	52.8*	21
G. morsitans brain extract		8.3±1.7	15.2*	11
L. migratoria brain extract		6.5±2.9	-9.7*	12

***Percentage based on untreated resting controls**

for the first three larvipositions. Their F_1 females were allowed to mate and feed routinely. The milk glands of F_1 females that managed to develop a larva in the uterus were dissected out during different stages of larval development, and processed routinely for transmission electron microscopy. Similarly the glands from untreated sterile females as well as those from sterile females treated topically with ZR 515 (juvenile hormone analogue) were processed for electron microscopy.

The milk glands of sterile F_1 offspring were characterized by a cytoplasm consisting predominantly of free ribosomes. Stacks of rough endoplasmic reticulum (RER) that characterize an actively secreting cell, were completely lacking. Instead, ringed arrays of RER, some of which resembled finger-prints, surrounded the lipid globules in the cytoplasm. Nuclear and cytoplasmic shrinkages were common in such glands. The latter form of shrinkage was observed along the basal as well as lateral plasma membranes. The lateral plasma membranes, however, remained attached to each other at the points where they were tightly held by separate desmosomes (Fig. 21).

Lysosomal bodies, formed in the cytoplasm around the Golgi vacuoles and saccules, and their membranes became indistinct. The cytoplasm around the Golgi bodies appeared to disintegrate. This gave rise to enlarged reservoir-like opening in the cytoplasm (Fig. 22). Disintegration was also observed in the nucleus, with the chromatin and nucleolus falling apart.

Following treatment of the sterile females with the JH analogue, signs of recovery of the protein synthetic apparatus in the secretory cells were observed. This included the recovery of the Golgi membranes, the reappearance of stacks of RER in the cytoplasm, and the disappearance of lysosomal bodies. The nuclei became oval in shape and there was no sign of disintegration in the nucleoplasm.

In fertile F_1 female offspring, and in the mothers, the secretory cells of the milk gland were active. The RER were aligned into well developed stacks and abounded in the cytoplasm (Fig. 23). Lipid globules were also well formed.

This study has revealed that following precocene II treatment of the females, the synthetic activity of the milk glands of the F_1 generation were affected to a varied degree. In the treated mothers and fertile offspring, the milk glands were not affected at all, and appeared to support and provide the necessary nourishment to the larva. In sterile F_1 females, however, the milk glands were rendered inactive due to nuclear and cytoplasmic

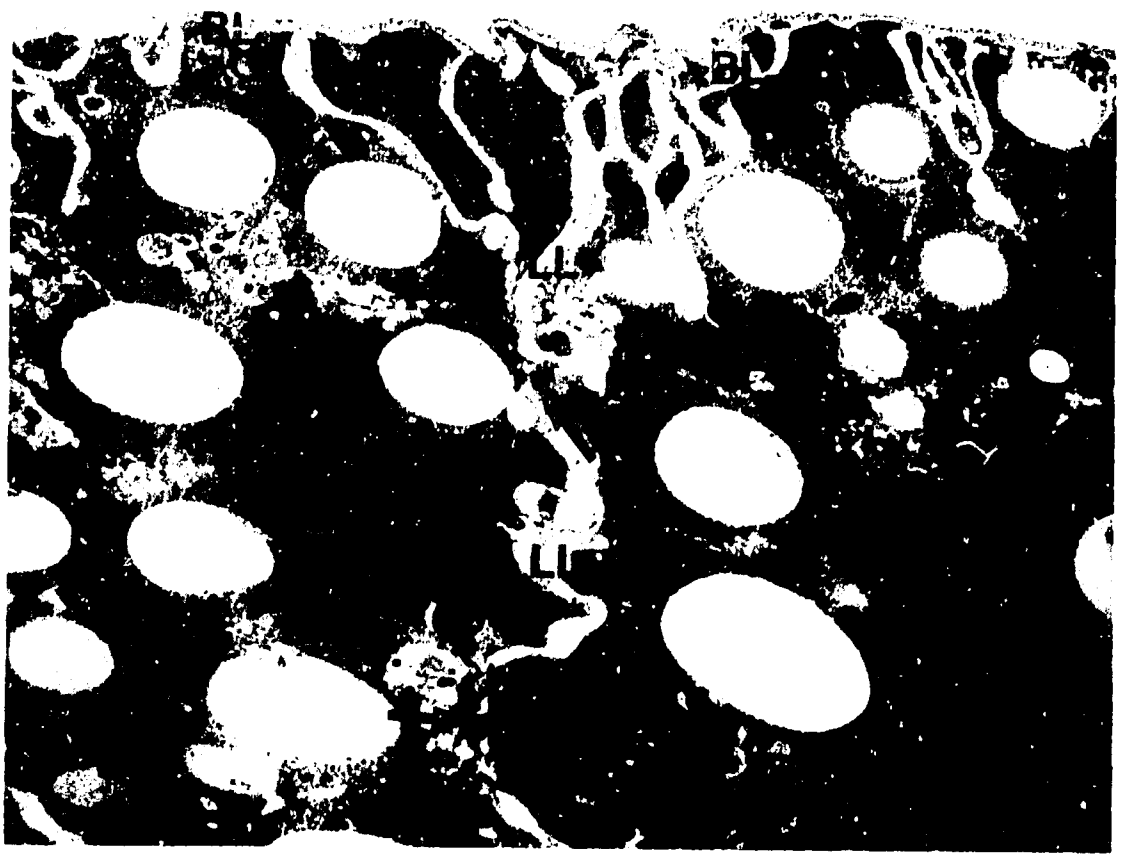


Figure 21. Milk gland of sterile female F1 of *G.m. morsitans* showing shrinkage of the basal (BL) and lateral (LL) plasma membranes. Note that the lateral plasma membranes are held together only by separate desmosomes (arrows). Note also the array of fingerprint-like rough endoplasmic reticulum (RER) N, nucleus; Ly, lysosome

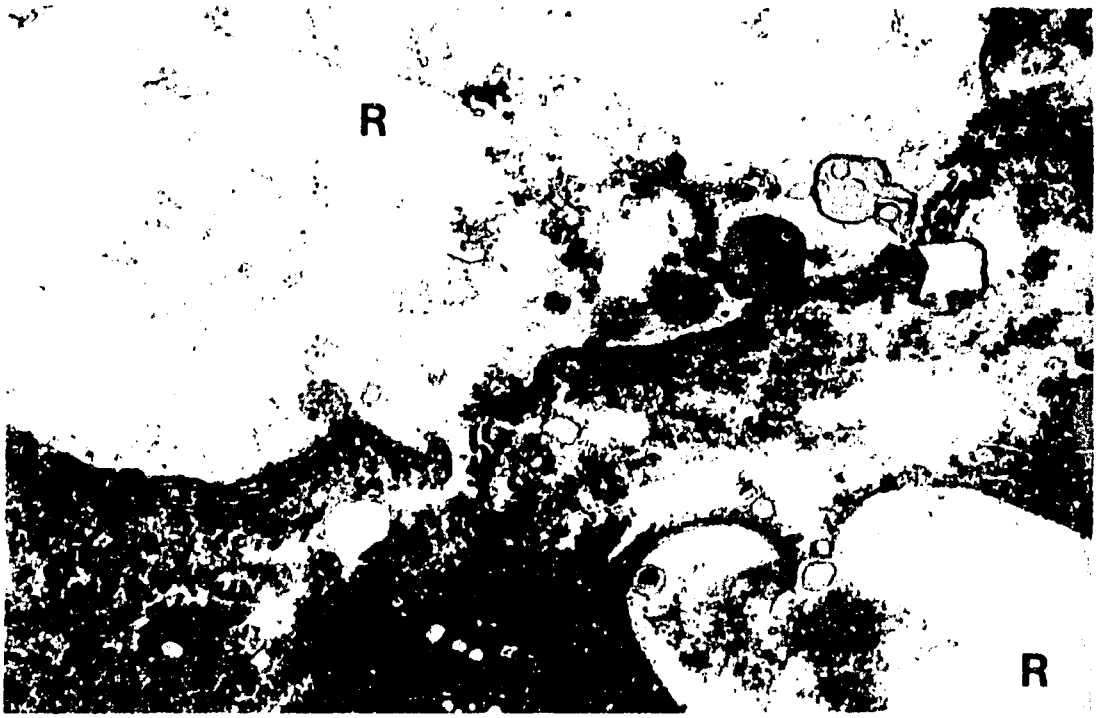


Figure 22. The disintegrating cytoplasm in the milk gland of a sterile F_1 female. Note the collection of cell debris (CD) into reservoir-like openings (R). G, Golgi apparatus; RER, rough endoplasmic reticulum.

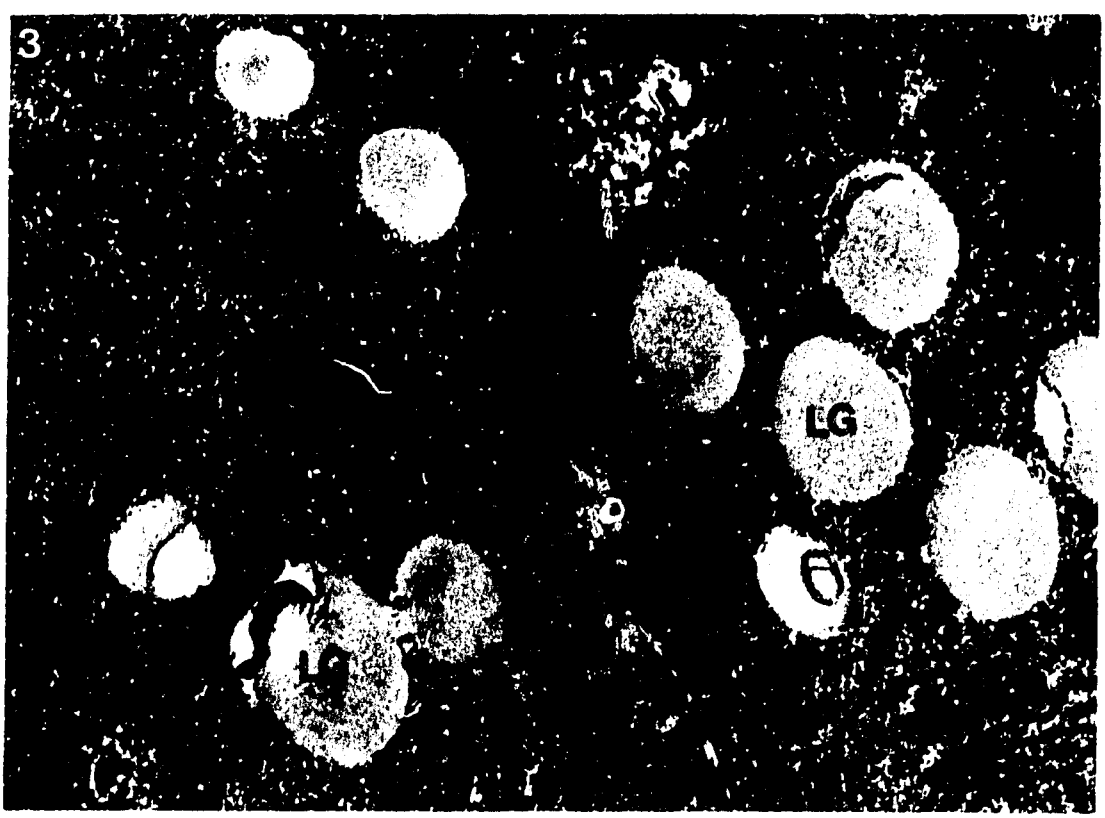


Figure 23. In a normal F_1 female the actively secreting gland cells have numerous stacks of well developed rough endoplasmic reticulum (RER) and lipid globules (LG).

shrinkage and disintegration. Although sterility itself was irreversible it was possible to reverse this inactivity of milk glands by treatment with a juvenile hormone analogue.

ULTRASTRUCTURE OF CULTURED EMBRYONIC TICK CELLS

J. R. S. Gatti, M. Nyindo, J. S. Dhadialla

Tick embryonic cells have been cultured for the production of antigens to be used for experimental immunization of cattle against infestation by the brown ear tick, *R. appendiculatus*.

Light microscopic observations of the cells in culture revealed the presence of two adherent cell types: epithelial type cells which are small and oval in shape are predominant while spindle shaped cells which are elongate are fewer.

This report concerns the ultrastructural observations of the two cell types. Cells which had been in culture for 30, 60 and 90 days were pelleted and transferred to cold glutaraldehyde fixative. The pellets were then processed for electron microscopy following standard procedures.

Ultrastructural observations confirm the presence of two cell types (cell type I and cell type II). The type I cells are predominant and are characterized by a plasma membrane which is thrown into folds, giving it the appearance of a microvilli brush border, hence the name epithelial cell type. In the type II cell, the plasma membrane has no indentations, but take a smooth course around the cytoplasm (Fig. 24). Often the cells clump together into a monolayer.

The cytoplasm of both cell types is characterized by the presence of rough endoplasmic reticulum. These occur as single short strands scattered evenly throughout the cytoplasm. The canaliculi of the RER are fairly wide and seem to contain a secretion. They resemble the documented RER of trophocytic fat body cells of some female ticks. The Golgi area of the cytoplasm is made up of flattened saccules with an electron-lucent core, and vesicles which are electron transparent. Condensing vacuoles with secretory granules have not been observed. Apart from the ribosomes that stud the RER, free ribosomes and polysomes occur in the cytoplasm. The nucleus occupies a central position. The chromatin in the nucleus is evenly dispersed, but condensed chromatin occurs along the inner membrane of the nucleolemma.

A number of intracellular inclusions occur in both cell types. Lipid inclusions have been observed in some of the cells. Also conspicuous in both cell types is the presence of membrane-bound, electron-dense granules. These appear in different sizes. The smaller ones have electron-dense grains resembling glycogen particles. The larger ones appear to be made up of similar grains cemented together by an electron-dense matrix. They resemble secretory vesicles and yolk granules observed in vitellogenic oocytes from ticks. Lysosomal activity manifests itself in two forms. The first form of this activity consists of numerous small membrane-bound vesicles within a larger membrane-bound enclosure.

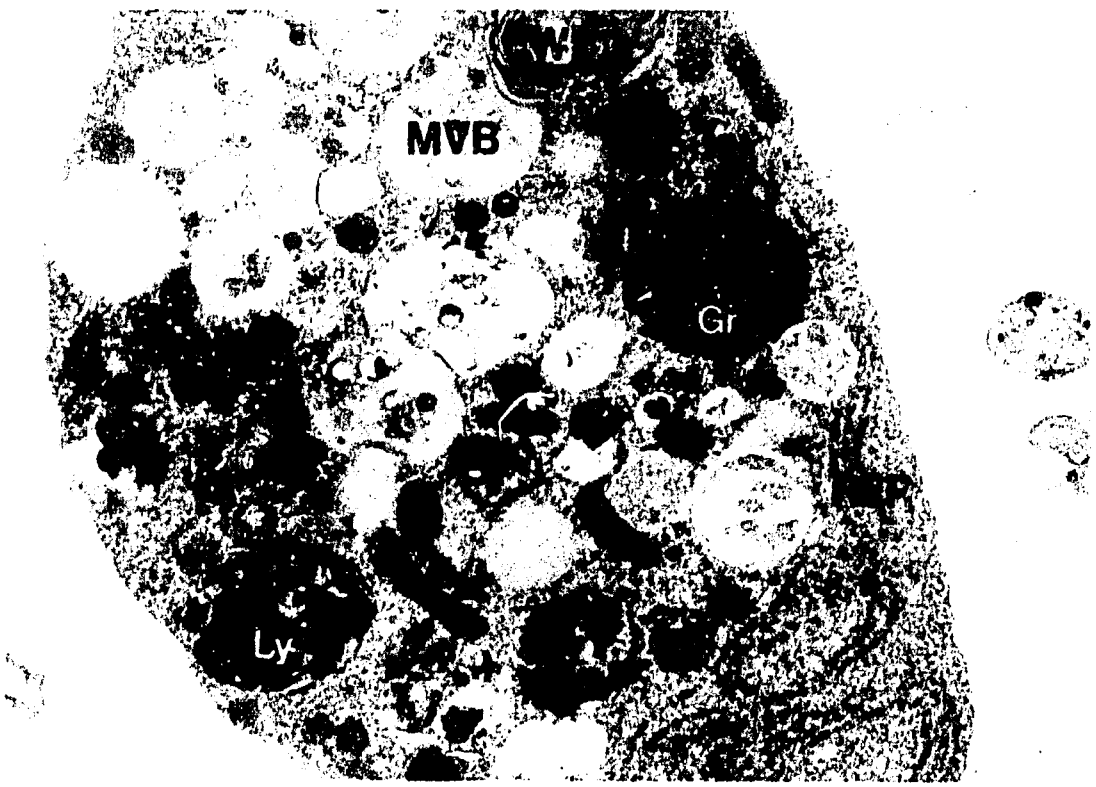


Figure 24. A type II cultured tick embryonic cell.

These are referred to as multivesicular bodies. The other form of lysosomal activity consists largely of whorled rings and other kinds of cell debris. All the intracellular inclusions observed in these embryonic cells are typical of those observed by other workers in tick oocytes during vitellogenesis.

The observations reported indicate that the tick embryonic cells are active in protein synthesis and also, possibly synthesize material similar to that observed in tick vitellogenic oocytes.

SENSORY ORGANS OF *CHILO PARTELLUS*

I. D. Kokwaro, S. Wakhaka, M. Chumiaw

Scanning electron microscope studies on the larval and adult antennae were conducted. It was observed that each larval antenna has a sensillum styloconicum and two types of sensilla basiconica. Electrophysiological data from sensillum styloconicum showed that the sensillum responds to temperature changes while three of the sensilla basiconica were sensitive to odour stimuli. Transmission electron microscopy on these sensilla is in progress. Furthermore behavioural tests to odour are being conducted in order to establish the relationship between behavioural responses and electrophysiological data.

Work on the morphology of the moth antennal sensilla has continued. It is now known that the grooved sensilla on the antenna of the male moth are innervated by two cells. One of these cells is sensitive to odours from the abdominal gland of virgin *Chilo* females. Transmission electron microscopy studies on the rest of the adult antennal sensilla are in progress.

TRICHOMES ON MAIZE LEAF SURFACES

I. D. Kokwaro, J. K. Ampofo

Based on ecological studies which showed that trichomes on maize leaf surfaces appeared to influence the selection of oviposition sites on resistant and susceptible maize genotypes, the scanning electron microscope was used to study various leaf surfaces in order to explain the role of trichomes in *C. partellus* oviposition. The lower surfaces where there are no trichomes or where they had been shed were more preferred for oviposition. On the other hand, where there was a mixture of trichomes and spines (Fig. 25), oviposition was hindered.

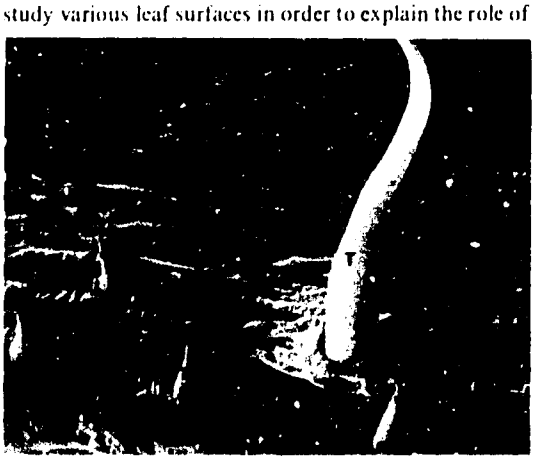


Figure 25. Scanning electron micrograph of the upper leaf surface of maize. X 725 T - Trichomes; Note stomata (S) in the background

These observations indicate that apart from insect sense organs plant texture plays part in guiding *C. partellus* to suitable ovipositional sites.

EFFECT OF LIGATION ON SPERMIOGENESIS IN *G. MORSITANS*

I. D. Kokwaro, S. Yagi

We have previously reported on the cellular organization of the pupal testes of the tsetse *G. morsitans* and recorded ultrastructural changes which characterize the transformation of cells during the process of spermatogenesis *(ICIPE Eleventh Annual Report, 1983)*. The objective of the present study has been to examine the effect of ligation on testicular development and spermiogenesis.

Gross morphological studies showed that ligation delayed pupariation, retarded testicular development and caused alterations in cellular components. The testes from ligated pupae failed to elongate, to coil and finally degenerated. The histological features most noticeable in the pupal testicular cells were mainly a reduction in the number of cysts and spermatids, development of thick intercystial tissue and the appearance of vacuoles of various sizes indicating that there may be a breakdown of cell contents. Future studies will aim at elucidating the hormonal control mechanism on spermatogenesis in *G. morsitans*.

FUNCTION OF THE CHORIOTHETE IN THE TSETSE, *G. M. MORSITANS*

J. A. Kongoto

The ventral part of the uterus of glossinids is characterized by the presence of a glandular structure known as the choriothete. The structure was so named by its discoverer, Jackson, in 1948, who suggested that it was responsible for the removal of the chorion from the newly-hatched first-instar larva and the exuvium from the newly-molt second-instar larva. Subsequent to Jackson's observations, however, there has been some controversy regarding the function of the organ, some authors suggesting that the choriothete is merely an organ for support of the developing embryo or larva.

The aim of the present study is to elucidate the function of the choriothete and to investigate the nature of its involvement in the removal of the chorion and exuvium.

Our observations on the secretory nature of the choriothete confirm those of other workers. It has been suggested by other authors that the secretion, which consists of at least proteins and mucopolysaccharides, acts as a cement to bind the chorion and the first larval exuvium in turn, during their removal. The choriothete cells are probably also involved in ionic exchange by virtue of their extensive plasmalemmal invaginations, which are associated with mitochondria (Fig. 26).

In 1983 we reported the presence of cuticular spine-like structures and rows of depressions on the choriothete.

Figure 26. Low power transmission electron micrograph of part of the choriothete showing plasmalemmal infoldings (asterisks) X 10,000.

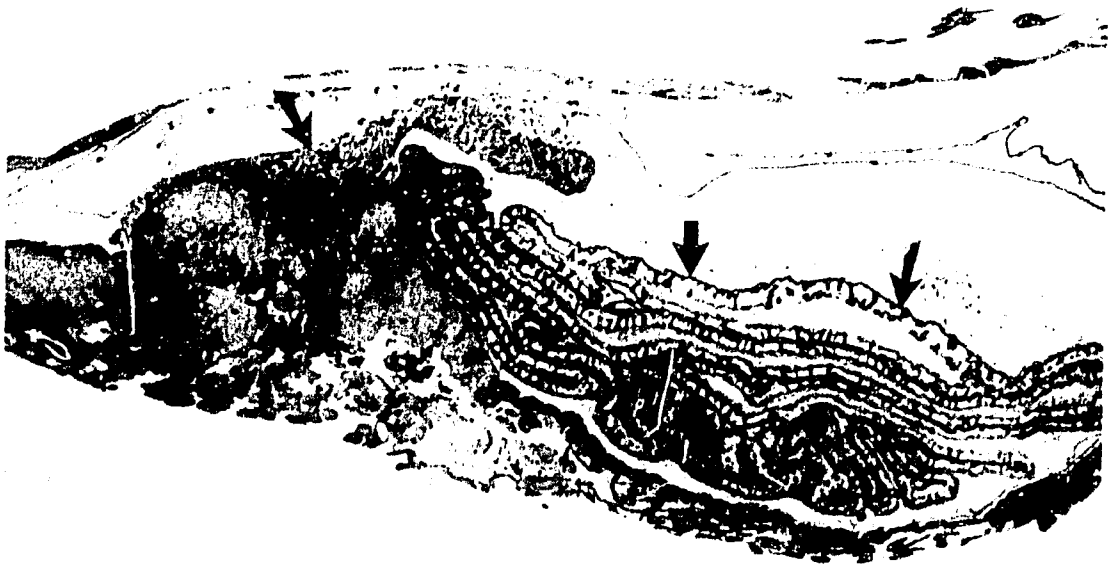


Figure 27. Photomicrograph showing the cast cuticular mass (arrows) in close association with the choriothete (C h) in a 16-day-old mated female *G m. morsitans* X 328.

Figure 28. Transmission electron micrograph showing part of the cast cuticular mass (arrows) in close association with the apical part of the choriothete. Cu, cuticular lining of the choriothete; Pi, plasmalemmal infoldings of the apical cell membrane; Mi, mitochondria; Cy, cytoplasm. X 15,280.

These features are probably involved in the removal of the chorion and exuvium.

Light and electron microscopic observations show that both the cast chorion and exuvium are closely associated with the choriothete (Figs. 27, 28). From observations made on the choriothete of flies both at an advanced stage of pregnancy and at younger stages, it appears that the choriothete probably changes its contour at different stages of development. These observations support the suggestion that the choriothete is involved in the removal of the chorion and first-instar larval exuvium.

Sensory Physiology

Gustation in the stem borer *C. partellus* 77
Antennal sensilla of *Chilo* larvae 80
Chemocommunication in tsetse flies 81

Sensory Physiology Research Unit

Since the Sensory Physiology Research Unit (SPRU) was set up over ten years ago, there has been an increase in the number of staff and scientific equipment. In order to create a better working environment it was necessary to renovate the laboratory and improve on the placement of electrophysiological set ups as well as other items in the laboratory. This exercise was started and completed early this year. This has contributed to a reduction in the mechanical and electrical interferences which used to affect experiments.

SPRU has continued to collaborate with the crop pests and tsetse research programmes and collaborative efforts with the sister units (chemistry and bioassay and fine structure) have continued. Studies on the tsetse fly have been focussed on behaviour, especially the antennal reaction to certain stimuli assumed to affect the fly's behaviour. Some of the stimuli tested are compounds of known composition and concentration. Other stimuli are of unknown composition but are mainly extracts of the host animal by-products such as buffalo urine which is supposed to be a good tsetse attractant. This work will soon be supported by electrophysiological test especially single cell responses from the antennal sensilla. With regard to crop pests, the chemical stimuli related to host plant resistance or susceptibility to stem borers are not well understood. We have therefore employed electrophysiological and behavioural bioassays on C. partellus *to investigate the effects of materials obtained from susceptible and resistant host plant genotypes. Since behaviour is an important aspect of the work, steps are being taken to develop an adequate feeding bioassay for* Chilo *larvae. So far most of the electrophysiological studies on olfaction have been done on the moths and very little on the larvae. Olfactory studies on the larvae have therefore been initiated and are beginning to yield results.*

GUSTATION IN THE STEM BORER *C. PARTELLUS*

S. M. Wakhile, H. M. Kahoro

During the period we investigated electrophysiological responses from the gustatory receptors especially sensilla styloconica on the larval mouthparts and the trichoid sensilla on *C. partellus* ovipositor and tarsi. Some functional morphology studies were done to verify the function of some of the sensilla studied. NaCl (0.01 to 0.1M) and D sucrose (0.01-0.1M) dissolved in 0.1M NaCl were used as standard stimuli on the taste hairs. The other stimuli of unknown composition were obtained from 4-week-old maize plants, (susceptible Inbred A and resistant CM 1 324). The stimuli from the above maize genotypes were as follows: the leaf exudate collected from the upper surfaces of the maize leaves, the other exudate was that which oozed from maize stems when the leaf-bearing part was cut off and the other stimuli were aqueous extracts obtained by passive extraction of maize stem tissues. Since there is no adequate feeding bioassay for *Chilo* larvae, we are developing a method suitable for such a bioassay.

Electrophysiology

Moth ovipositor tarsal and sensilla. Four sensilla on

the *Chilo* ovipositor are contact chemoreceptors. They are more sensitive to NaCl than sucrose. The presence of sucrose in the stimulating solution inhibits the effect of NaCl. It was observed that the leaf exudates from both susceptible and resistant plants were as good a stimulant as NaCl solution. This does not necessarily imply that the stimulating component in the leaf exudate is NaCl. It is however apparent that the presence of the leaf exudate can be detected by the ovipositor and the intensity of response to the above exudate is stronger than that recorded from the tarsal sensilla. It is also interesting to note that when the leaf exudate is forming on the upper surface of the leaves, the moths are actively looking for oviposition sites on the under-surface of the leaves. It will be worthwhile to establish the chemical components in the leaf exudate and to find out whether the presence of the exudate on the upper surface of the leaves is one of the factors which inhibit oviposition on that surface. It has been reported, however, that moths supplied with leaf exudate from any of the cultivars had a shorter longevity than those supplied with distilled water. This implies that female moths which imbibe leaf exudate are affected physiologically in terms of survival and egg-production. As reported in our earlier studies on the tarsal sensilla of Pyralidae moths, each tarsomere on the first pair of legs has three pairs of trichoid sensilla. The medial and medio-lateral sensilla (formerly pairs number 1 and 2) are bimodal in that they are innervated by mechanochemoreceptive cells. On the other hand the lateral sensilla (formerly pair number 3) is sensitive to mechanostimulation only. Recently, transmission electron microscopy showed that the medial and medio-lateral sensilla shafts contain four dendrites and a fifth dendrite terminates at the base of each sensillum. On the other hand the lateral sensilla are each innervated by two cells. The dendrite of one of the cells extends into the sensillum lumen while the other terminates at the sensillum base. Electrophysiological tests showed that more than one cell in the medio-lateral sensilla are sensitive to sucrose whereas in the medial sensilla only 1 cell is sensitive. Other workers have reported that on the white butterfly, *Pieris brassicae*, the tarsal contact chemoreceptors have been implicated in the selection of oviposition sites. The available information on *Chilo* suggests that the tarsal and ovipositor contact chemoreceptor sensilla are likely to play a crucial role when moths are selecting oviposition sites.

Larval mouthparts sensilla. Although the external features of the medial and lateral sensilla styloconica are similar, the lateral styloconicum sensillum respond with increasing spike frequency to increasing concentrations of sucrose but the medial styloconicum sensillum does not. (Fig. 29). As shown in figure 30, stimulation with aqueous extracts from the susceptible and resistant cultivars evoked rapidly adapting responses from the lateral styloconicum sensillum, whereas responses from the medial styloconicum sensillum adapted less rapidly. The reverse was true when the two sensilla were stimulated with exudates collected from the stems. The effect of the tested stimuli on the styloconicum is shown in table 54 in terms of average impulses frequencies generated in the first 200 ms of stimulus application. Adding sucrose to NaCl almost quadrupled the spike frequency from the lateral styloconicum but had almost no effect on the medial one. Response in the medial sensillum was mainly caused by NaCl and among the stimuli tested the leaf exudate had the least effect on the two sensilla.

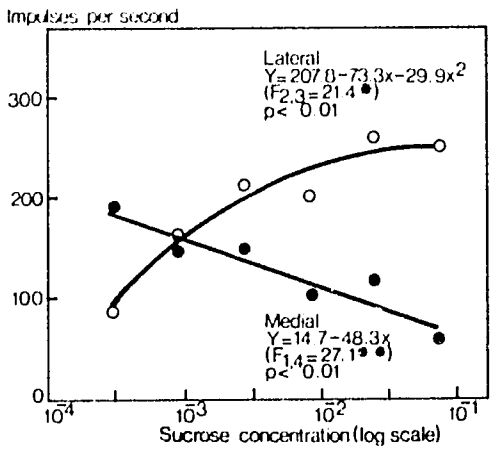


Figure 29. Graph showing the relationship between sucrose concentration and impulse.

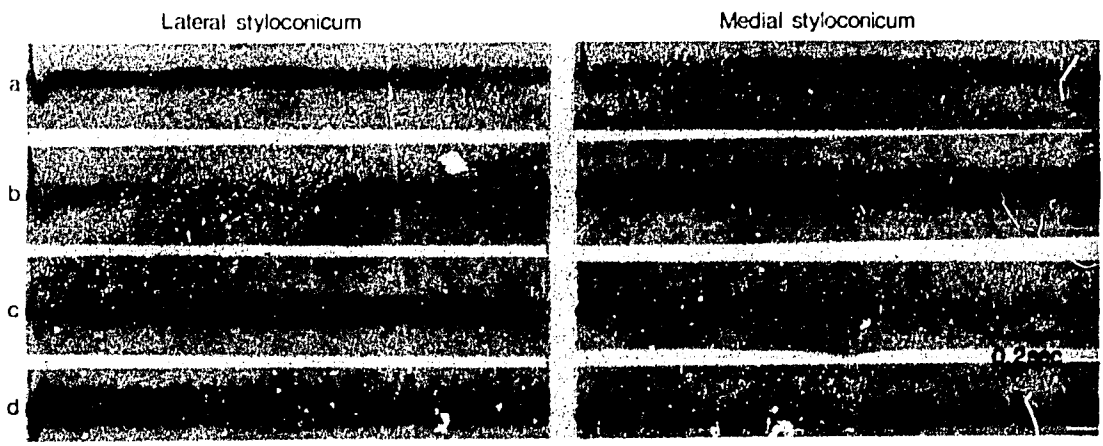


Figure 30. Responses from the lateral and medial maxillary sensilla styloconica of *C. partellus*.

Table 54. Average impulses per second in the first 200ms of stimulus application

Stimulus	Lateral styloconicum	Medial styloconicum
10^{-2}M NaCl	50	115
10^{-2}M NaCl & 2.4 × 10^{-2}M sucrose	189	113
Inbred A dew	25	40
CMI 324 dew	32	31
Inbred A stem exudate	140	45
CMI 324 stem exudate	171	82
Inbred A aqueous extract	98	132
CMI 324 aqueous extract	98	126

It is known that an important part of the sense of taste in lepidopterous larvae is localized in the maxillae where each maxilla bears a pair of sensilla stylocomica. Hence the need to get more information about the sensitivity of those sensilla to known compounds and plant extracts. The same ought to be done on the maxillary palpi sensilla but it is relatively difficult to obtain meaningful electrophysiological data from these. Results reported here and those of other workers show that the sugar sensitive cells in the lateral stylocomica of *Chilo* are more sensitive than their counterparts in the medial stylocomica. This is also the case for other lepidopterous larvae studied. Under normal circumstances the fourth- or fifth-instar larvae used in this study would not come in contact with the leaf exudate as they would already be burrowing in the stem. It is therefore not surprising that the leaf exudate had the least stimulating effect on both the maxillary stylocomica sensilla. On the other hand the stem exudate and extracts are important components of the medium in which such larvae grow and develop. The stimuli in those components can have far-reaching consequences on larval behaviour and it is interesting to note that the two stylocomica sensilla were affected differently by the two components. It is essential to establish the chemical stimuli responsible for the observed differences.

Feeding bioassay

For the feeding bioassays it was essential to use a porous substrate which could absorb the phagostimulant solutions and is also amenable to *Chilo* larvae feeding behaviour. Mature piths from maize and papyrus stems were selected. Water soluble and organic compounds in the two piths were extracted by soaking the piths in two changes of methanol for at least two days. The piths were then washed in running water for another two days, freeze-dried and weighed. Piths so prepared readily absorbed the test solution. A pair of piths were soaked in each of the test solutions (D-sucrose, diluted aliquots of the aqueous and organic maize extracts). Since the organic extracts were dissolved in acetone, the solvent was allowed to evaporate from the piths before they were made dump by soaking in limited amounts of water. All the treated piths were then dropped in labelled vials containing two fourth to fifth-instar larvae which fed on the piths for seven days. At the end of the feeding period the larvae were removed and the intact pith together with the saw dust (feacal material plus pith crumbs) were dried in the oven at 60° C. After drying, the sawdust debris on the pith was shaken off and the weight of the sawdust and frass produced by larval activity as well as that of the intact pith taken. The amount of the original pith weight which was converted to sawdust or frass was taken as a measure of larval feeding activity. This was expressed as a percentage of the initial pith weight.

The maize and papyrus piths served as suitable substrates for feeding *Chilo* larvae. As shown in table 55, the larvae displayed more activity on the maize piths than on the papyrus piths. This is possibly due to the fact that the maize pith has a firm texture and does not soak up as much moisture as the papyrus pith. Most of the larvae used were recovered alive at the end of the experiment. Those larvae recovered from the maize piths which had been treated with the organic extracts showed a marked degree of growth and a few pupated. This suggests that the organic extract had more nutrients than the aqueous extracts.

Table 55 Percentage of pith weight converted to sawdust by laval activity (feeding)

Stimulus on pith	Percent converted	
	Maize stem	Pypyrus stem pith
Control	5.2	2.6
Inbred A aqueous extract	5.8	3.3
CMI 324 aqueous extract	10	3.9
Inbred A organic extract	10	4.8
CMI 324 organic extract	11	7.9
Sucrose 8.1 x 10^{-3}M	14	1.4

Feeding bioassay reaffirmed the fact that sucrose is a good phagostimulant for the larvae. The piths treated with sucrose induced far much better feeding activity than the controls (table 55). More tests have to be done before any conclusive statement can be made on the effects of other stimuli currently under investigation. Feeding bioassay for *Chilo* are a necessity because they provide an opportunity to observe what happens when larvae are presented with one or more stimulants under specified conditions. Furthermore the observed behaviour reflects the interaction of different sensory cells namely those on the maxillary galea, the epiphryngeal surface of the labrum and possibly the mandibles. Feeding is one of the factors which determine establishment of insects on different plants. It has been suggested that insect feeding activity on plants has two main aspects: the acceptance of a diet for feeding and the degree of food ingestion from the accepted diet. During the initiation and continuation of feeding the observed behaviour results from the message gathered by the sensory cell. This message comes from the combined effect of several chemical stimuli in plant tissues. Observations by other workers experimenting on other lepidopterous larvae showed that insects prefer certain combinations of phagostimulants. This implies that plants with the right combinations may be susceptible whereas those lacking the combinations may be resistant. In order to identify such difference in various cultivars electrophysiological and behavioural methods will be

very useful. However, it is important to point out that collaboration with the chemists is indispensable.

ANTENNAL SENSILLA OF *CHILO* LARVAE

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

There is very little electrophysiological data on the olfactory sensilla of lepidopterous larvae. This is probably due to the fact that those larvae are very clumsy and difficult to handle. The larval antenna are situated in sockets where they can be completely retracted thus making it impossible to approach their sensilla. However, when the antenna are forced out of their sockets the use of metal electrode as a means of obtaining electrophysiological responses has limited application because the antennal structure has a tendency to recede

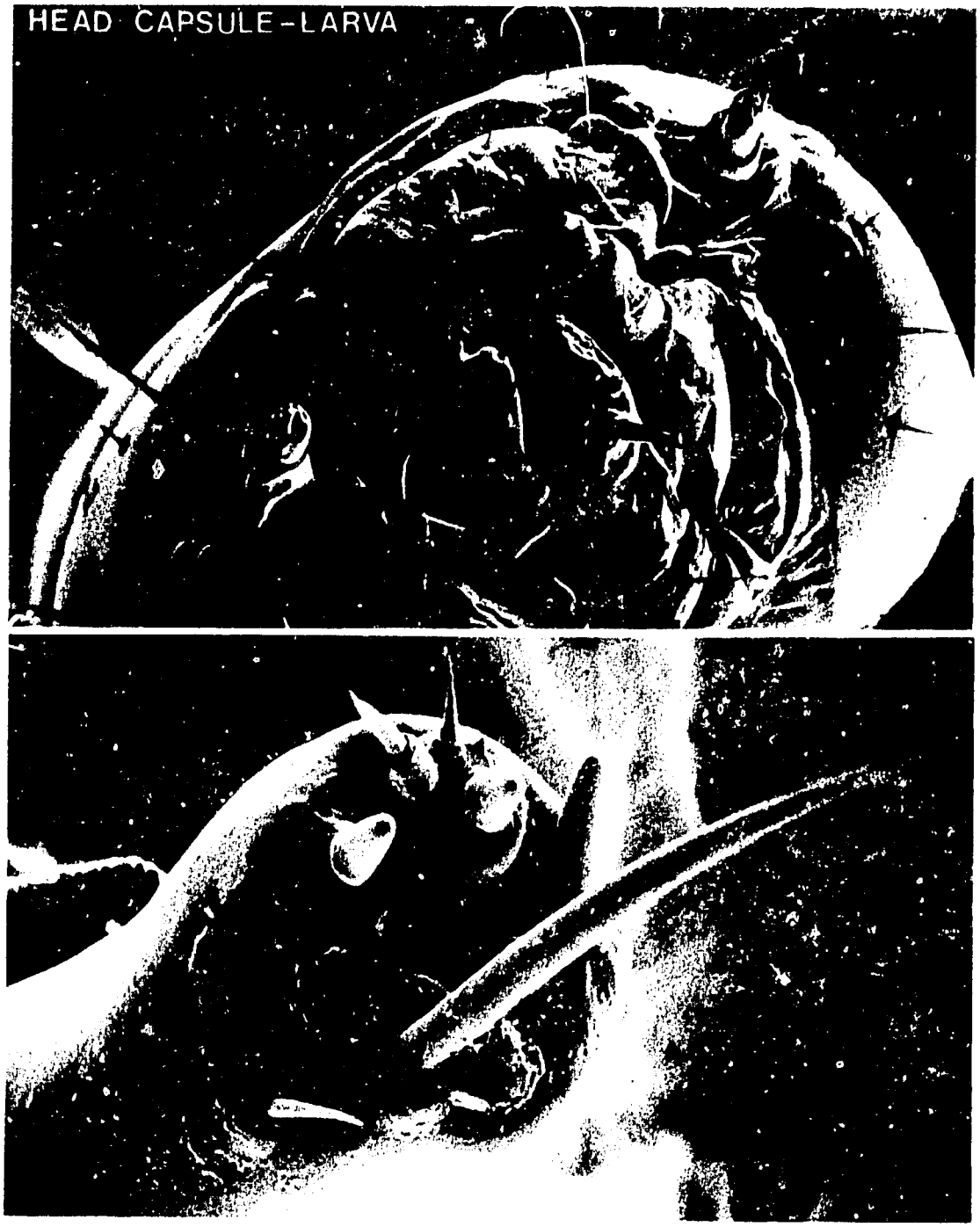


Figure 31. Scanning electron micrograph of the head capsule of *C. partellus* larva.

while the electrode is being implanted at the bases of the sensilla. Therefore, in our electrophysiological studies we used the cut-tip recording method, which proved to be much more applicable, and it was possible to obtain reproducible results.

Scanning electron microscopy showed that the *Chilo* antenna has three different sensilla types; the blunt tipped sensilla basiconica, the sensilla styloconica and a lone sensillum basiconicum with a sharp pointed tip (Fig. 31). No electrophysiological data has been obtained from the latter sensillum. Data obtained from sensillum styloconicum shows that it is a cold receptor while the blunt tipped sensilla basiconica are olfactory receptors.

It is interesting to note that the styloconica sensilla on the *Chilo* larvae has a collar which marks the borders of the platform from which the sensillum shaft projects like a missile. The same is true for sensilla styloconica on the adult *Chilo* antenna but the significant difference is that the larval styloconica sensilla are three times longer than those of the adult. Furthermore both larval and adult antennal styloconica are cold receptors responding to low temperatures with increasing spike frequency, and are inhibited by light and rising temperature (Fig. 32).

So far, we have more electrophysiological data from the olfactory hair (A) situated close to the styloconicum sensillum. This sensillum has at least three active cells producing high medium and low amplitude spikes (Fig. 33). Analysis of the spike frequencies from those cells showed that sorghum and maize odours affect those cells differently (Fig. 34). This suggests that *Chilo* larvae are capable of distinguishing between sorghum and maize odour.

These kinds of electrophysiological data from the larval antenna are encouraging in that we may be able to use electrophysiological bioassays to find out whether susceptible and resistant genotypes differ in their odour components. For this work to be meaningful it will have to be done in close collaboration with the chemists who have to isolate the pertinent chemical compounds from these plants.

CHEMOCOMMUNICATION IN TSETSE FLIES

R.K. Saini

Experiments to screen various synthetic and natural chemicals in order to identify attractive and repelling chemicals are in progress. The perception of the cuticular female sex pheromone (15, 19, 23-trimethylheptatriacontane) was also studied electrophysiologically and behaviourally.

Electrophysiological recordings of responses from tarsal, tibial and even femoral chemoreceptive hairs on any of the legs of *G. m. morsitans* gave no indication of the stimulatory effect of morsilure. However, electroantennograms (EAG's) were recorded during stimulation with the odour of morsilure indicating that the pheromone was perceived by the insect via olfactory receptors on the antennae (Fig. 35). The amplitudes of the EAG's ranged from about 0.1 mV on stimulation with 5 μl of morsilure to about 1.0 mV on stimulation with 20 μl. In comparison, alcohols and ketones evoked much higher EAGs. In fact on stimulation with the odour of only 5 μl of a substance like 2 butanone, an EAG of about 3 mV may occur.

The fact that the flies were able to smell the pheromone was further confirmed by behavioural experiments in which antennal movements on stimulation with the odour of synthetic pheromone were studied. These experiments also indicated that male responses to morsilure increased with increasing starvation (Fig. 36). The observed increase in male responsiveness to morsilure with increasing starvation is in agreement with other behavioural activities such as spontaneous flight activity, visual responsiveness and probing responsiveness which also increase with increasing starvation.

Experiments to screen various chemicals for attractancy or repellency indicate formaldehyde, pentanal, acetone, methylvinylketone, methylethylketone

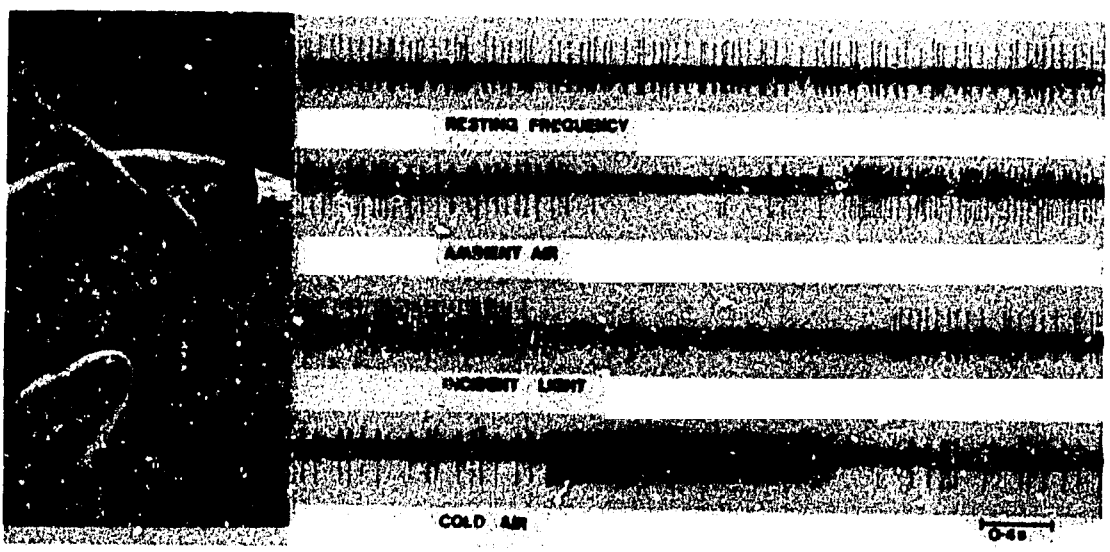


Figure 32. Scanning electron micrograph of the antennal styloconicum sensillum (SS) and the adjacent sensilla.

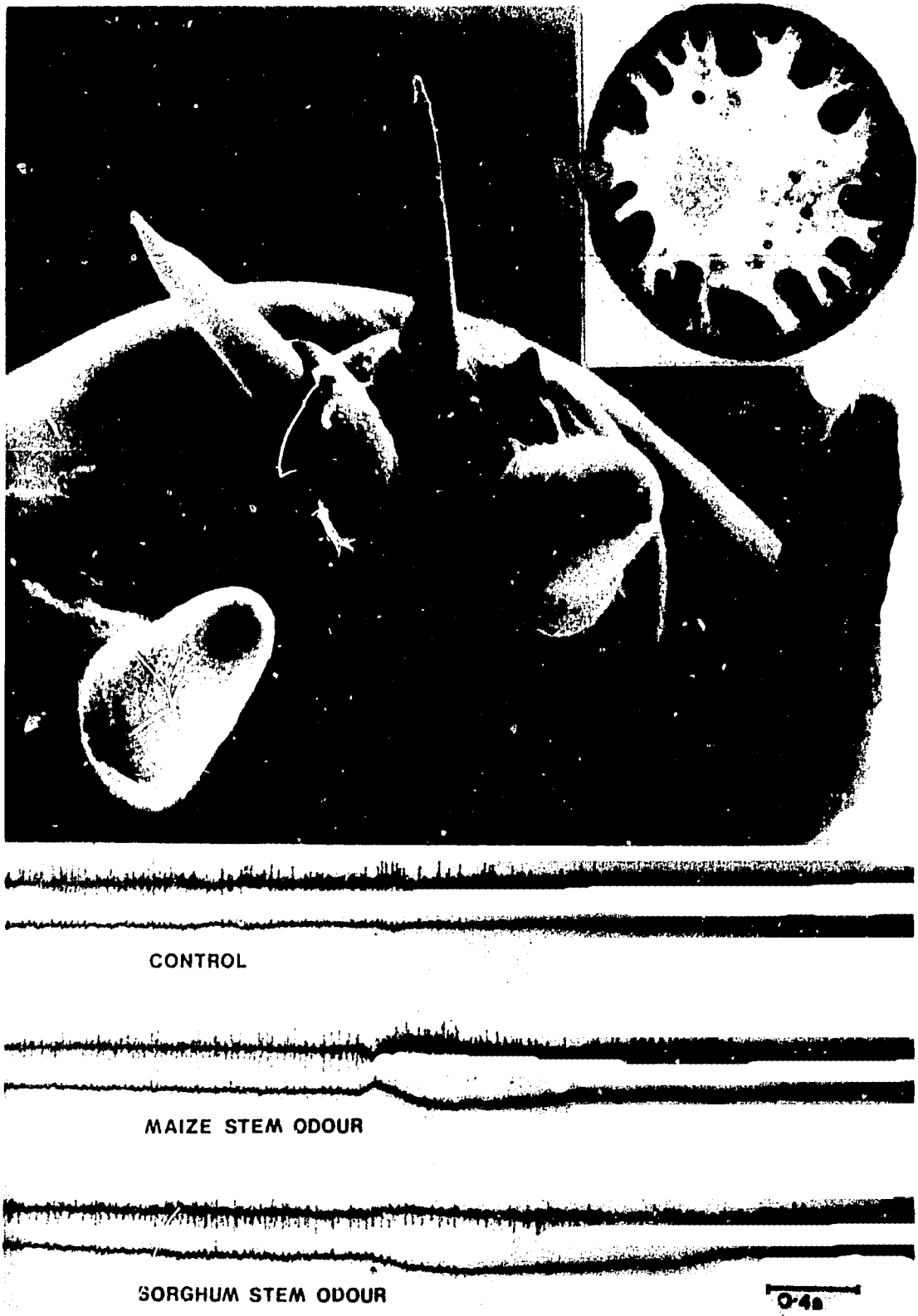


Figure 33. Scanning electron micrograph of the antennal distal end of *C. partellus* larva.

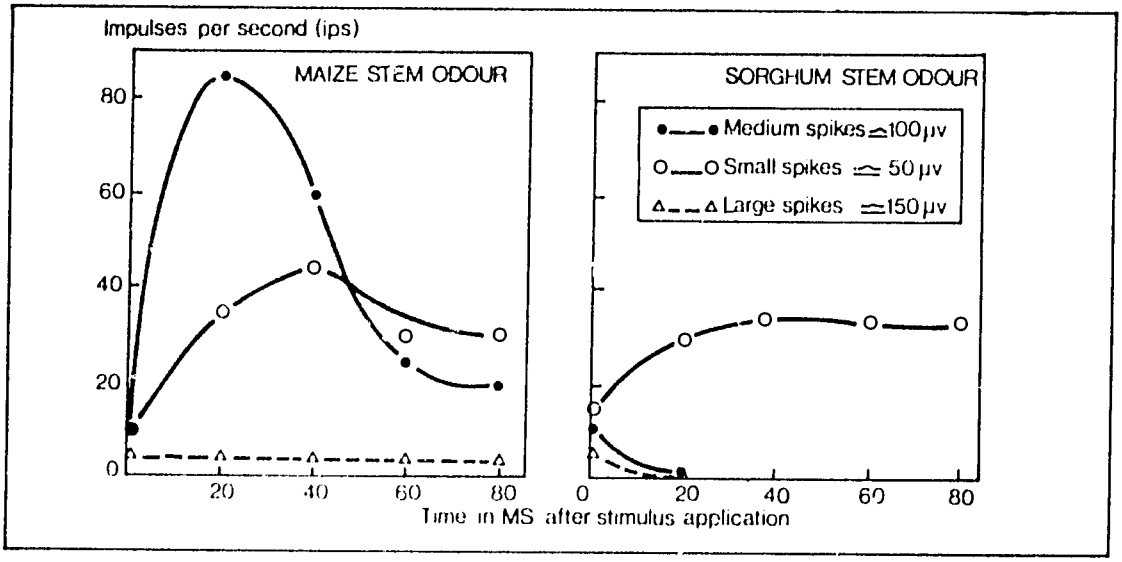


Figure 34. Graph showing the effect of maize and sorghum plant odours on the cells innervating antennal sensillum 'A' of *C. partellus* larva

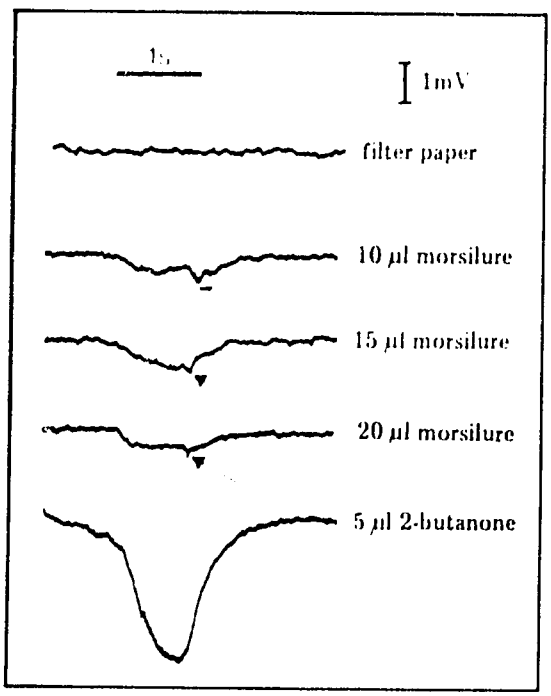


Figure 35. EAG recordings from 12-day-old *G.m. morsitans*

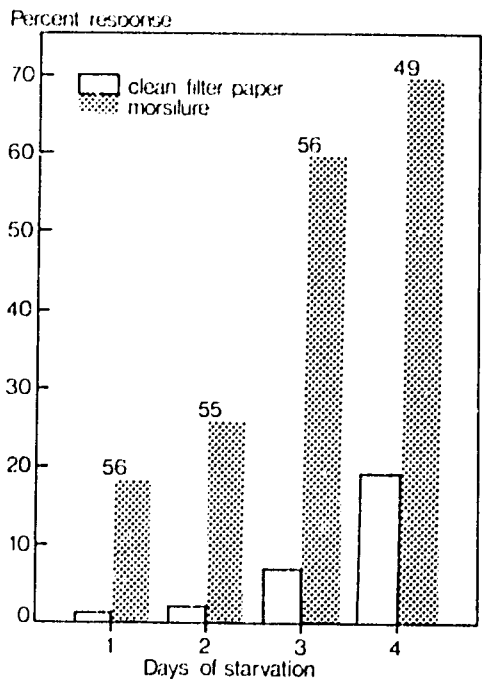


Figure 36. Behavioural (antennal) responses of 4 groups of 7-day-old *G.m. morsitans*

and 1-octen-3-ol to be attractants. Overall responses of males to these chemicals were significantly more than those of females. The amplitude of the EAG's on stimulation with 10 µl of the above chemicals ranged from about 3 mV on stimulation with octenol and methylethylketone to about 1 mV on stimulation with pentanal. Studies are also in progress to determine the attractive substances in buffalo urine.

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Biostatistics and Computer Services

COMPUTER PROGRAMMING, CONSULTATIONS, ASSISTANCE, SHORT COURSES AND WORKSHOP

H.F. Mizeki

Scientific Usage

Programme. Nineteen computer programmes were written and tested with data from the research scientists by Dr. Magalit. Five programmes from *Biometry* by F.J. Rohlf (HOMOV, NESTA, BASTAT, BINOM, and NEGBIN) were rewritten and tested.

To enhance the computing capability of the scientists, programmes such as balanced incomplete block (BIBD), multiple regression (MULREG), correlation with missing value (CORRE), 2-way frequency (FREQ), Latin Squares with Covariance (LATINANO), SORT (sorting of data), LATIN (Latin square), and 2 factors (FACTOR02) are presently being developed.

Some standards are being set up in BCSU for writing FORTRAN programmes and are included in another report. Sixteen programmes from R.G. Davies, *Computer Programming* were rewritten and tested.

A senior programmer, was hired in October 1984 to maintain the foregoing programmes and write more scientific and simulation programmes in the unit. He is currently testing a programme in the Wang PC to estimate the mortality rates of the tsetse fly. He will help supervise the operations of the computer systems of the Centre including the Wang PC's which were acquired late this year.

Consultation and Assistance. Computer Programming consultations and assistance on data analysis and word processing is being extended to the Mbita Point Research Station staff. These services are presently done at Duduville but will be shifted to MPFS as soon as the Wang PC's are installed there.

An operator programmer was hired in August 1984 for Mbita and is currently being trained at Duduville before assignment. He is presently converting the FORTRAN programmes, under the supervision of the Data Systems Manager, in the VS 80 for the Wang PC's which will be installed at MPFS. He is also testing the MSTAT, a statistical package, which will be used by the research staff at MPFS.

Consultations and assistance on data processing and analysis, and word processing were extended to 29 staff members. The 19 programmes mentioned above were written as a result of these consultations. Assistance on word processing was extended by Mrs. Ssebunya to other members of staff using the VS 80 at Duduville. Dr. R. Dransfield continues to give assistance to the research staff on experimental design, data processing and analysis. His assistance is very valuable to the BCSU.

Computerized data system. This was developed for the Tsetse Ecology Project in March 1984 and similar systems will be developed for the other projects later this year. Several test programmes were written for testing the above system. Programmes to summarize the tsetse data are being developed and tested. The data entry and editing, re-editing and merge programmes were developed for the above data system and more will be written to analyze it. We finished analyzing six large data sets for Dr. Seshu Reddy. In collaboration with Dr. Dransfield, we also completed analysis of data for Dr. Unnithan. There were many other analyses done for other ICIPE staff on a limited scale.

Training

A one week course in computer programming was given to ARPPIS students and some ICIPE staff in May 1984.

Another one on biostatistics was given by Dr. Dransfield for one week. A one-hour lecture on data analysis and computers was given to the ICIPE/UNEP group training course participants.

A lecture-demonstration (3 h) on computers and word processing was conducted for participants of a workshop on Scientific Technical Editing, sponsored by International Development Research Centre (IDRC) on August 27, 1984.

The Data Systems Manager attended the workshop on Microcomputer Applications in Agricultural Research

and the Training on Database Software for Microcomputers, held at the International Rice Research Institute, Los Banos, Philippines, on 24 September to 5 October 1984. He presented two papers entitled: 'Microcomputer applications in analyzing experimental data', and 'Computing experience of ICIPE'. The MSTAT, a statistical package, was given to him at the end of the workshop.

Word processing. BCSU staff extend training and assistance regularly to secretarial staff on Word Processing using the OIS 105, VS 80 and the PC.

A computerized mailing list for our library and publications department and a list of the 1983 staff publications and research are now in our mainframe. Accounting records, purchases and sales report for the Duduville International Guest Centre are being prepared in the mainframe using word processing.

An OIS supervisor was hired in October 1984. She takes over the responsibility of training and supervising the secretarial staff on word processing. She will supervise the operations of the OIS 105 system and the PCs at Chiromo.

Computer hardware. The VP 2200 system was adequate for many scientific applications. However, because of the limited memory (32KB) and the limited number of computer programmes in this system, new programmes are being initiated and written for the VS 80 system as mentioned above.

It was found difficult to write and maintain BASIC-2 programmes for the 2200 VP. The 2200 system programmes were also found to be cumbersome and difficult to maintain. More than 80% of scientific applications are now in the VS 80 and this usage will increase as more programmes are written for the VS 80 system. The 2200 VP will be phased out in 1985 and will be replaced by the PCs. There were frequent breakdowns in the printers (daisywheel and band) of the VS 80. These were due mostly to mechanical and heating (inadequate air-conditioning) problems.

Preventive maintenance on the system helped a lot to stabilize the operators of the printers. The OIS at Chiromo had frequent breakdowns early this year and the repairs had to be supervised closely. The operations of both the VS 80 and the OIS are now in stable condition. The VS 80 30 MB hard disk was found insufficient for the storage space requirement of the system.

A maintenance agreement between ICIPE and Computer Applications Limited (CAL) was finalized. The maintenance capability of CAL was studied carefully and found adequate. For a while, the word processing documents of the VS 80 cannot be read at the OIS in Chiromo. It was discovered later to be a hardware problem, i.e. improper disk alignment. Another work station and a new printer were installed in Chiromo in 1983.

Three WANG personal computers (PC) were ordered; 2 for Mbita research station and 1 for the administration division at ICIPE House.

The following upgrading of the VS 80 were carried out in 1984:

- Disk drive from 30 MB to 90 MB in January 1984.
- One work station from 32 KB to 64 KB - October 1984.
- 96 character print-band was installed to include small letter capability to the band printer - October 1984.
- Purchase of high-density matrix printer 5577V.

This will give the VS 80 graphics capability and high speed quality/draft letter print. The 40 CPS Daisy Printer of the VS 80 was installed to the OIS 105 (November 1984) to upgrade the printing speed of the OIS and the 20 CPS Daisy Printer was connected to the VS 80.

Two PCs, one for the use of the Chiromo scientific staff and one for the administration division were acquired towards the end of the year.

Outreach and Training

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Outreach and Training Unit

The creation of the Outreach and Development Programme in 1984 added a new dimension to ICIPE's activities. It provides an outlet to national research systems for information, methodologies and technology developed by the Centre and for training opportunities for national manpower development. This strengthened ICIPE's commitment to existing collaborative linkages in research and development and paved the way for establishing new linkages with more national research and extension services, international agencies and advanced research laboratories for the purpose of sharing information and technology. The programme was restructured during the same year into a unit composed of two sections: Collaborative Research and Development, and Training.

The objective of ICIPE's Outreach and Training Unit is to facilitate further testing and development of integrated pest and vector control strategies at the national level through national programmes as well as regional and international networks. It is also aimed at developing national capabilities in order to facilitate and enhance ICIPE's mandate and its objectives and the application of pest management technology, and consequently, creating foci for collaborative network in national research programmes. The programme is meant to assist in building local scientific and technological capacities of tropical developing countries, particularly in Africa, where the focus is on strengthening national research programmes to make them better equipped for transfer of pest management technologies.

To achieve these objectives, the Unit was engaged in activities to strengthen existing collaborative links and missions to explore areas and institutions where ICIPE might, through collaborative work, have mutual benefit in research development and training.

COLLABORATIVE RESEARCH AND DEVELOPMENT

Z.M. Nyira

Collaborative activities with other institutions continued in Kenya on investigations of resistance to ticks in sheep and goats (Kenyatta University College), research on leishmaniasis (Ministry of Health) and on maize and sorghum tolerance to the stem borers, and in Ivory Coast, on research and training in insect sensory physiology and fine structure.

At the invitation of national agencies, ICIPE exploratory missions visited Somalia and Uganda to assess what role ICIPE might play in the development and strengthening of national research and extension systems in pest management and training. As a result, an agreement was signed between ICIPE and the Ministry of Agriculture of the Somali Democratic Republic for ICIPE to assist the Somali Agricultural Research Institute in investigating the biology and ecology of stem borers of sorghum and maize in rainfed as well as irrigated areas of Somalia. In Uganda, the ICIPE team identified a number of areas in which collaboration with national institutions could result in mutual benefit. Of particular interest were research on African trypanosomiasis and on integrated pest management of sorghum stem borers and the training of Ugandans in these areas.

Contacts were made with Tanzania regarding research and training on tsetse; with Zambia, on research and

training on tsetse and ticks; and with Sudan, on research and training on livestock ticks. During 1984, in addition to the on-going collaboration with the International Rice Research Institute (IRRI), ICIPE consolidated its long-standing linkage with the International Institute of Tropical Agriculture (IITA). New areas where ICIPE and IITA might undertake cooperative research and training were identified. Also, ICIPE maintained active interaction with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), the International Centre for Maize and Wheat Improvement (CIMMYT) and the International Laboratory for Research on Animal Diseases (ILRAD). Contact was established between the Centre and the Commission for Scientific and Industrial Research Organization (CSIRO), Australia, and the International Livestock Centre for Africa (ILCA) in Addis Ababa, Ethiopia.

The Centre continued to have useful collaborative links with United Nations institutions and with other centres of excellence such as the University of Neuchatel (Switzerland) and Kuvin Centre for the Study of Infectious and Tropical Diseases (Israel). The University of Khartoum also expressed interest in collaborating with ICIPE in research and training on leishmaniasis.

ICIPE was represented at a number of international fora deliberating on aspects of bilateral or multilateral cooperation which afforded the Centre opportunities to assess what role it might play in the application of insect science and pest management technology.

THE AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE (ARPPIS)

M.E. Smalley

The African Regional Postgraduate Programme in Insect Science (ARPPIS) continued to establish itself during 1984 as an important and effective contribution by ICIPE to the training of insect scientists in Africa. The programme is a collaborative venture between ICIPE and African universities which began in 1983. Students register with a participating university for the Ph.D. degree of that university, but carry out their research work under the supervision of ICIPE senior scientific staff, using the facilities and expertise of ICIPE.

The 1984 ARPPIS class started their studies on March 1984. The eight students, who came from 5 African countries, were: Miss D. Adabie, Ghana; Mr. I. Bahana and Mr. M. Ogenga-Latigo, Uganda; Mrs. U. Elneima, Sudan; Mr. L. Kantiki, Malawi; and Mr. C. Maranga, Mr. J. Nderitu and Mr. J. Omollo from Kenya.

The class spent their first two weeks visiting relevant research institutes in and around Nairobi, thereby becoming familiar with the local scientific community. They then began a six-month period of coursework designed to bring all the students to the same high level of understanding within areas of insect science considered essential. Where possible, the courses were taught by staff seconded from a participating university so as to increase the exposure of ARPPIS students to as wide an academic background as possible. The six courses, all compulsory, were: Insect Taxonomy and Insect Functional Morphology both taught by Professor R. Kumar of the Rivers State University of Science and Technology, Nigeria; Insect Ecology, presented by Professor D. Griffiths of the University of Dar es Salaam, Tanzania; and Insect Pathology, Insect Physiology and Biochemistry; and Biostatistics and Computer Science, taught by ICIPE scientists.

Following their coursework, the 1984 class began their research projects. Together with the 1983 class this resulted in 16 postgraduate students from ARPPIS, working alongside ICIPE scientists. The 16 students were attached to ICIPE research programmes as follows: 3 in the Tsetse Research Programme, 1 in the Medical Vectors Programme, 6 with the Plant Resistance to Insect Attack and Bionomics and Applied Ecology sections of the Crop Pests Programme, 2 in the Insect Pathology and Pest Management Programme and 1 student working on a termite project jointly supervised by the ICIPE and the National Museums of Kenya.

During the year the first ARPPIS seminar was held. This was a highly successful meeting organized by the ARPPIS students themselves at which the discussion of each student's research was both robust and valuable. The seminar, twice yearly meetings of all ARPPIS staff and students, and regular informal contacts made possible by having all students working in and around ICIPE facilities have produced a sense of community and common purpose which is a valuable feature of the ARPPIS.

Mr. Richard Bagine, a member of the 1983 class, spent six months working in the United Kingdom at the Tropical Development and Research Institute, the British Museum (Natural History), the Chemical Laboratory Unit, University of Southampton and the Department of Applied Biology, Imperial College, London University. His visit was to study collections of East African *Odontotermes* and to learn techniques of morphometric analysis and the application of isoenzyme analysis to termite taxonomy. He was funded by the British Council.

The ARPPIS Academic Board met twice during 1984, in June and December, both times in Nairobi. The meetings continued to guide the programme by resolving the concerns and problems inevitable in such a new and unique postgraduate training venture as ARPPIS. The December meeting also selected the eight new students for the 1985 class.

During 1984, three more universities signed the Memorandum of Agreement with the ICIPE thus recognizing the rationale and objectives of ARPPIS, and so becoming Participating Universities. They were the universities of Malawi, Zambia and Sierra Leone. There are now eleven participating universities within ARPPIS.

At the end of September, Professor J. Okedi left the ICIPE and returned to his post as Head of Department of Zoology, Makerere University, Uganda. Professor Okedi had been on leave of absence from his University since December 1982, when he became the first Academic Coordinator of ARPPIS. On 1 October 1984, Dr. M.E. Smalley joined as Academic Coordinator.

POSTDOCTORAL RESEARCH FELLOWSHIPS

M. E. Smalley

The postdoctoral research fellowship scheme has again drawn to ICIPE young scientists wanting to develop their research skills and work in a tropical environment. During 1984, eight postdoctoral fellows were working with ICIPE research programmes and units; three with Crop Pests, two with Tsetse and one with Livestock Ticks Research Programmes, and two with the Chemistry and Bioassay Research Unit. The eight came from Ghana, Tanzania, Uganda, Kenya, India and the USA.

GROUP TRAINING COURSE IN PEST AND VECTOR MANAGEMENT SYSTEMS

J. F. Omange

The seventh International Group Training Course on Components Essential for Ecologically Sound Pest and Vector Management Systems which was postponed in 1983 was finally conducted in 1984, from 22 July to 10 August. Twenty eight trainees from 11 countries participated. This brings the total number of participants to 187 from 36 countries, from the time the course started in 1977. The distribution of participants by country is as shown below.

The Food and Agriculture Organization of the United Nations (FAO) sponsored two Ethiopian Pest management specialists to the 7th International Group Training Course on Pest and Vector Management Systems followed by a two-week training course in termite biology, ecology and control, organized by ICIPE. Four members of ICIPE staff of the former Termite Programme conducted the course which involved extensive field excursions to semi-arid parts of southern and northern Kenya.

STAFF DEVELOPMENT TRAINING

J. F. Omange

Staff development scheme is intended to provide staff with opportunities for upgrading their skills or acquiring job aids that would enable them to become more effective in carrying out their duties and responsibilities at the ICIPE. Several institutions outside the host country, Kenya, provided at the postdoctoral, postgraduate, technical and managerial levels. Besides ICIPE, major sponsors of the staff under the scheme were: The International Development Research Centre (IDRC), the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), The International Atomic Energy Agency (IAEA), the

Country distribution of participants at ICIPE International Group Training Course in Pest Management, 1977-84

Country of origin	1977	1978	1979	1980	1981	1982	1984	Total
Brazil				1	1			2
Colombia		2	1		1	1	1	6
Egypt						1	1	2
Ethiopia			2	1	1		3	7
Ghana	3	1	2	1	1	1	1	10
India	1	1		1		1	1	5
Israel		1				1	—	2
Ivory Coast		1		—	—		—	1
Jordan		1		—	—		1	2
Kenya	7	7	6	5	6	7	8	46
Lebanon		1		—	—	—	—	1
Lesotho					1	1	1	3
Malaysia			—			—	1	1
Malawi	2	1	—	3		1	1	8
Mauritius		2	3	2	4	1	—	4
Nigeria		2	3	2	4	1	—	12
Pakistan		—	1	—	—	—	—	1
Philippines		—	2	1	1		—	4
Rwanda		1	—	—	—	—	—	1
Saudi Arabia		1	1	—	1	—	—	3
Senegal			—	—	1	—	—	1
Sierra Leone				—	—	1	—	1
Somalia	1			1	1	—	—	9
Sudan		1	2	—	1	2	3	9
Swaziland		—		1	1	—	—	2
Taiwan		1		—	—	—	—	1
Tanzania	—	3	2	2	2	2	—	11
Thailand		1	—	—	—	—	—	1
Togo	—	1	—	—	—		—	1
Uganda		2	4	3	2	2	6	19
United Arab Emirates		—		—	2	2	6	2
Zaire		2		—	—	—	1	3
Zambia	1	1	—	2	1	3	1	9
Zanzibar			—		1	—	1	2
Zimbabwe					2		2	4
TOTAL	15	34	29	26	35	28	39	196

United Nations Development Programme (UNDP) and the John Pringle Fellowship Scheme.

Locally, five ICIPE technical staff were sponsored for training in laboratory technology at the Kenya Polytechnic, one secretary was sponsored for training at Kianda College and an accountant was part-sponsored at Strathmore College to complete some courses for an ACCA certificate. Within the centre, secretarial staff were trained in the use of the word processor.

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Management

The management and administration of core research programmes and units of the Centre as a whole was significantly strengthened during the year through recruitment for all the key positions previously vacant as follows:

Deputy Director	Dr. Mathew Paton Cunningham
Manager for Communications Systems	Mr. Leonard Okola
Administrative Manager	Mr. Semei Nyarzi
Programme Leaders	
Medical Vectors	Dr. Mutuku John Mutinga
Tsetse	Dr. Leonard Harrison Otieno
Unit Heads	
Chemistry and Bioassay	Dr. Ahmed Hassanali
Histology and Fine Structure	Dr. Elizabeth Dorothy Kokwaro
Acting Unit Head	
Sensory Physiology	Dr. Samuel Mukasa Waladde

Steps to streamline administrative procedures and strengthen management systems were implemented to promote greater efficiency, effectiveness and economies.

International professional staff

The international professional staff level has been maintained within the limit of 40-man years decided upon by the Governing Board.

The full staff list appears at the end of this report.

AMENITIES AND SOCIAL WELFARE UNITS

General. Supervision by the Administrative Manager of the operations of the International Guest Centre System, the Mbita Point International School, and the Mbita Point Field Station Clinic was formalized during the year with a view to refining clear lines of integration and communication with the Centre's management.

Mbita Point International School. Extensions to the School were 90% completed and the new Standard 8 class established. A Deputy Principal was appointed, bringing the total establishment of teachers to 9 against a total enrolment of 64 pupils.

Mbita Point Field Station Clinic. Over 90% of extension work to construct a maternity wing was completed. The clinic treated an average of 300 cases per month during the year.

International Guest Centre System. Construction work on the Guest Centre at Mbita Point Field Station commenced during the last quarter of the year. It will have a capacity of 16 study-bedrooms and a conference room for 150 people. Meanwhile the 4-bedroom temporary Guest House at the Station was intensively utilized by the ICIPE staff travelling to the Station on duty and by the centre's visitors.

Average occupancy at the Duduville International Guest Centre was 60% for the year. Its expansion programme is incorporated in the Duduville Phase II Capital Development.

CAPITAL DEVELOPMENT

Mbita Point Field Station. Priority work on the laboratories, senior staff housing and office complexes were completed and became operational during the year. Work carried forward to 1985 within the 1984 budget concerns the guest centre complex and completion of fittings and furnishing of the school and clinic extensions.

Duduville Phase II Development. Efforts to raise funds for this project continued throughout the year. In view of the acute space problems at Chiromo, the Board decided to allocate modest funds annually from core funds for this development. Towards the end of the year, the World Bank signified its support of the programme by some US$ 2.5 million and the Kenya Government contributed US$ 0.286 million, leaving a balance of US$ 3.714 million yet to be raised.

RELATIONS WITH THE HOST GOVERNMENT

The Centre's relations with the Government of the

Republic of Kenya continued on an excellent basis. In view of the growth of the ICIPE research, scientific, training and outreach activities, and the decision to construct an expanded headquarters complex, formal discussions on a new headquarter agreement were initiated with the host government. These cordial discussions were carried forward to 1985.

Communication and Information

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Communication and Information Division

The Communication and Information Division provides critical professional support to the ICIPE as a whole in the area of publications, graphics, photography, library and documentation, public relations and conference and liaison services.

During the year under review the Division took firm steps to strengthen its capability in all these areas. In particular, the Division began to introduce a wider range of publications, and to improve the quality of existing ones. New methods of information dissemination are being explored and towards the end of the year a well-known film-maker was commissioned to make the first video-tape documentary on the work of the Centre. An introductory history of the ICIPE was also commissioned and will be published shortly.

The Open Day held at the Mbita Point Field Station on 31 May 1984 was a major challenge to the staff of the Division, but one that was met with great success. Other new ventures included the establishment of the ICIPE Archives. The Division is also pushing ahead with the gradual change of ICIPE's corporate image, which started during the year with the introduction of a new logo.

LIBRARY AND DOCUMENTATION

N.M. Ntabuga

Acquisitions

The Library accessioned 203 books and monographs during 1984. Current journals were 130 titles out of which 94 were subscription and the rest from donations and exchange.

Services

Due to staff shortages, some of the services did not reach expected levels. However, they were all maintained. Current awareness service continued on a moderate pace relying much on published and other outside tools. The full staff establishment achieved towards the end of the year helped to lay ground work for a more vigorous service during 1985. Profiles will then be fully developed so that selective dissemination (SDI) starts in earnest.

The library continued to procure reprints of ICIPE staff publications and work was started to improve on their indexing for quick retrieval and effective utilization. Through purchases from the British Lending Library (BLL) and cooperation with other libraries and institutions around Nairobi and beyond, photocopies of publications and where necessary originals were acquired for the users throughout the year.

A mailing list is maintained for the distribution of ICIPE publications through donations and exchange arrangements. The publications include *Insect Science and Its Applications* (for exchange) *Dudu*, *ICIPE Annual Report*, and occasional publications. The mailing list, still growing, with about 500 addresses at the end of 1984, was computerized for easy manipulation.

Through the assistance of the Commonwealth Agricultural Bureau and the Commonwealth Institute of Entomology, the library was able on behalf of its users to do nine retrospective computer searches during the year. Because of its limited budget, charges for computer time and postage of results have always been passed on to the requester programmes. Other services included loans of books and reader enquiry service.

Mbita Point Field Station Library

New shelves and other furniture were acquired for the library which is now fully operational, to extend the above listed services to the Field Station staff.

PUBLICATIONS

S. Mwanyecky, J. Mukanyange, B. Otuoo

The department continued to give editorial, graphic and photographic services to the Centre. A list of staff publications appears at the back of the report. For the first time in many years, the Annual Report was produced entirely in-house and was published in time for the Annual Research Conference and meetings of the Governing Board.

Other publications included recommendations of the two workshops held during the year. The International Study Workshop on Host Plant Resistance and its Significance in Pest Management and the International Study Workshop on Leishmaniasis Epidemiology. Recommendations from the Planning Group for ICIPE's Biological Control Research were also published. A brochure for Mbita Point Field Station was also published as well as the ARPPIS Calendar and Annual Report for that year.

The journal, *Insect Science and Its Application*, continues to publish mini-reviews, original research papers, book reviews, information on new patents related to insect control, and obituaries of prominent insect scientists. The journal, now in its fifth volume, became bi-monthly from the beginning of the current year and all the six issues have been on schedule and were completed this year. Two special issues (of Volume 5), were issued during the year. The first, on the theme: 'Perception and Management of Pests and Pesticides', based on the International Meeting on Perception and Management of Pests and Pesticides, was supported by FAO, UNESCO Man and the Biosphere (MAB) Programme, UNEP, and the United States Agency for International Development-funded Consortium for International Crop Protection (CICP). The second, was on the theme, 'Tsetse Behaviour and Population Ecology' and based on the International Study Workshop on Tsetse Behaviour and Population Ecology convened by the ICIPE.

The total pagination of the volume was 534, close to the anticipated pagination of 600 for 1984. Efforts are continuing to diversify the content of the journal, and to reach a wider readership. A computer Software Survey Section is underway for Volume 6 (1985).

The book series, *Current Themes in Tropical Science*, with its third volume entitled, *Caste Differentiation in Social Insects (with emphasis on termites)*, edited by J.A.L. Watson, B.M. Okot-Kotber and C. Noirot, which is expected to appear in bookshops early in 1985, has continued to receive assistance from guest editors. The volumes have been basically proceedings of international workshops convened by ICIPE, although the manuscripts are now made more comprehensive for the book readership. However, future plans will highlight specific publications in selected topics.

Dudu. The Dudu newsletter has taken a new direction from being an internal organ to a more international orientation. The emphasis is now on reports on research and related activities. All staff news and announcements will be covered by the internal newsletter ICIPE News.

Staff Activities. Mrs. Winnie Oyuko, the graphic designer, completed a 6-months training course at the International Rice Research Institute (IRRI), Los Banos, Philippines. Her training involved all aspects of graphic design and book production. Her newly acquired skills will be a great boost to the publications programme at the Centre.

Mrs. Sarah Mwanyecky, Associate Editor, and Miss Joy Mukanyange, Communications Officer (Editor), participated in the planning and coordination of a 2-week workshop on 'Technical Scientific Editing'. The workshop was attended by some 30 participants from Eastern, Central and Southern Africa. It was organized within the framework of the International Development Research Centre's (IDRC) project to strengthen publishing capabilities within the region.

Future Plans

Within the coming year the department intends to acquire a phototypesetting machine and to import high quality printing paper in order to improve the general standard of our publications.

CONFERENCE AND LIASON

R. Washika

International Study Workshops

International Study Workshop on Host Plant Resistance and Its Significance in Pest Management. This was held at Duduville International Guest Centre (DIGC). The workshop attracted approximately 80 participants and observers from various national and international crop research and plant protection institutes. The themes discussed included:

- Types and mechanisms of host plant resistance (HPR).
- Factors influencing the expression and stability of host plant resistance.
- Screening techniques and methodologies for Host Plant Resistance, and
- Host Plant Resistance in Pest Management.

Useful recommendations emanating from this workshop were published and circulated to all relevant institutes. Proceedings of the workshop are being published in a special issue of the journal, *Insect Science and Its Application* vol. 6 (1).

International Study Workshop on Leishmaniasis Epidemiology. This workshop took place at Duduville International Guest Centre from 10-13 September 1984

and attracted approximately 26 participants from various national and international research institutes.

The broad areas discussed included:

- Review of advances in the status of leishmaniasis in different regions of the world
- Vectors and reservoirs in the epidemiology of the disease
- Epidemiological parasitology and chemotherapy and treatment of leishmaniasis

The participants visited one of ICIPE's field research sites for leishmaniasis epidemiology at Marigat, Baringo District, in Western Kenya. Field discussions were devoted to formulating recommendations, taking into account recommendations of the WHO Expert Committee on the Leishmaniases (WHO Technical Report No. 701, 1984).

Papers presented at this workshop and recommendations are being published in a special issue of *Insect Science and its Application*.

Planning Group for Biological Control Research at ICIPE. A panel of six prominent scientists from IITA, Texas A and M University, CIBC Pakistan, CIBC Kenya and FAO Rome with special expertise in pest management research, particularly biological control, met at Duduville from 11-20 June 1984 to assist ICIPE in formulating research strategies for the Biological Control Section of the Crop Pests Research Programme. The panel was required:

- To provide expert opinion, views, suggestions on the development of biological control components for an integrated pest management system for crop borers
- To identify major gaps within the ongoing research
- To formulate a work programme for the period 1984-1986
- To suggest areas of collaboration with other institutes on research and training

14th Annual Research Conference

ICIPE's 14th Annual Research Conference was held from 15 to 18 April 1984. Medical Vectors Research Programme (Leishmaniasis) and the Crop Pests Research Programme (section on Plant Resistance to Insect Pests) were reviewed in-depth. The [illegible] the research on tsetse, livestock ticks, chemistry and bioassay, fine structure, and sensory physiology and training were reviewed through poster presentations.

A wide cross-section of the scientific community in Kenya, and from other countries including science administrators, donor agencies, policy makers and national and international collaborating institutions participated in the conference and, as in the past, offered useful advice and opinion to ICIPE's research staff. The traditional Annual Public lecture was given by Professor Peter Miller of the University of Oxford. The theme of the lecture was 'Sperm Competition in Dragonflies'.

Open Day

An Open Day Field Day was organized at Mbita Point Field Station in May 1984. The aim was to acquaint farmers, extension workers and national research personnel with the role of an international research centre, such as the ICIPE, in promoting agricultural and animal production. This awareness will promote active dialogue between ICIPE scientists and farmers leading to improved collaboration with national agricultural research programmes and extension systems.

Over 3000 guests including scientists, institutional executives, government officials, administrators, farmers and students attended. The official opening was performed by Honourable W.M. Arap Saina, Assistant Minister for Agriculture and Livestock Development. In view of the success of this event, it is planned to organize similar ones biannually.

VISITORS

During the year a number of distinguished persons visited the Centre. They included the following among others:

Professor Marini Bettolo, Chairman of the Department of Chemistry, University of Rome and a member of the Governing Board of the Italo-African Institute which maintains scientific and cultural exchange with African countries. He gave a series of seminars both at ICIPE and the University of Nairobi.

A 4-man mission to Kenya from the International Service for National Agricultural Research (ISNAR), the Hague, Netherlands. They held wide-ranging discussions with staff of the Tsetse, Livestock Ticks, Crop Pests and Training programmes.

His Excellency Joao Augusto de Medeiros, Brazilian Ambassador to Kenya. He was also the chief guest at the opening of the International Study Workshop on Leishmaniasis Epidemiology.

Dr. J.W. Koehring, Director of the East African Regional Economic Development Services Office (REDSO) of the United States Agency for International Development. REDSO have given ICIPE a five-year grant to support research on plant resistance to pests.

Mr. Robert Bell, Programme Manager, Sub-Sahara African Programme, US National Science Foundation, Washington.

Dr. Richard W. Lyman, President of the Rockefeller Foundation, New York.

Dr. E.H. Hartmans, Director General, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

His Excellency J.R. Gaechter, Swiss Ambassador to Kenya, June 1984.

Honourable Dr. Mohamed Ali Nour, Minister for Livestock, Republic of Somalia.

Dr. Luigi Chiarappa, Chief, Plant Protection Services, FAO, Rome.

His Excellency Emile Tydemann, the Royal Netherlands Ambassador to Kenya.

His Excellency Zoran Zagar, the Ambassador of the Socialist Federal Republic of Yugoslavia to Kenya.

His Excellency Gian Luigi Valenza, Italian Ambassador to Kenya, accompanied by Mr. Gian Ludovico Pennacchio, UNDP Resident Representative.

Professor Abdus Salam, 1979 Nobel Laureate for Physics and Director of the International Centre for Theoretical Physics, Trieste, Italy — March 1984.

Dr. Nyle C. Brady, Senior Assistant Administrator for Science and Technology, Agency for International Development, Washington, D.C.

1984 Seminars

Speaker	Titles
Dr. Michel Brossard University of Neuchatel Switzerland	Immunity in rabbits against the tick *Ixodes ricinus* L.
Professor P.N. Campbell Courtauld Institute of Biochemistry University of London U.K.	The application of recombinant DNA for the identification of new biologically active peptides
Professor A.G. Gatehouse University College of North Wales U.K.	Genetic and environmental factors controlling flight performance in *Spodoptera exempta*
Dr. Isaac Jondiko ICIPE, Nairobi	A new approach to synthesis of Rethrolones, the alcohol portion of pyrethrins
Professor G.B. Marini-Bettolo Chemistry Department University of Rome	Recent research in African medicinal plants
Professor G.B. Marini-Bettolo Chemistry Department University of Rome	Natural chemical factors in plant protection
Dr. Alan McCaffery Tropical Pesticides Research Institute London	The role of the corpora allata and measurement of their activity during adult development in Orthoptera
Dr. M.F. Mutinga ICIPE, Nairobi	Progress on the investigations on the vector of *Leishmania major* in Baringo District
Mrs. M.A. Oketch ICIPE, Nairobi	Isolation and culture of termite microorganisms for potential use in bioconversion processes
Dr. R.F. Rhoades International Potato Centre (CIP) Lima, Peru	Farmer-back-to-farmer: An interdisciplinary model for the generation of acceptable agricultural technology
Professor K.N. Saxena ICIPE, Nairobi	Interaction among host and non-host plants determining the establishment of the leafhopper *Amrasca devastans*
Dr. D.A. Turner ICIPE, Nairobi	Recent progress in cultivation of the African trypanosome in-vitro
Dr. D.A. Turner ICIPE, Nairobi	The status of tsetse and trypanosomiasis on Zanzibar Island
Mrs. R.M.W. Vundla ICIPE, Nairobi	The production of antibodies to *Rhipicephalus appendiculatus* egg antigens in mice and rabbits, using different immunization methods

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Personnel

OFFICE OF THE DIRECTOR

Professor J.R. Ochamobo, *director*
Mrs. G.M. Ochola, *secretary*
Miss M. Wafula, *senior secretary*
Mrs. J.K. Ayobo, *secretary*
Mr. J.K. Kibor, *driver*
Mr. Otieno Oyalo, *driver*
Mr. J.M. Mwangangi, *driver*
Mr. D.O. Aoko, *clerical assistant*
Mr. A.O. Wete, *messenger*

Office of the Deputy Director

Dr. M.P. Cunningham, *acting deputy director*
Miss S.M. Kagondu, *senior secretary*

Planning and Development Unit

Mrs. R.A. Odingo, *principal planning officer*
Dr. V.O. Musewe, *research management officer*
Miss M.H. Bugembe, *associate planning officer*
Mr. V.S. Mutsya, *internal auditor*
Miss B.A. Nanja, *senior secretary*

ADMINISTRATION DIVISION

Mr. S. Nyanzi, *administrative manager*
Mr. J.G. Awich, *principal administrative officer*
Mr. M.M. Mounde, *senior administrative officer*
Mr. J.E. Okiro, *senior administrative officer*
Miss R. Sugaba, *senior secretary*
Mrs. S.G. Okwirry, *secretary*
Mrs. E.J. Gombe, *secretary*
Miss M.A. Awino, *secretary*
Miss G.M. Wachuru, *secretary*
Mrs. G. Weya, *senior telephonist receptionist*
Mrs. M.S. Mvenco, *telephonist receptionist*

CROP PESTS RESEARCH PROGRAMME

Plant Resistance to Insect Pests Section

Professor K.N. Saxena, *principal research scientist/PI*
Dr. T.C. Saxena, *principal research scientist (IRRI)*
Dr. R.S. Pathak, *senior research scientist*
Dr. J.K.C. Ampofo, *research scientist*
Mr. S.H.O. Okech, *associate scientific officer*
Dr. A.M. Alghali, *postdoctoral research fellow*
Dr. H. Kumar, *postdoctoral research fellow*
M. James P.O. Odero, *postgraduate research fellow*
Mr. F.O. Onyango, *technician*
Mr. P.F.W. Njoroge, *junior technician*
Mr. M.D.O. Bungu, *junior technician*
Mr. L.L.K. Kidiavai, *junior technician*
Miss A. Ragot, *technical assistant*
Mr. M.D. Arwa, *technical assistant*

Bionomics and Applied Ecology Section

Dr. K.V.S. Reddy, *senior research SH*
Dr. G.C. Unnithan, *senior research scientist*
Dr. F.G. Omolo, *senior research scientist/agronomist*
Professor S. Yagi, *research associate*
Dr. M. Botchey, *postdoctoral research fellow*
Dr. T. Connelly, *postdoctoral research fellow*
Mr. A. Dissemond, *graduate research scholar*
Mr. J.C. Olela, *principal technician*
Mr. S.M. Othieno, *senior technician*
Mr. M.C. Lubega, *technician*
Mr. C.O.J. Jakomito-Simbi, *technician*
Mr. P.O. Ollinga, *technician*
Mr. P.O. Agwaro, *junior technician*
Mr. S.O. Paye, *junior technician*
Mr. G.O. Amala, *technical assistant*
Mr. J.G. Kibuka, *technical assistant*

Biological Control Section

Dr. G.W. Otoo, *senior research scientist/SH*
Dr. W.A. Otieno, *research scientist*
Dr. M.O. Odindo, *research scientist*
Mr. J.B. Okeyo-Owuor, *postgraduate research scholar*
Mr. J. Kilori, *senior technician*
Mr. P. Mwamisi, *junior technician*
Mr. R. Okello, *junior technician*
Mr. S. Mutti, *junior technician*
Mr. R.C. Odhiambo, *junior technician*
Mr. P. Amutalla, *technical assistant/driver*

Insect Mass Rearing Section

Dr. R.S. Ochieng, *research scientist/Section head*

LIVESTOCK TICKS RESEARCH PROGRAMME

Dr. M.P. Cunningham, *principal research scientist/PL*
Dr. R.M. Newson, *senior research scientist*
Dr. N.B.A. Nyindo, *senior research scientist*

Dr. C.K.A. Mango, *research scientist*
Dr. J.J. de Castro, *research scientist*
Mr. D.K. Punyua, *scientific officer*
Mr. O.A. Mongi, *postdoctoral research fellow*
Mr. A. Chiera, *research assistant*
Mr. C.A. Ayanyo, *chief technician*
Miss R. Chesang, *technician*
Mr. R. Ojowa, *technician*
Mr. P.O. Ngoko, *technician*
Miss W. Nderitu, *secretary*
Mr. P. Muteria, *technical assistant*
Mr. J.N. Ndungu, *technical assistant*
Mr. G.T. Thuo, *technical assistant*
Mr. M.G. Kimondo, *technical assistant*
Mr. G.M. Hindi, *technical assistant*
Mr. F. Wanyangu, *technical assistant*
Mr. S. Mulindo, *technical assistant*
Mr. N.R. Tome, *technical assistant*
Mr. O. Opere, *technical assistant*
Mr. J. Mugane, *technical assistant*
Mr. S.G. Onyinyo, *technical assistant*

MEDICAL VECTORS RESEARCH PROGRAMME

Dr. M.J. Mutinga, *senior research scientist/PI.*
Dr. B.M. Okoe-Kotber, *research scientist*
Dr. J.B. Kaddu, *research scientist*
Mrs. L. Rogo, *scientific officer*
Professor T. Gemetchu, *research associate*
Miss I. Irungu, *graduate research scholar*
Mr. C.C. Kamau, *research assistant*
Mr. B.N. Otiero, *chief technician*
Mr. M.P. Nyamori, *senior technician*
Mr. J. Mwandandu, *junior technician*
Mr. D.M. Omogo, *junior technician*
Mr. F.M. Kyai, *technical assistant*
Mr. R. Musyoki, *technical assistant*
Mr. K. Kiswili, *technical assistant*
Mr. S. Mutua, *technical assistant*
Mr. J. Ndambuki, *technical assistant*
Mr. D. Mbavu, *technical assistant*
Mr. P.B. Chepkoimet, *technical assistant*
Mr. P. Munguti, *technical assistant*
Mr. P. Munywoki, *technical assistant*
Mr. P. Matuvo, *technical assistant*
Mr. P. Wa-Ndei, *technical assistant*
Mr. W. Mulwa, *technical assistant*
Mr. S. Singi, *technical assistant*
Mr. J.S.D. Tawuo, *technical assistant*
Mr. R. Muoki, *technical assistant*
Miss M.I. Okulo, *technical assistant*
Miss R.I. Ndururu, *technical assistant*

TSETSE RESEARCH PROGRAMME

Dr. I.H. Otieno, *senior research scientist/PI.*
Dr. T.K. Golder, *senior research scientist*
Dr. M.F.B. Chaudhury, *senior research scientist*
Dr. D.A. Turner, *senior research scientist*
Dr. G.P. Kaaya, *research scientist*
Dr. S.R. Tarimo, *postdoctoral research fellow*
Mrs. M. Owaga, *scientific officer*
Dr. D. Yuichi Chigusa, *scientist-in-residence*
Miss N.F. Darji, *senior research assistant*
Mr. R. Brightwell, *research assistant*
Mr. E.I. Onwe'uzo, *graduate research scholar*
Mrs. M. Olutatwa, *secretary*
Mr. P.O. Agutu, *senior technician*
Mr. I.G. Onyango, *technician*
Mr. E. Mpanga, *technician*
Mr. D. Uvyn, *junior technician*
Mr. M.R. Mutuaruhiu, *junior technician*
Mr. S. Marumba, *technical assistant*
Mr. D.K. Mungai, *technical assistant driver*
Mr. J.M. Muchiri, *technical assistant*
Mr. J.A. Makau, *technical assistant*
Mr. J.K. Kiilu, *technical assistant*
Miss E.N. Mwangi, *technical assistant driver*
Mr. Stephen S. Wakape, *technician*

CHEMISTRY AND BIOASSAY RESEARCH UNIT

Dr. A. Hassanali, *senior research scientist/UH*
Dr. T.S. Dhadialla, *research scientist*
Dr. P.G. McDowell, *research scientist*
Dr. H. Osore, *research scientist*
Dr. D.A. Otieno, *research scientist*
Dr. J.I. Jondiko, *postdoctoral research fellow*
Mrs. M.A. Okech, *scientific officer*
Mr. W. Lwande, *postgraduate research scholar*
Mr. E. Osir, *graduate research scholar*
Mrs. R.M.W. Vundla, *scientific officer*
Mrs. R.A. Okoth, *secretary*
Mr. M.S. Rajab, *research assistant*
Mr. A. Chapya, *chief technician*
Mr. E.N. Ole Sitayo, *senior technician*
Mr. E. Nyandat, *technician*
Mr. L. Moreka, *junior technician*
Mr. G. Ochieng', *technical assistant*
Mr. G.O. Adul, *research assistant*
Mr. P.O. Amoke, *technical assistant*

HISTOLOGY AND FINE STRUCTURE RESEARCH UNIT

Dr. E.D. Kokwaro, *research scientist/UH*
Dr. L.R.S. Awiti, *research scientist*
Mr. M.B. Chimtawi, *chief technician*
Mr. P. Lisamulla, *principal technician*
Mrs. J.K. Muriithi, *senior technician*
Mrs. J.A. Kongoro, *research assistant.*
Mr. N.T. Ogoma, *junior technician*

SENSORY PHYSIOLOGY RESEARCH UNIT

Dr. S.M. Waladde, *research scientist/Ag. UH*
Dr. R.K. Saini, *research scientist*
Mr. P.G.N. Njagi, *graduate research scholar*
Mr. H. Kahoro, *senior technician*
Mr. S.A. Ochieng, *technician*
Mr. P. Ahuya, *technical assistant*

BIOSTATISTICS AND COMPUTER SERVICES

Dr. H.F. Magalit, *data systems manager*
Mrs. W.N. Ssebunya, *senior systems analyst*
Mr. F.C. Obeya, *computer programmer*
Mr. J.K. Maina, *senior computer programmer*
Mrs. P.M. Alila, *office systems supervisor*

INSECT AND ANIMAL BREEDING SERVICE

Mr. J. Wanyonje, *principal technician/UH*
Mr. H. Bandah, *technician*
Mr. J. Kagoiya, *technician*
Mr. J. Ongudha, *junior technician*
Mr. E.O. Awuoche, *junior technician*
Mr. G.M. Birir, *junior technician*
Mrs. R. Kariuki, *technical assistant*
Mr. J.K. Gitegi, *technical assistant*
Mr. S.M. Mhugua, *technical assistant*
Mr. G. Mburu Nganga, *technical assistant*
Miss M.G. Wanjiru, *technical assistant*
Mr. M. Mwikya, *technical assistant*
Mr. J.O. Omolo, *technical assistant*

OUTREACH AND TRAINING UNIT

Dr. M.P. Cunningham *coordinator*
Dr. L.O. Abe, *principal training officer*
Mr. J.F. Omange, *senior training officer*
Mrs. M. Antao, *senior secretary*

ARPPIS Programme

Dr. M.E. Smalley, *academic coordinator*
Mrs. M.U. Arara, *senior secretary*

Mrs. Y. Obiero, *secretary*
Miss D.A. Adabie, *postgraduate research fellow*
Mr. L.A. Abdullahi, *postgraduate research fellow*
Mr. J. Bahana, *postgraduate research fellow*
Mr. R. Bagine, *postgraduate research fellow*
Mrs. U.M. Elneima, *postgraduate research fellow*
Mrs. W.S. Forawi, *postgraduate research fellow*
Mr. L.M. Kantiki, *postgraduate research fellow*
Mr. S. Kyamanywa, *postgraduate research fellow*
Mr. M.O. Latigo, *postgraduate research fellow*
Mr. C. Maranga, *postgraduate research fellow*
Mr. J. Nderitu, *postgraduate research fellow*

FINANCE DIVISION

Mr. E.J. English, *financial manager*
Mr. R.M.P. Okura, *principal accountant*
Mr. G.W. Kanza, *senior accountant*
Mr. A.A.M Oguda, *accountant*
Mr. R. Otieno, *accountant*
Mr. G.O. K'Owino, *supplies officer*
Mr. C.M. Oloo, *assistant supplies officer*
Mrs. A.A. Okumali, *senior secretary*
Miss F. Ojode, *senior secretary*
Mr. M.A. Kawaka, *assistant accountant*
Mr. E.K. Ongola, *assistant accountant*
Mrs. L. Kimani, *accounts assistant*
Mr. D.M. Owino Olalo, *storekeeper*
Mr. F.K. Cheserek, *clerical assistant*
Miss S. Adoyo, *accounts clerk*

COMMUNICATION AND INFORMATION DIVISION

Mr. L. Okola, *manager for communication systems*
Miss R. Washika, *senior communication officer*
Mr. N.S.M. Nsubuga, *senior librarian*
Miss J. Mukanyange, *communication officer (Editor)*
Mrs. S. Mwanycky, *associate editor*
Mrs. W. Oyuko, *graphic artist*
Mr. S.F. Odoi, *scientific illustrator*
Mrs. R.D.P. Ortega, *documentalist*
Miss E. Kahuhu, *library assistant*
Mrs. M.R. Opande, *senior secretary*
Mrs. F. Opere, *secretary*
Miss A.W. Muhato, *secretary*
Miss H. Githinji, *typesetter*
Mr. F.J. Shikhubari, *technician*
Mr. A. Shisoka, *clerical assistant*

WORKSHOPS AND LABORATORY MANAGEMENT

Mr. A. Mando, *principal controller for technical services*
Mr. F.M. Suchicki, *electronics and instrumentation engineer*
Mr. A.R. Bhaloo, *assistant electronics engineer*
Mr. H.N. Rai, *refrigeration technologist*
Mr. P.O. Nyacheo, *senior technician*
Mr. J.J.O. Ocholloh, *senior technician (maintenance)*
Mr. J.N. Mtei, *senior technician*
Mr. J.M. Maina, *technician*
Mr. S.O. Obiero, *technician*
Mr. J.O. Ogallo, *technician*
Mr. J.B. Omullo, *technician*
Mr. J.O. Onyango, *plumber/mason*
Mr. P. Oluya, *junior technician/glass blower*
Mr. F.N. Nyoike, *junior technician/glass blower*
Mr. J.K. Gadonya, *junior technician (maintenance)*
Mr. K. Kinuthia, *technical assistant*
Mr. J. Omondi, *technical assistant*

SECURITY, TRANSPORT AND JANITORIAL SERVICES

Mr. P.M. Arrumin, *controller for security, transport and janitorial services*
Mrs. P.A. Oriwa, *security officer*
Mr. J.O. Oduol, *senior mechanic*
Mr. F. Ombija, *senior mechanic*
Mr. F.O. Hamala, *mechanic/driver*
Mr. P.N. Mahogo, *senior driver*
Mr. Akhaya, *assistant janitor*
Mr. J.M. Kiarie, *technical assistant/driver*
Mr. J.B. Oyondi, *driver*
Mr. G.M. Kinyanjui, *driver*
Mr. P.O. Owuor, *driver*
Mr. S.N. Achochi, *driver*
Mr. S.N. Rukungu, *driver*
Mr. M.M. Zablon, *driver*
Mr. S.K. Langat, *driver*
Mr. G.K. Too, *driver*
Mr. S.O. Araka, *driver*
Mr. M.O. Ombech, *driver*
Mr. D.I. Isoso, *driver*
Mr. P. Otiende, *driver*
Mr. L. Kisutia, *cleaner*
Mr. A.M. Buhusi, *cleaner*
Mr. D. Chege, *cleaner*
Mr. E. Asami, *cleaner*
Mr. E. Okulo, *cleaner*
Mr. J.O. Opondo, *cleaner*
Mr. E.E.O. Obuya, *cleaner*
Mr. S. Obondo, *gardening assistant*
Mr. N. Okumbe, *cleaner/messenger*
Mr. L.L. Ayekba, *gardening assistant*
Mr. J. Elegwa, *gardening assistant*
Mr. W. Achiroma, *security guard*
Mr. D.O. Singa, *security guard*
Mr. J.D. Nyawalo, *security guard*
Mr. R.K. Milgo, *scurity guard*
Mr. A. Makola, *security guard*
Mr. J.A. Laban, *security guard*
Mr. E.H. Otieno, *security guard*
Mr. A.M. Ouma, *security guard*
Mr. A.A. Muyanda, *security guard*
Mr. S.M. Kibati, *security guard*
Mr. T.S.A. Ekisa, *security guard*
Mr. M.O. Yongason, *security guard*
Mr. F.M. Muinde, *security guard*
Mr. J.N. Nzioka, *security guard*
Mr. C. Mulela, *security guard*
Mr. T.M. Otieno, *security guard*

DUDUVILLE INTERNATIONAL GUEST CENTRE

Mr. J.A. Achilla, *senior business and catering controller*
Mrs. P. Owitti, *senior secretary*
Mr. J.E. Mwangi, *head cook*
Mrs. J.A. Musiga, *housekeeper*
Mr. A.I. Okapesi, *assistant head cook*
Mr. A. Lweya, *cook*
Mr. D.K. Yaem, *stores assistant*
Mrs. L.A. Nyamora, *assistant accountant*
Mrs. E. Kwach, *telephonist/receptionist*
Mr. P.O. Omollo, *barman/waiter*
Mr. A.M. Mutwoli, *room steward*
Mrs. P.A. Ochola, *room steward*
Mr. H.M. Kibisu, *assistant launder*
Mr. C.M. Lumati, *assistant launder*
Mr. G. Gichuru, *kitchen assistant*
Mr. J.M. Mwakisha, *kitchen assistant*
Mr. R. Gathu, *driver*
Mr. A.M. Mugone, *driver*
Mr. J.O. Mukhobi, *janitorial assistant*
Mr. M.O. Songa, *security guard*
Mr. A. Muyanda, *security guard*
Mr. E. Kiprono, *security guard*

MBITA POINT FIELD STATION

Dr. Z.M. Nyiira, *principal research scientist/SM*
Mr. S.M. Kimaita, *senior administrative officer*
Mr. Z. Orwa, *security transport officer*
Miss M.W. Mathai, *librarian*
Mr. F.A. Ahanga, *accountant*
Mrs. G. Kwanya, *secretary*
Miss E. Afandi, *secretary*
Miss F.B. Esalako, *secretary*
Miss S.M. Digolo, *secretary*
Mrs. M.N. Okach, *assistant secretary*

Mr. P.O. Ngugi, *accounts assistant*
Mr. M.E.N. Asudi, *accounts assistant*
Mr. J. Ongonga, *clerical assistant*
Mr. C.N. Keli, *supplies assistant*
Miss D.A. Achieng', *receptionist/telephonist*
Miss A.W. Makoko, *copy typist*
Mr. J.O. Ohato, *mechanic/driver*
Mr. J.N. Asanyo, *mechanic/driver*
Mr. P.O. Mbuya, *driver*
Mr. A.A. Olwoko, *driver*
Mr. L.O. Odongo, *driver*
Mr. J. Mokaya, *driver*
Mr. S. Ogechi, *driver*
Mr. W. Jayatileka, *driver*
Mr. N. Ouya, *driver*
Mr. R.O. Okello, *junior technician*
Mr. B.O. Ogal, *technical assistant*
Mr. J. Atiche, *janitor*
Mr. R.R. Nyaridi, *senior machine operator messenger*
Mr. Z.O. Nyandere, *cleaner*
Mr. E.K. Ogutu, *cleaner*
Mr. C.O. Okello, *gardener*
Mr. B.S.K. Masyanga, *farm controller*
Mr. P.O. Ouma, *farm assistant*
Mr. P.O. Outa, *farm assistant*
Mr. J. Sagini, *farm assistant*
Mr. S.O. Odero, *farm assistant*
Mr. J.W. Achola, *farm assistant*
Miss P. Nyagaka, *farm assistant*
Mr. F.O. Arum, *farm assistant*
Mr. E.K. Ong'onge, *farm assistant*
Mr. J.O. Osuimba, *farm assistant*
Mr. J.O. Odero, *security guard*
Mr. E. Sonye, *security guard*
Mr. R.C. Joshi, *station site engineer*
Mr. D. Oyoto, *security guard*
Mr. B. Mogendi, *security guard*
Mr. A.O. Ogaja, *security guard*
Mr. J.K. Opere, *security guard*
Mr. A.N. Makori, *security guard*
Mr. D.L. Debe, *security guard*
Mr. M. Wasulwa, *security guard*
Mr. P.L. Rakwach, *security guard*
Mr. J.J. Okach, *security guard*
Mr. J.J. Okach, *security guard*
Mr. A. Agoro, *security guard*
Mr. H. Abura, *security guard*
Mr. P.M. Aliarda, *plumbing and water works technician*

MBITA POINT INTERNATIONAL SCHOOL

Mrs. P.A. Ogada, *principal*
Mr. F.O. Omolo, *teacher*
Mrs. C.O. Ndiege, *teacher*
Mr. D.P. Makachola, *teacher*
Mr. Y.M. Koko, *teacher*
Mr. H.M. Mulwa, *teacher*
Mr. A.M. Sentamu, *teacher*
Mr. D.B.E.O. Okong'o, *teacher*
Miss S. Omune, *school attendant*

MBITA POINT FIELD STATION CLINIC

Dr. J.B. Odhiambo, *institutional doctor*
Miss Z.M. Macharia, *staff nurse*
Mr. C. Munafu, *laboratory technologist*
Mr. F.O. Kirowo, *pharmaceutical technologist*
Mr. C.O. Ngutu, *clerical assistant*
Mr. L. Odongo Otieno, *driver*
Mrs. L.A. Abuya, *janitorial assistant*
Mr. John H. Odoyo, *clinical officer*

MPFS GUEST HOUSE

Mr. C.B. Oyieyo, *MPGS Guest House supervisor*

NKURUMAN OUTREACH STATION

Mr. J.M. Kobaai, *Security Guard*

MUHAKA OUTREACH STATION

Mr. S. Abdalla, *Security Guard*

Abbreviations

PL	Programme Leader
SH	Section Head
UH	Unit Head
SM	Station Manager