THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

THE
INTERNATIONAL
CENTRE OF
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AND ECOLOGY

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Contents

Foreword

Over the last decade and a half, the International Centre of Insect Physiology and Ecology (ICIPE) has endeavoured to push vigorously for two components of its mandate. First, to undertake the necessary mission-oriented research that would lead to the design, development and demonstration of cost-effective, environmentally acceptable, culturally sensitive long-range integrated pest management (IPM) systems for the crucially important pests and disease vectors in Africa (and other tropical regions of the world). And, second, to assist in developing and nurturing the national capacity in pest management research and development, through training programmes, visiting appointments, study workshops, and similar means. Both of these objectives have been pursued vigorously and in a concentrated manner.

Because of the success being achieved in these two areas, a strong contextual framework has now been established for the ICIPE to initiate the third component of its mandate - the establishment of an interactive research and development (R & D) relationship between the ICIPE and selected national programmes in the tropical developing regions of the world. In this respect, and as a deliberate start in this problem area, the ICIPE has launched a dialogue in recent months with policy - and decision-makers in Eastern and Southern Africa on plans to establish an African Regional Pest Management Research and Development Network (PESTNET). Such planning was only possible because of the operation of four main factors:

The ICIPE has accumulated a large body of information on certain pests (crop borers, livestock ticks, and tsetse, especially) which forms a vital knowledge base that could be utilized for the design, development and demonstration of integrated pest management systems. The present Annual Report gives a glimpse of this diversity and vitality of the various advances in scientific work at the Centre.

The ICIPE and the Rockefeller Foundation have jointly put together a Social Science Group specifically to interface with the biological sciences, with the intention of assuring the appropriate effectiveness of new technological advances within a researched traditional knowledge base and within a socio-economic context which can respond to such advances.

The ICIPE has a strong programme of training for scientific leadership, through its youthful but dedicated Postdoctoral Research Fellowship scheme, ARPPIS (African Regional Postgraduate Programme in Insect Science), and FAMESA (Financial and Administrative Management of Research Projects in Eastern and Southern Africa).

The recent decision by the ICIPE that it will rely on national programmes to provide expertise in plant breeding, agronomy, and similar applied science areas in its interactive relationships with national programmes is a force for building together a continuing association and a give-and-take relationship founded on mutual respect for each party's contribution to a common goal - which is IPM.

During the course of the year 1986, these plans will mature; and we expect that PESTNET will represent a new quantum jump in ICIPE programme activities from early 1987.

In the midst of the ongoing African crisis, the Centre (and similar R & D institutions dedicated to the long-range solutions to Africa's multifarious problems) brings some hope - through mission-oriented research, and through its insistence that the continent must create the means to solve its own problems within the framework of international cooperation.

THOMAS R. ODHIAMBO, *Nairobi, 1 September 1986.*

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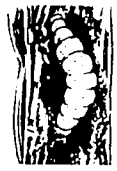


Crop Pests Research Programme

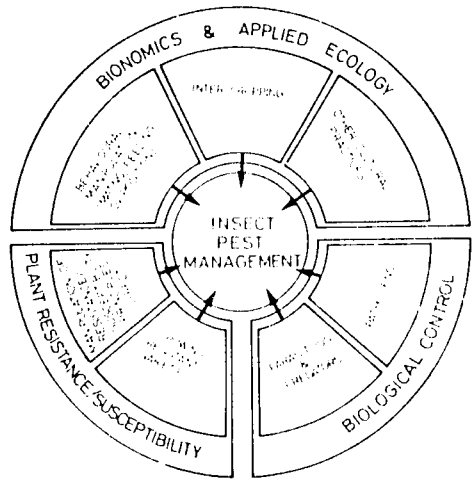
The Crop Pests Research Programme (CPRP) of the ICIPE is aimed at developing strategies for the management of key insect pests of important crops by methods which are environmentally safe and technically as well as economically feasible for resource-poor, subsistence farmers in Africa and other developing countries in the tropics. As these farmers can neither afford nor handle pesticides which pose hazards, the pest management strategies being developed are non-pesticidal. The crops presently under study are sorghum, maize and cowpea. The insect pests of these crops currently under investigation include: sorghum and maize stem borers (Chilo partellus, Busseola fusca, Eldana saccharina *and* Sesamia calamistis) *and the cowpea pod borer* Maruca testulalis.

The investigations undertaken are concentrated mainly on Chilo partellus *and* Maruca testulalis. *However, when other species of insects including borers and trips attack the target crops interacting with* C. partellus *or* M. testulalis *they are also engaged for study.*

In order to achieve the stated goal, research is being carried out under 4 main sections: Bionomics and Applied Ecology (BAE), Plant Resistance to Insect Pests (PRIP), Biological Control (BC), and Insect Mass Rearing Technology (IMRT). The broad categories of pest management components being developed by these sections are schematically summarised in Figure 1.

The methodologies developed or standardised and the information generated to develop these components are summarised here: yield losses caused by C. partellus *in sorghum and maize in relation to number of larvae infesting the plants at different stages of their development; cultural practices including intercropping different crop varieties in relation to the pest attack; reproduction and behaviour of the borers in relation to their management; levels of resistance in additional sorghum and maize genotypes and cultivars obtained from various sources including exotic ones; profiles of colonising responses of* C. partellus *to different crop cultivars determining their resistance or susceptibility; biology and reproductive potential of certain previously identified, promising parasitoids of the borers; culture and use of certain pathogens, particularly the protozoan* Nosema, *for the control of the target pests and refinement of simple techniques for mass rearing of target insects for these studies.*

PROPOSED COMPONENTS OF PEST MANAGEMENT



BIONOMICS AND APPLIED ECOLOGY

Bionomics and Applied Ecology has been engaged in investigations on crop losses caused by the target crop borers, their population biology, intercropping and other cultural practices as means to reduce the pest attack, and reproduction and behaviour of the borers to provide elements which can assist in pest management. The results of the work done during the year are discussed below.

Yield Losses in Sorghum

K V. Sesha Reddy

The yield losses caused by the stem borer, *Chilo partellus*, in sorghum (cv. Serena) were assessed during the year by methods standardised for this study. Sixty sorghum plants were grown in plots of 2 X 4 m each and covered with a white nylon netting, immediately after emergence, to prevent external infestation. Sorghum plants of 6 growth stages viz. 10, 20, 30, 40, 50 and 60 days after emergence (DAE), were artificially infested with 5 and 10 neonate larvae of *C. partellus* per plant. At harvest, grain yields from these plants were taken separately and compared with those of the uninfested control.

The results showed that the grain-yield loss in sorghum caused by *C. partellus* larvae was a maximum of (74.4-87.8%) when the plants were infested at 10 DAE. As the plants' developmental stage advanced at the time of larval infestation, the yield loss declined reaching as low a level as 2-13% at 60 DAE. There were no significant differences between the plants infested at 10 DAE and 20 DAE nor between 20 and 30 DAE nor between 30, 40, 50 and 60 DAE. However, significant differences in the yield losses were observed between the levels of infestation, 10 larvae/plant causing a higher loss than 5 larvae/plant. Severe foliar damage, dead hearts and stem-tunnelling were associated with the infestation and subsequent yield losses during the first 3 growth stages.

These observations suggest that early and simultaneous planting of sorghum can reduce yield losses, since the plants would be both old at the time when maximum borer infestation occurs and likely to have a more or less evenly low-borer infestation. It may also be noted that, for assessment of crop losses by insect, it is extremely important to take the plant population at the start of the crop cycle after thinning and to assess the stand loss, the number of unproductive and productive plants at the harvest.

Yield Losses in Maize

M A. Botchey

Crop losses caused by *C. partellus* in a maize cultivar (Inbred A) were assessed. The maize plants were infested at 3 weeks and 8 weeks after emergence (WAE) with 2, 4 and 8 neonate *C. partellus* larvae. The results showed that infestation of the plants at 3 WAE by the larvae reduced the yield, expressed as cob weight/plant, by about one-fifth to one-fourth that of the uninfested control. The number of internodes tunnelled was found to increase significantly as well with the level of infestation. But, postponement of infestation of the plants till 8 WAE caused much less yield loss than the infestation at 3 WAE. With reference to the number of larvae infesting the plants, the yield loss increased only slightly with the increase in the number of larvae from 2 to 8 per plant.

Intercropping as a Component of Pest Management

Previous reports have shown that certain crop combinations in an intercropping system, e.g., sorghum-cowpea or maize-cowpea, reduce the attack and damage to the plants by the the stem borers whereas certain other combinations, e.g., sorghum-maize, suffer from increased damage. On the basis of this information, further studies on the intercropping systems involving incorporation of certain borer-resistant sorghum cultivars in the mixed cropping were carried out, as well as studies of the effect of mixed cropping on thrips. The results achieved are presented below.

Role of Resistant/Tolerant Cultivars in Intercropping Systems

E.O. Omolo

In 1984, the role of resistant/tolerant cultivars in the best and worst combinations was studied at Mbita Point Field Station and on Rusinga Island. The 2 tolerant sorghum cultivars were the ICIPE's ICS1 and ICS2 and the resistant cultivar from ICRISAT was IS-4660. Two susceptible sorghum cultivars (Serena and Ochuti-local) served as controls. In the case of maize, the resistant variety was the ICIPE's maize line ICZ1 and the susceptible control was Katumani maize. The parameters observed were: oviposition by *C. partellus* on plants in different crop combinations, percentage of plants attacked by the crop borers, number of the borer larvae, pupae infesting single plants, and grain yield per plant.

The results indicated that there were significantly more egg batches on Serena intercropped with the ICIPE's cowpea ICV2 than on the resistant IS-4660 and tolerant ICS1 and ICS2 sorghum intercropped with ICV2. Similarly, there were significantly more egg batches on Serena Katumani than on Serena/ICZ1 combinations. When Serena was grown alone 45% of the plants were attacked, and when grown with cowpea only 40% showed signs of damage. Another susceptible sorghum cultivar Ochuti, planted alone, suffered an attack on 54%, and when intercropped with cowpea the attack was reduced to 40%. When the tolerant cultivar ICS2 was planted alone the attack was 37%. But, it was significantly reduced to 28% when grown with cowpea. The damage to ICS1 was also reduced to 26% when grown with cowpea. The attack by the pod borer *M. testulalis* on cowpea was significantly reduced when cowpea was intercropped with ICS1 and ICS2. Even susceptible sorghum Ochuti and Serena kept the attack by the pod borers and other pod bugs down, but not as effectively as ICS1 and ICS2. These experiments demonstrated that intercropping of different plant species lowers the pest-population level, and incorporation of resistant and tolerant cultivars further lowers this level.

The mean infestation during the entire plant growth period was significantly higher on Serena (2.28) and IS-4660 (2.27) intercropped with ICV2 than on ICS1 (1.68) and ICS2 (1.93) intercropped with ICV2. The grain yield of Serena was not significantly affected, possibly because of its high capacity for tillering and tolerance. The grain yield of ICS2 was low and that of IS-4660 was extremely low. This could have been due to a heavy attack of sorghum shootfly after which serena recovered but not IS-4660. Some of these resistant lines may not be good agronomically, but may serve as sources of resistance. However, this study clearly demonstrates that resistant/tolerant sorghum cultivars interacted significantly with the pest population, cropping patterns and grain yield. The data indicated that the use of resistant and tolerant cultivars improved the situation on the worst combination of maize-sorghum. This is helpful to the subsistence farmers who plant maize and sorghum together. The yield of maize was much better than that of sorghum because the former was not affected by the shootfly. The cultivars ICS2 and IS-4660 protected the cowpea from *M. testulalis* attack more successfully than Serena.

It is clear, in view of the above results, that incorporation of resistant cultivars in an intercropping system offers an advantage in reducing pest attack.

Ecological Factors Governing Flower Thrips (*Megalurothrips sjostedti Trybom*) Population Density in Cowpea/Maize Mixed Crop System

S. Kyamanywa

The results of the earlier experiments (Twelfth Annual Report, 1984) indicated that the population density of *M. sjostedti* on cowpea plants was significantly lower in the cowpea/maize mixture compared to the sole crop of cowpea. We previously studied: (i) the influence of maize/cowpea mixture on colonisation of cowpea plants by *M. sjostedti;* (ii) responses of *M. sjostedti* to maize and cowpea odours, and (iii) light-intensity variations in the mixture and sole crop and its influence on distribution and settling behaviour of *M. sjostedti.*

Field studies, using white and green coloured water traps indicated that there were no significant differences in the number of thrips per trap between the mixture and sole crop during the colonisation phase. This suggested that the cowpea-maize system did not interfere with the initial number that colonised the crop, and that the differences observed between the two cropping systems were due to other factors and not disruption of thrips colonisation.

Choice experiments under cages, indicated that significantly fewer thrips settled on cowpea plants which were in association with maize than those that were alone. This indicated that close-range orientation of thrips to host plant was reduced by the presence of maize. Studies of the responses of thrips to pure and mixed odours of cowpea and maize plants, indicated that the presence of maize odour interfered with responses of thrips to cowpea plant odour.

Light intensity was monitored using a quantum radiometer. The results indicated that there was significantly less light reaching the cowpea canopy in the mixture than in the sole crop. When artificial shading was used to study the behaviour of *M. sjostedti* it was observed that the pest preferred unshaded cowpea plants to shaded ones and in fact the thrips migrated from shaded to unshaded cowpea plants. This suggested that shading resulting from maize plants may have significantly contributed to the reduction of thrips in the mixture.

Reproductive Biology of *Busseola fusca*

G.C. Unnithan

Studies proceeded on aspects of the development, reproductive biology and behaviour of *B. fusca.* Inquiry into larval development showed that it was possible to rear *B. fusca* throughout the year on young sorghum stems without any intervening diapause. Larval feeding on mature stems resulted in the induction of diapause.

B. fusca males mated 3.2 ± 1.7 (mean $\pm$ SD) times during their lifetimes. Only 90% of the females kept with an equal

number of males mated; the mated females were inseminated 1.2 ± 0.4 times. There was no significant difference in the longevity, fecundity and fertility among *B. fusca* from different seasons/sources (first generation moths from diapausing larvae, long and short-rain sorghum crops and laboratory-reared moths). *B. fusca* showed strong preference to sorghum for oviposition. In three-choice tests in cages, with sorghum (Serena), maize (Katumani) and *Pennisetum macrourum*, and an alternative host the proportion of the total eggs laid on these were 0.64:0.18:0.18, respectively. This preference for sorghum for oviposition may be a reason for the higher *B. fusca* infestation on sorghum than on maize, observed in farmers' fields.

Infestation and Carry Over of Stem Borers in Farmers' Fields

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

Investigations were performed in the farmers' fields on Rusinga Island during 1984-85 which involved: (a) monitoring of stem-borer infestation and larval/pupal populations on long and short-rain sorghum (Serena) crops at Kaswanga location, and short-rain maize (Katumani) and sorghum crops at Sienga location on Rusinga Island and, (b) the role of crop residues in the carry-over of stem borers.

B. fusca was the major stem borer affecting sorghum with maximum infestation of 100% and 90% on 1984 and 1985 long-rain crops. Infestation by *C. partellus* was extremely low, averaging only 2% and 3.5%, respectively. However, the short-rain maize and sorghum crops were subjected to very heavy infestation by both *B. fusca* and *C. partellus*. Average infestation by *C. partellus* on maize and sorghum were 82% and 67.9%, compared to 22.5% and 64.7% infestation by *B. fusca*. The heavy infestation of the short-rain crop by *C. partellus* was probably due to the presence of the small, active population of the pest which survived on the stubbles of the long-rain crop. The absence of other sorghum or maize crops in surrounding areas, because the farmers on Rusinga Island do not grow sorghum or maize during the short rains, likely contributed as well to the heavy infestation.

B. fusca and *C. partellus* populations (larvae and pupae) surviving on crop (sorghum) residues in the farmers' fields, in Kakrigu, Sienga, Kaswanga and Wasaria, were determined during the 1984-85 off-season. *B. fusca* survived as diapausing larvae after the harvest of the long-rain crop (August) until the beginning of the following long-rain season (February). Pupation of the diapausing larvae started in mid-November and continued until the end of the following February (Table 1). *B. fusca* egg-laying on the short-rain sorghum crop coincided with the pupation and emergence of the diapausing larvae. Even after land preparation in February, for the 1985 long-rain crop, the stubbles scattered around contained diapausing larvae and pupae which appeared to be the source of infestation of the newly planted crop. Burning the crop residues before or during land preparation would certainly reduce the number of first generation *B. fusca* and, hence, would be an effective cultural control. *C. partellus* survived as an active as well as diapausing population on stubbles, but their numbers were extremely low (Table 1).

Pheromone Biology of *Chilo partellus*

G.C. Unnithan and K.N. Saxena

In order to effectively use the female moths to trap the males of *C. partellus* for monitoring the adult population, it was considered important to study the various factors influencing the trap catches to standardise the trapping techniques. The following factors have been studied: distance between traps; larval diet; number of females per trap, and the age and mating status of the females.

The results indicated that traps with virgin females set 40 m apart attracted more males than those set 20 m apart. Virgin females reared on an artificial diet attracted males as effectively as those reared on the natural feed (sorghum). The number of males trapped increased with the increased number of virgin females per trap up to a maximum of 4 females/trap, and thereafter, there was no increase in the number of males trapped. The attractancy of the virgin females declined with advance in age. After the females were mated their attractancy was reduced and remained low for the 5 days during which the tests were conducted.

PLANT RESISTANCE TO INSECT PESTS

A component of pest management being developed under Plant Resistance to Insect Pests programme involves plant resistance to crop borers. Research is being conducted in the following areas: (1) Evaluation of various sorghum, maize and cowpea cultivars obtained from different sources for resistance to the crop borers; (2) Principles determining resistance or susceptibility of these cultivars; and (3) Genetics of plant resistance to insects. It is the intension of these studies to provide information to be used by plant breeders to develop plant varieties with increased resistance

Table 1. *Busseola fusca* and *Chilo partellus* off-season larval and pupal populations on crop (sorghum) residues in the farmers' fields in Rusinga Islands

	Population/50m²(x±SE)			
	B. fusca		*C. partellus*	
Sampling dates	Larva	Pupa	Larva	Pupa
Sept. 20 - Nov. 1, 1984	116.2±25.2	0.0	11.90±3.10	1.20±0.60
Nov. 15, 1984 - Feb. 21, 1985	29.9± 9.0	4.2±1.2	0.36±0.17	1.04±0.21

*Off-season populations were determined every 2 weeks starting September 20, 1984

in combination with other desirable characteristics. The previous repo . has given information on the resistance/susceptibility levels of different sorghum, maize and cowpea cultivars to their borer pests, the colonising responses of the pests to a few cultivars related to their resistance or susceptibility, and modes of inheritance of resistance characteristics. Further work done on these aspects during 1985 is reported upon here.

Evaluation of Additional Cultivars of Target Crops for Resistance to the Stem Borers

Evaluation of additional cultivars of sorghum and maize for resistance against their crop borers continued during 1985 in order to identify new sources of resistance.

The methods for evaluation were standardised, using 2 categories of parameters: colonising levels of insects on different plant cultivars; and degree of damage suffered by them. The parameters for the colonising levels were: oviposition (percent eggs laid), and number of larvae/pupae per plant or per 10 plants. The damage parameters included: the primary damage expressions e.g., foliar lesions (visual rating on 1-5 or 1-9 scale), stem-tunnelling (percent of stem height), dead heart (percent plants showing the symptom), and secondary damage expressions, e.g., stalk-breakage, tassel-breakage (maize) or head-breakage (sorghum), ear-drop (maize). The comparisons of these parameters among the test genotypes or cultivars were made under natural and artificial infestations with adults as well as neonate larvae. The results briefly follow.

Evaluation of Sorghum Cultivars for Resistance to *Chilo partellus*

K.N. Saxena, K.V. Seshu Reddy

The following cultivars received from different sources (ICRISAT and USA) were compared with respect to their resistance or susceptibility to *C. partellus*: IS nos. 18520; 18363; 2146; 1044; 3962; 4660; 10370; 10711; 18676; 4213; 4405; 4881; 10364; 12447; 18323; 18326; 18517; Tx 28; Tx 38; S-118; S-178. Of these, IS-18363 has been previously reported to be highly susceptible, IS-18520 (Serena) tolerant, IS-2146, IS-4660, IS-2205 and IS-1044 resistant to *C. partellus* (Dabrowski and Kidiavai, 1983; Pathak and Olela, 1983). However, the methods and parameters used for comparing their resistance or susceptibility levels have not been uniform for all the cultivars tested. It was, therefore, necessary to evaluate these again, along with the other hitherto unevaluated cultivars, by infesting them artificially and uniformly with larvae for larval damage and with adults for oviposition. The methods used for infestation of the plants and assessment of larval damage and adult oviposition were developed and described in the Twelfth Annual Report, 1984.

The results indicated that, for ovipostion by the adults, the most suitable cultivars were IS-18363; IS-2146; IS-4660; IS-4213; IS-10364; IS-18323; IS-18326; IS-18517. The cultivars moderately suitable in this respect included: IS-18520; IS-18676; IS-3962; IS-12447; S-118; Tx 28, Tx 38. The least suitable were IS-1044· IS-10370; IS-4405; S-178.

The larval/pupal infestation levels in most of the tested cultivars were quite similar to those in the tolerant check IS-18520. But, in a few cultivars e.g., IS-1044; IS-4660; IS-3962; IS-10370; IS-4881; S-178, these levels were nearly one-half and in the case of IS-10711 one-third of those in the tolerant check.

With respect to the larval damage to the plants, the foliar-damage rating was highest for IS-18363; IS-18520; IS-2146; IS-4405; IS-10364; IS-12447; IS-18326; IS-18517; S-118; lowest for IS-1044 and medium in the remaining cultivars tested. The stem-tunnelling was high in IS-18520; IS-18363; IS-2146; IS-4405; IS-10364; IS-12447; IS-18517; Tx 28, medium in IS-18676; IS-10370; IS-18323; IS-18326; Tx 38; S-118 and low in IS-1044; IS-4213; IS-4881; IS-3962; IS-4660; IS-10711; IS-178. The 'dead hearts' were observed in the maximum percentage of plants (15-20%) in Tx 28; Tx 38; medium percentage (10-15%) in IS-18363; IS-1044, and in a low percentage (less than 5%) in the remaining cultivars tested.

Our observations revealed that the value of certain parameters for a cultivar could be greater and other parameters lower than the corresponding parameters for another cultivar. In order, therefore, to compare the overall resistance or susceptibility of different cultivars, the ratio of each parameter value for a cultivar to that for the check IS-18520 was calculated. The ratios for all the parameters for each cultivar were then added together and averaged to give the overall resistance or susceptibility index (ORSI). This index for the check IS-18520 would be 1.0. The lower the ORSI value for a cultivar (than 1.0), the greater would be its overall resistance. Comparison of these indices (Table 2) shows that IS-18363; IS-10364; S-118 were much more susceptible than the check IS-18520 which was similar to IS-2146; IS-4213; IS-4405; IS-12447; IS-18323; IS-18326; IS-18517; and Tx 28. On the other hand, Tx 38; IS-4660; IS-3962; IS-10370; IS-10711; IS-4881 were resistant and IS-1044 as well as S-178 highly resistant to the borer relative to the check IS-18520.

Table 2. Overall resistance or susceptibility indices (ORSI) for certain sorghum cultivars

Cultivar	ORSI*	Cultivar	ORSI*
IS-18520 (Serena) (check)	1.00		
IS-18363	1.98	S-178	0.44
IS-2146	1.30	S-118	1.54
IS-1044	0.40	IS-4213	1.00
IS-4660	0.64	IS-4405	0.84
IS-3962	0.54	IS-4881	0.60
IS-10370	0.52	IS-10364	1.50
IS-10711	0.50	IS-12447	1.10
IS-18676	0.56	IS-18323	0.88
Tx 28	1.02	IS-18326	0.92
Tx 38	0.70	IS-18517	1.28

*The parameters measured were: per cent oviposition, no. larvae/pupae per plants, foliar damage rating, per cent length tunnelled, and per cent plants with 'dead heart'. Ratio of each parameter value to that for the check IS-18520 was calculated. The ratios of all the 5 parameters for each cultivar were averaged to give the ORSI values

Evaluation of Additional Maize Germplasm for Resistance to Stem Borers, Particularly *Chilo partellus*

J.K.O. Ampofo

One hundred and five maize accessions from Pools 19; 21; 27; 32; and 47 were obtained from CIMMYT for evaluation with *C. partellus* and other stem borers. The parameters compared among the plants were the same as previously described.

The results revealed that mean foliar damage rating among the accessions ranged from 2.1 in acc. no. 47 to 5.5 in acc. nos. 21, 24 (all from pool 19) and 89 (Pool 21). Individual plant ratings, within accessions, varied beyond this range. The percentages of dead plants and broken stalks ranged from 0 in several accessions to 15 (dead plants) in acc. no. 69 and 20 (broken stalks) in acc. no. 68 (both from Pool 32). The mean severity of rust among the accessions ranged from 1.8 to 4.3.

Accessions with a mean foliar damage rating of 3.5 or less and with 0% dead plants and stalk breakage were considered resistant. Accessions with foliar damage rating between 3.5 and 4, but with percent dead plants and stalk breakage between 5 and 10, were considered moderately susceptible. Accessions falling outside these categories were considered susceptible. Within each accession, plants that scored foliar damage ratings of below 3.5 were selected irrespective of whether the accession was considered resistant or not.

Table 3 lists a grouping of maize plant accessions according to the resistance categories described above. On the whole, 11 accessions were selected as resistant, 13 as moderately resistant, 7 showed a good level of resistance to foliar damage and stalk breakage but also showed high susceptibility to leaf rust.

Table 3. A grouping of maize plant accessions in terms of resistance to stem borers and disease

Accession Numbers			
Resistant	Moderately resistant	Resist. to insect susceptible to disease	Susceptible
34(19)	29(19)	71(21)	18(19)
45(19)	16(19)	84(21)	19(19)
44(19)	35(19)	85(21)	20(19)
47(19)	42(19)	95(11)	21(19)
49(32)	45(19)	96(21)	22(19)
50(32)	46(19)	99(21)	24(19)
51(32)	48(32)	100(21)	
58(32)	52(32)		
64(32)	55(32)		
67(32)	56(32)		
101(21)	65(32)		
	72(32)		

Figures in parentheses indicate the pool nos. from which these accessions were extracted

Relationship Between Borer Damage to Different Plant Cultivars and their Yield

H. Kumar, K.N. Saxena

Although several reports proffer information on the nature and extent of damage caused by *C. partellus* and other borers to maize and sorghum, we do not have adequate knowledge of the effects of different types of borer damage to different cultivars of these crops on their yield. This aspect was, therefore, studied with reference to maize at Mbita, and is being reported.

During the long rainy season of 1984, two experiments were conducted using the maize lines Inbred A (susceptible), and Katumani, ICZ2-CM (resistant). These cultivars were grown in 2 solo and mixed planting patterns. For the solo pattern, each cultivar was planted in a plot of 5.25 × 5.25 m in 8 rows 75 cm apart; each row being 5.25 m long, having 10 plants 30 cm apart. The solo plots of the 3 cultivars were replicated thrice and were arranged in a complete randomised-block design. For the mixed pattern, the 3 cultivars were planted together in a plot of 6 × 5.25 m having a total of 9 rows 75 cm apart; each row being 5.25 m with 10 plants 30 cm apart. Each row of 1 cultivar was planted on either side by the 2 cultivars; the distribution of the 3 cultivars among these rows being randomised. The plants were allowed to receive natural infestation by *C. partellus* and other borers. 'Dead heart' damage was expressed as the percentage of plants showing this symptom. Foliar damage in the form of lesions was rated on a 1-5 scale for each plant. Stem-tunnelling was expressed for each plant as the percentage of the stem length.

The results showed that the foliar and stem damage in Inbred A were much higher than those in Katumani or ICZ2-CM, whereas the cob damage was almost equally low in all. However, the stem damage to Inbred A in the solo-planting pattern was much greater than in the mixed-planting pattern. No such differences were observed in the other 2 cultivars nor in the other types of damage for any of the 3 cultivars. Thus, mixed planting of the 3 cultivars reduced the stem damage to the susceptible Inbred A.

In order to discern the relationship between the damage to a plant and its grain yield, simple correlation coefficients were computed, using the standard statistical procedures. In the susceptible maize line Inbred A, there was a significant negative linear correlation between the yield of a plant and the tunnel length ($r^2 = 0.24^*$). On the other hand, in ICZ2-CM and Katumani maize lines the relationship between this pair of characters was not significant ($r^2 = 0.004$ to $0.01)^{NS}$. The regression of grain yield on foliar lesions in 3-5 week old crops revealed no significant correlation in any of the 3 maize lines. Nevertheless, when the crop was 7 weeks old, foliar lesions in Inbred A showed a significant negative correlation with the yield ($r^2 = 0.13^{**}$). But, the correlation coefficient for the same pair of characters was not significant for ICZ2-CM or Katumani.

Principles Determining Resistance or Susceptibility of Different Crop Cultivars

Approach and Methodology. The approach followed for this study was the same as previously explained. 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 colonisation of a plant is governed by the following 6 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 consideration of the above, our present study is being undertaken in 2 parts. The first involves a comparison of the above-mentioned responses of the target insects to the susceptible and resistant genotypes of its plants. The second involves the study of the role of the plant characters in determining these responses.

The methods developed for measuring these colonising responses of the insects to the plants and their characters have been described in the Eleventh and Twelfth Annual Reports 1983 and 1984. The results of the work done during the year are presented below.

Principles Determining the Resistance or Susceptibility of Sorghum to *Chilo partellus*

A. N. Saxena

The sorghum cultivars taken up for this study included: IS-18520 Serena; IS-18363; IS-18463 (Swarna); IS-2146; IS-1044; IS-4660; and IS-2205. Of these, the first one is a tolerant cultivar commonly grown by the farmers in parts of Kenya. The second and third cultivars are believed to be susceptible and the remaining ones to show varying degrees of resistance to *C. partellus*. The above colonising responses of the insects to these cultivars were compared by the methods developed before and described in previous reports.

The ovipositional response of *C. partellus*, involved in the initial selection or rejection of a plant cultivar by the insect, was compared among the 7 test cultivars in the specially designed 3-sector chamber (210 × 80 × 80 cm) described before (Eleventh and Twelfth Annual Reports, 1983 and 1984; Kumar and Saxena, 1985). These cultivars were offered in a 2-choice situation, 1 of the 2 cultivars in each test being IS-18520 (Serena), which served as the standard reference, and the other selection varied. The oviposition, expressed as the percentage of total number of eggs laid on both the cultivars, was high on IS-4660, low on IS-1044 and IS-2205 and medium on the remaining cultivars including the tolerant check IS-18520.

The role of distance-perceivable characters of these cultivars in oviposition was also compared by placing a wire-net barrier between the moths in the chamber and the plants outside, as previously described. Under this situation, the ovipositional response to IS-18363; IS-2146 was greater than that to IS-18463; IS-1044; IS-4660; IS-2205 and less than that to the reference IS-18520. These observations suggest that certain distance-perceivable characters of the test cultivars can contribute to the differences in the insect's oviposition on them, provided that their contact-perceivable characters, considered below, do not influence the oviposition by the insect after its arrival on the plants.

The role of contact-perceivable characters of different cultivars in oviposition by *C. partellus* was studied in specially designed chambers described before (Eleventh and Twelfth Annual Report, 1983 and 1984; Kumar and Saxena, 1985). The leaves of 2 cultivars, 1 being the reference IS-18520, were offered such that insects would remain in maximum contact with them during the test period. The percentage of eggs laid on IS-18363; IS-18463; IS-1044; IS-4660 and IS-2205 was almost the same as that on the reference IS-18520. But, on IS-2146, the oviposition was greater than that on the reference IS-18520.

Thus, the above observations suggest that oviposition by *C. partellus* may be influenced by the distance-perceivable characters of some cultivars and contact-perceivable characters of others.

With reference to the **larval responses** to the test sorghum cultivars, the **orientation** of the first instar larvae emerging from the eggs laid on a plant would involve their **arrest** and settling on it for feeding or departure therefrom. Such an arrest was lower on IS-1044 and IS-4660 than on the remaining cultivars, including the check IS-18520 on which the larval arrest was medium. However, the larval attraction from outside the plants to IS-18363 and IS-18463 was higher than that to the other cultivars including IS-18520. Differences in the arrest and attraction of the larvae to different cultivars would seem to contribute to those in their resistance. The **larval feeding** on the leaves was high on IS-18363; medium on IS-18520 (check); IS-18463; IS-2205; low on IS-2146 and very low on IS-1044; IS-4660. The larval feeding on the stem was, however, lower on IS-1044 and IS-2205 than on the rest. The **larval development** was high on IS-4660, medium on the check IS-18520 and low on the remaining cultivars.

On the basis of the parameters cited above, a profile of all the measured colonising responses of the insect to each cultivar was constructed. The profile of none of the 7 cultivars was similar. These profiles revealed different colonising responses in different cultivars. Such information will prove helpful to plant breeders in developing cultivars with increased resistance.

Principles Determining Resistance or Susceptibility of Maize Cultivars to *C. partellus*

The study of these principles was undertaken initially in 3 maize cultivars: Inbred A, ICZ1-CM, ICZ2-CM, the first

being susceptible and the remaining resistant to *C. partellus*. Our observations on the colonising responses of the insect to these cultivars in relation to their resistance or susceptibility have been presented in the Eleventh and Twelfth Annual Reports, 1983 and 1984.

Our observations have shown varying degrees of differences in oviposition as well as larval arrest, attraction, feeding and development on 3 cultivars contributing to differences in their susceptibility or resistance to the insect. With these findings as the basis, further investigations were conducted during 1985 on aspects of larval behaviour on maize cultivars as well as a few additional resistant cultivars. The aspects and the results are herewith reported.

Chilo partellus Larval Movement, Survival and Development in Relation to Resistance or Susceptibility of Different Maize Cultivars

J.K.O. Ampofo

Previous studies at the ICIPE showed that *C. partellus* neonate larval colonisation starts in the inner-whorl tissue and spreads to the other tissues as both plant and larvae grow older. However, a knowledge of the details of the larval movement and duration of stay within different tissues has been scanty. Experiments were designed during the year to investigate *C. partellus* larval movement, survival and growth on resistant and susceptible maize plants in the whorl stage of development.

Six maize cultivars with different levels of leaf feeding resistance to *C. partellus* were planted in pots in the screenhouse at the ICIPE Field Station, Mbita Point. The cultivars were MP701; MP702; MP704; ICZ1-CM; ICZ2-CM and Inbred A. Twenty-three days after emergence (DAE), each plant was infested by placing 10 freshly emerged *C. partellus* neonates in the inner whorl region and allowing them to settle. Ten plants from each cultivar were randomly selected each week (7 days) and were rated individually for foliar damage. The plants were then dissected and larval location within plant, as well as feeding activities such as cavities within stems and leaf midribs, were measured and recorded. The larvae were weighed individually and head capsules were measured at their greater widths. The head capsule widths were used to determine instars of the larvae collected. Plant dissection was continued for 5 weeks at which time most of the larvae were in the ultimate instar or pupal stage.

The mean percentage of larvae recovered from the maize cultivars 7 days after the infestation ranged from 85 in MP701 to 42 in MP702. Larval establishment in MP701 and MP704, however, declined sharply during the next 7 days compared with ICZ2-CM, Inbred A and MP702 in which larval establishment appeared relatively stable. The percentage of larval cadavers found in the plants was low

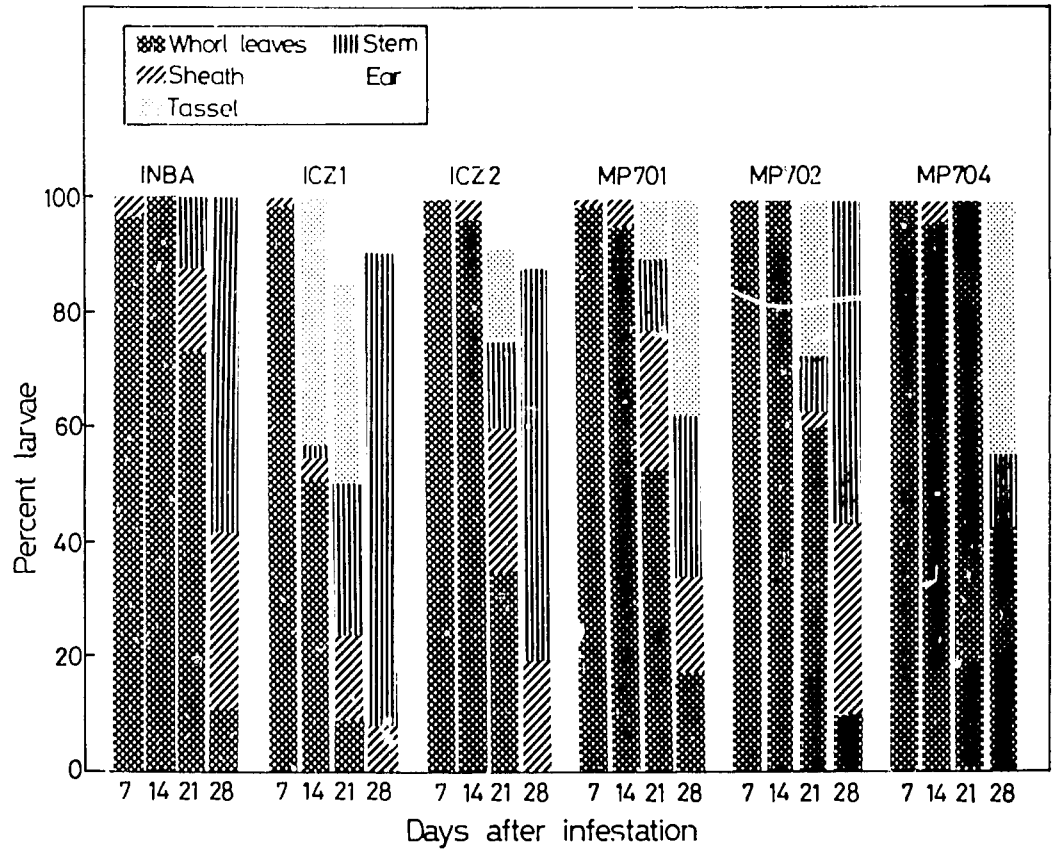


Figure 2. Larval distribution (location) within plant

and ranged between 0.6% in MP704 and 4% in ICZ1-CM. The majority of dead larvae were found in the last sample 35 DAE when most of the larvae were in fifth and sixth instars. Mean larval weight and instar distribution monitored over the various time intervals showed growth and development to be faster on Inbred A and the ICZ cultivars than on the MP cultivars.

In all cultivars, plant damage started within the inner whorls where the neonate larvae settled and fed on the young leaves. Nearly all the larvae recovered from the plants 7 days after the infestation were from this region and larval instar ranged between first and second. As the larvae turned into third instar they tended to migrate from the whorl to other tissues as these became differentiated. Thus, if the green tassel was ready within the whorl the larvae colonised it, otherwise they bore into the midribs of the whorl leaves or migrated through the leaf sheaths to reach the lower stems. Larval presence in the sheaths was considered as transitional since very little feeding was observed in such tissues.

In cases where feeding was observed within the sheaths, the lesions or tunnels were formed as an extension of the midrib tunnels. In the faster developing cultivars, e.g., ICZ1-CM, 44% of the larvae were found in the tassel within the whorls 14 days after the infestation compared with the other cultivars (Figure 2) which had the majority of larvae still in the whorl leaves. Fifth and sixth instars as well as pupae were consistently found in the stems and developing ears in plants where these tissues were differentiated. The larval instar at 14 days after the infestation ranged from first to fourth with the distribution varying from one cultivar to the other. The mean larval instar was third in ICZ1-CM, ICZ2-CM and Inbred A, compared with second in the MP material. At 21 days after infestation, the larvae were generally found in the green tassels, sheaths and stems. In MP704, however, all the larvae were recovered from the whorl tissue and in Inbred A 75% of the larvae were still in the midribs of the whorl leaves compared with only 9% of the larvae in whorls of ICZ1-CM. Larval instar ranged from first to fourth in MP701 and MP704 and from third to sixth in ICZ1-CM and Inbred A. At 28 DAI (51 DAE) nearly all the larvae were recovered from outside the whorls except in MP704 which had about 45% of the larvae still in the whorls.

The results indicate that plant-growth characteristics, rather than the level of resistance, appear to be the major factor influencing the movement of larvae within plants. However, the level of plant resistance influenced larval survival, growth and development.

Principles Determining Resistance in Certain Cowpea Cultivars to *Maruca testulalis*

S.H.O. Okech

During the period under report, studies continued on larval orientation, feeding, growth and development on the pods of the 3 cowpea cultivars namely, Vita 1 (susceptible), Vita 5 (resistant), TVu 946 (resistant). Some plant-morphological characteristics influencing susceptibility/resistance of the resistant TVu 946 cultivar to flower and pod damage were also studied.

Larval movement from one cultivar to another, that is, Vita 1, Vita 5 and TVu 946 planted in alternate rows in a plot, was studied at peak flowering time. When the first instar larvae were released on the susceptible Vita 1, in a combination with the resistant TVu 946, significantly more larvae remained on Vita 1 and did not move to TVu 946. But, when the larvae were released on TVu 946, surrounded by Vita 1, there was a very high emigration from TVu 946 to Vita 1. Similarly, when Vita 1 was combined with the resistant Vita 5, there was a higher emigration from Vita 5 to Vita 1. Emigration from TVu 946 and vice versa was not significant. The results indicate that Vita 1 is more attractive and has a stronger arrestant which causes larvae to cease locomotion once they arrive on the plant. TVu 946 and Vita 5 may be less attractive and may be lacking the arrestants; most of the larvae tend not to settle and, hence, move away from the plant.

Feeding responses of the fourth instar larvae on the pods of the above 3 cultivars showed that consumption of the susceptible Vita 1 and the resistant TVu 946 was similar. Vita 5 was consumed more than the 2 other cultivars. However, absorbability of TVu 946 was poorer than Vita 1 or Vita 5. Assimilability and nutritive value of Vita 5 was also poorer than the susceptible Vita 1. Feeding on flowers by the first instar larvae was similar. Similarly, the area of the leaf consumed by the first larvae was almost identical for the 3 cultivars, but Vita 1 and Vita 5 have thicker leaves than those of TVu 946. So, the total quantity of leaf tissue consumed on Vita 1 and Vita 5 might be more than that consumed in TVu 946.

When the pods of the 3 cultivars were split into husk and green seed and fed to the third instar larvae, Vita 1 husk was more suitable for larval development than the husk of Vita 5 and TVu 946 cultivars. The larval development on the seeds was quite different from that on the husk. Vita 5 seeds were significantly better for larval development than those of Vita 1 and TVu 946 seeds. When a comparison was made between the larval development on the husk and seeds of the same cultivar, significantly more larvae developed to pupa on the husk than on the seeds of Vita 1.

These results indicate that significantly lower larval development on TVu 946, previously observed on larvae reared on complete pods (husk + seed) from first instar to adult emergence, was mainly due to poor husk quality. In Vita 5, poor husk quality is supplemented with suitable seeds and, hence, no difference from Vita 1 exists in the suitability of the pod for larval development.

The resistant TVu 946 cultivar has long peduncles which bear flowers and pods above the leaf canopy. However, flowers are normally formed while the peduncle is still within the canopy. The peduncle grows very fast and in 2-3 days, the flowers are pushed above the leaf canopy, therefore, it is possible to have flowers at different positions within the plant. Pods are usually 15-25 cm above the leaf canopy. Experiments were conducted to test whether this morphological characteristic of TVu 946 would influence susceptibility/resistance of this cultivar.

Tests on the position of flowers were conducted using the first instar larvae, while the fourth instar larvae were used for tests on pods. The treatments were such that in some plants flowers or pods were maintained 15-25 cm above the

canopy while in others peduncles were folded to maintain flowers or pods inside the canopy. In flowers, there were no significant differences in percent infestation between flowers which were raised above the canopy and flowers which were within the canopy when plants were infested by releasing first instar larvae on the leaves. However, damage on pods was significantly higher on those which were within the leaf canopy than on those which were above the canopy.

It was reported earlier that flowers are the most preferred part of cowpea plant for the first instar *M. testulalis* and that flowers of TVu 946 were less infested by larvae than the other cultivars. These results indicate that first instar larvae are quite mobile and can reach any part of the cowpea plant; there is no barrier in TVu 946 plants which can hinder first instar larvae from reaching the flowers. The position of flowers within the plants of TVu 946 (or morphology/architecture) may not be responsible for lower infestation of flowers of TVu 946 compared to the susceptible Vita 1 cultivar. It may, therefore, be concluded that higher emigration from TVu 946 plants, as reported above, results in fewer larvae staying on the plants and, hence, lower infestation of the flowers.

Observations in the laboratory and field showed that a majority of the older larvae (fourth instar) hardly moved up along the peduncles of TVu 946. Most of those larvae (75%) which moved upwards dropped down just before reaching the pod. It may, therefore, be concluded that fourth instar larvae (which mostly attack pods) may be bulky and cannot walk as efficiently as first instar larvae to enable them to reach the pods. By having long erect peduncles which carry the pods above the leaf canopy, TVu 946 cultivar has made its pods less accessible to *M. testulelis* larvae than those other cultivars like Vita 1 which have their pods inside the leaf canopy. Also, those larvae which manage to encounter pods of TVu 946 cultivar do not develop as efficiently as those in Vita 1 pods due to adverse effects of the husk of TVu 946 which they have to eat through before they reach the seeds.

Genetics of Plant Resistance to Insects

R.S. Pathak

Gene Effects for Sorghum Resistance to the Stem Borer Chilo partellus. Understanding the nature and magnitude of various kinds of gene effects helps formulate breeding procedures. To estimate the gene effects on a five-parameter model, a trial consisting of five generations, P_1; P_2; F_1; F_2; and F_3 or the cross between tolerant cultivar Serena (P_1) and resistant cultivar IS-2146 (P_2) was conducted in a randomised-block design with 4 replications.

The plant characters examined were: dead heart, stem-tunnel, peduncle tunnel, peduncle breakage, chaffy head, leaf feeding, plant height, and grain yield. The results (Table 4) showed that in a 5 parameter model, the estimated gene effects were: constant (m), additive (d), dominance (h), dominance × dominance (l) and additive × additive (i). Generation-mean analysis showed that the parameter m was significant for all the characters. None of the components showed significance, indicating an important role of non-allelic (epistasis) interactions for percent dead heart in addition to additive (d) and dominance (h) gene effects. However, the magnitude of dominance (h) and dominance × dominance (l) were higher than additive (d) gene effects. This suggests that non-additive gene effects (h) and (l) are more important in inheritance of dead heart. The low estimate of additive (d) gene effects for dead heart suggests slow progress under selection.

The additive gene effects (d) were significant for all characters, except dead heart, and were higher in magnitude than dominance (h) gene effects. The epistasis component (l) was significant for peduncle tunnelling and peduncle breakage while additive × additive (i) was significant for peduncle tunnelling, peduncle breakage, plant height and grain yield.

The presence of significant additive (d) gene effects and complementary epistasis for stem tunnelling suggests that improvement would be expected through standard selection procedures and that the progress would be faster than for dead heart. For peduncle damage, additive (d) and epistasis gene effects (l and i) were more important than dominance (h) gene effects. However, peduncle tunnelling and peduncle breakage showed duplicate and complementary epistasis, respectively, suggesting that selection based on peduncle breakage will be more effective than selection based on peduncle tunnelling. Significant additive (d) gene effects and non-significant dominance (h) and epistatic gene effects for chaffy head indicates the fixable nature of gene action for this character.

Although additive gene effects (d) were significant for leaf feeding, the magnitude of dominance (h) and epistatic gene effects (l and i) were also considerable. This type of gene action is an indication of obtaining desirable cross. Presence of significant additive (d) and additive × additive (i) gene effects, which are fixable, associated with complementary

Table 4. Estimates of gene effects for resistance/tolerance to stem borer, *Chilo partellus* in sorghum, the ICRISAT, 1985

Character	m	d	h	l	i
Dead heart (%)	24.75**	0.68	-1.03	-11.63	0.65
Stem tunnel (%)	32.95**	1.16	-2.62	12.08	2.80**
Peduncle tunnel (%)	15.08**	4.10	-0.42	5.52** 8.01	
Penducle breakage (%)	25.80**	7.65**	-1.87	-21.87**	14.91**
Chaffy head (%)	24.60**	4.03	0.55	2.61	0.55
Leaf feeding (1-9)	9.98**	-0.50**	0.37	0.31	-0.33
Plant height (cm)	151.30**	-16.35**	-0.68	-67.84	-35.30*
Grain yield (g/hill)	14.40**	7.49**	-5.35	-1.39	11.49**

*Significant at 5% level
**Significant at 1% level

epistasis was very encouraging for both plant height and grain yield. Selection in this situation would be highly effective for increased grain yield and desirable plant height through standard breeding procedures.

The present study has indicated the importance of epistatic gene effects for most characters and thus shows that this type of gene action cannot be ignored in formulating breeding procedures. Considering the importance of both additive and non-additive (dominance and epistasis) in the inheritance of stem-borer resistance and tolerance and grain yield, recurrent-selection procedure seems the best available to meet the requirements in the present case, as it will utilise all 3 kinds of gene effects.

Genetic Variation of Cowpea Resistance to the Legume Pod Borer, Maruca testulalis. *Maruca* causes damage to stem, flowers, pods and seeds — the later two being the most devastating. Previous studies have shown that cowpea resistance to pod borer is polygenic and the level of resistance usually low to moderate. Further studies were undertaken to measure the genetic variation of pod damage and seed damage in a cross between the resistant cultivar TVu 946 and susceptible cultivar ICV 5. Continuous variation in parents as well as F_1 and F_2 progenies confirmed that resistance to *Maruca* is polygenic in inheritance. The parental cultivars involved in the cross represented a fairly wide range of character expression for growth habit, flowering time, days to maturity, number of peduncles per plant, peduncle length, canopy height, peduncle height above canopy, 100-seed weight and grain yield per plant.

Degree of dominance, minimum number of genes, heritability and expected genetic advance for percentage pod damage and percentage seed damage were estimated. Means and degree of dominance of F_1 and F_2 populations suggested partial dominance of susceptibility over resistance. Gene action governing the expression of resistance was largely additive. The observed F_2 mean was in close agreement with both calculated arithmetic and geometric F_2 means. The degree of dominance in F_1 and F_2 were similar except for pod damage in F_1. The degree of dominance was greater in F_1 than in F_2 for both characters. The h_2 values were lower than h_1, indicating confounding effect of epistatic gene action. A minimum of 4 gene pairs were involved in the expression of resistance. Low estimates of heritabilities (30 to 34%) and genetic advance (2.83 to 7.14% of mean) suggested slow progress under selection.

Inheritance of Cowpea Resistance to the Cowpea Aphid, Aphid craccivora. Two resistant cultivars involved in the inheritance studies were (1) TVu 310 - an IITA cultivar, and (2) ICV12 - a mutant cultivar obtained through irradiation of ICV1. These resistant cultivars were crossed with the common susceptible cultivar ICV1. F_1 showed complete dominance resistance over susceptibility in both crosses. The F_2 progenies segregated in ratios of 3 resistant: 1 susceptible, indicating that resistance was conferred by a single dominant gene (monogenic). Back-cross breeding may be appropriate to transfer resistance genes to agronomically acceptable cultivars which are susceptible to aphids. Further studies will include the confirmation of monogenic inheritance in back-crosses, and an investigation to see whether the resistance gene in TVu 310 is the same as that in the mutant cultivars ICV11 and ICV12.

BIOLOGICAL CONTROL

Another category of components being developed for the management of the crop borers in this project involves the use of natural enemies for biological control. The previous report (Twelfth Annual Report, 1984) presented information on the incidence of various parasitoids and pathogens of the crop borers in mono- and intercropping-systems. Work on these natural enemies was continued, with a view toward utilising them for the control of the target pests.

Parasitoids of the Crop Borers as Components of Biological Control

The previous reports have mentioned that the following parasitiods infest the stem borers of sorghum and maize: *Pediobius furvus, Trichogramma sp. Apanteles sp.* and *Dentichasmias busseolae*. In order to use these parasitoids for biological control of the crop borers, it would be important to have a detailed knowledge of their biology. Aspects of their biology were studied during the present reporting period and the observations made are described below.

Progeny Production, Development, Sex Ratio and Longevity of *Pediobius furvus*

G.W. Otoo

These studies were conducted on mated *Pediobius furvus* females reared singly in a cage with 1-day old to 5-day old *C. partellus* pupae. The parasitoid was fed a 20% sucrose solution. There were 40 cages for each treatment and the experiment was carried out in the laboratory at a mean

Table 5. Performance of *Pediobius furvus* on 1- To 6-day old *Chilo partellus* pupae

Age of host pupae (days)	Percent (%) parasitism	Progeny production	Incubation period Sex-ratio (male:female)	period* (±SD)
1	33.0	74.7±32.0	1:6	21.7±1.4
2	15.0	79.1±33.8	1:15	21.1±1.3
3	32.5	71.5±43.1	1:4	20.8±0.7
4	16.5	62.5±34.1	1:6	20.6±0.7
5	1.3	102.0**	1:7	22.0
6	0	—	—	—

* Days, oviposition to emergence

**Progeny from a single female

temperature of 25°C. Observations were kept on percent parasitism, progeny production, sex-ratio of the offspring, and the incubation period of eggs in the hosts (Table 5). It may be observed that the percentage parasitism of pupae older than 4 days was much less than that of younger pupae.

Comparative Tests on Progeny Production and Sex-Ratio in Mated and Unmated Female *Trichogramma sp.*

G.W. Oloo

Forty mated female *Trichogramma sp.* were reared singly in cages with freshly oviposited *C. partellus* eggs. A similar experiment was conducted using unmated female *Trichogramma*. The observations indicated that there was no significant difference in incubation period between mated females (9 days, n = 40) and the unmated (8.8 days, n = 40). The sex-ratio of mated females was found to be 1.5 as compared to the unmated 1.3, while parasitism levels for mated (48.4%) was higher than the unmated (38.4%) females.

Development, Progeny Production, and Sex-Ratio in *Trichogramma sp.* Reared on *Chilo partellus* and *Busseola fusca*

G.W. Oloo

Forty mated female *Trichogramma sp.* were reared singly with freshly emerged *C. partellus* eggs. A second group of 40 females were also reared with freshly emerged *B. fusca* eggs. There was no significant difference in sex-ratio between mated females of *Trichogramma sp.* reared on *C. partellus* (1.5) as compared to mated females reared on *B. fusca* (1.4) with the balance in favour of female offspring. Similarly, in both cases, the average incubation period was 8.6 days, percent parasitism being 45.3 for *C. partellus* and 41.9 for *B. fusca* as the hosts.

Parasitism by *Apanteles sp.* on *Busseola fusca* Larvae

G.W. Oloo

Newly emerged *B. fusca* adults were mated, and females allowed to oviposit on wax paper. The eggs were incubated in the laboratory and allowed to hatch into first instar larvae. At fourth and sixth instar, 30 larvae were kept individually with single, mated female *Apanteles sp.* Records were kept on the number of larvae parasitised, apparent parasitism, mortality during larval development, emergence of *Busseola* adults, production of progeny, rate of development and sex-ratio of the progeny. There was no significant difference in percentage parasitism between fourth instar (50%) and sixth instar (53.8%) host larvae, or in their respective sex-ratios (1.2). However, the incubation periods and progeny production for the fourth instar host (21.6 days; 21.8 offspring) were different from sixth instar host (17.3 days; and 34.0 ± 7.0 offspring).

Dentichasmias busseolae, the Pupal Parasite of *Chilo partellus*

J.W. Bahana

Pure stands of sorghum (Serena) and maize (Katumani) were planted at 2 sites with the objective of studying population fluctuations of the stem borer *C. partellus* and percentage parasitism due to its pupal parasitoid *Dentichasmias busseolae* Heinrich.

One site selected was Opapo, 18 km on Homa Bay — Kisii Road, where the experiment was conducted on farmers' fields, and under natural rainfall. The second site was at MPFS, where the crops were grown under natural rainfall supplemented by irrigation.

Infestation by *C. partellus* at both sites was first recorded in the third week after plant emergence (APE). However, pest numbers at Opapo were very low throughout the short rainy season (1984). At MPFS, the *C. partellus* larvae population rapidly built up to a peak in the sixth week APE. Thereafter, low numbers were recorded until the twelfth week, when a second peak was reached. The pupal population was first recorded seventh week APE and reached a peak thirteen week APE.

Parasitism of *C. partellus* was first recorded eleventh week APE and reached a peak at 60% 2 weeks after. This, however, dropped to 15% in the 14th week, rising thereafter to a maximum of 70% in the 16th week. This also represented the maximum level of parasitism during the season.

Pathogens of the Crop Borers as Components for Biological Control

The previous report provided information on the incidence of certain pathogens, e.g., bacteria, fungi, protozoans and nematodes which infect and kill the crop borers of sorghum and maize. During this past year, further studies on the following aspects of these pathogens were conducted.

Table 6. Larval mortality of the spotted stalk borer *Chilo partellus* caused by pathogens in different instars in the field (long rainy seasons, 1985)

Intercrop pattern	Crop	Instars I	II	III	IV	V	PUPA
Maize-Sorghum-Cowpea	Maize	-	2	2	4	3	4
	Sorghum	-	-	3	5	4	5
Maize-Cowpea	Maize	-	-	3	5	2	-
Maize-Sorghum	Maize	-	-	1	8	1	2
	Sorghum	-	1	2	5	2	1
Sorghum-Cowpea	Sorghum	-	1	6	11	2	1
TOTAL		(0%)	(14.7%)	(19.8)	(44.19%)	(16.28%)	(15.12%)

Incidence of Pathogens of Crop Borers in Relation to their Mortality in Different Intercropping Systems

W.A. Otieno

Results from field experiments at both the ICIPE Field Station and farmers' plots during the long rainy season 1985, further confirmed and earlier preliminary observation that microbial activities are greater in certain crop combinations than in others. Highest mortality (13.38%) of the crop borers due to pathogens was confirmed in the sorghum-cowpea mixture and the lowest (7.75%) in the maize-sorghum combination.

Observations on the mortality of the stem borers in different instars within the mixed cropping system indicated that the highest mortality (44.19%) in *C. partellus* occurs during the fourth larval instar, no diseased larvae in the first instar having been recorded (Table 6).

Of the various pathogens recovered from the stem borer larvae, 2 hold promise for use as bio-control agents: a nematode (*Panagrolaimus sp.*) and a protozoan (*Nosema sp.*). We are briefly reporting on the studies conducted on these pathogens.

Nosema sp. as a Bio-Control Agent

M.O. Odindo

Tests were conducted in the screenhouse to determine the utility of this pathogen as an agent for the biological control of *C. partellus*. For these tests, sorghum was planted in the plots A, B and C in the screenhouse; there were 4 replicates. At 25 days after plant emergence, plots A and B were infested with neonate *C. partellus* at 20 larvae per plant, and placed directly inside the funnels of the main plant.

The pathogen inoculum was prepared and purified from recently dead *C. partellus* larvae which had been infected in the laboratory with *Nosema*. Three days after infestation of the plants, the previously infested plot A, was treated with the *Nosema* suspension of 10 drops of inoculum placed into the funnel. Plot B, which had also been infested, was treated with sterile distilled water. The third plot (C) was the non-treated control.

All the plants on the 2 plots which had been infested with neonate *C. partellus* larvae showed foliar damage at 7 days after infestation. Treatment of plants in plot A with *Nosema* stopped further damage to plants in this plot. In the plants inoculated with distilled water, the leaf damage by *C. partellus* continued so that at 40 days after plant emergence 67.5% of the plants displayed foliar damage. Plants in plot C remained infestation-free throughout the early period of the experiments, but by the fifty-first day after plant emergence some plants were also infested with second generation larvae. Similarly, formation of dead hearts was reduced by 60% at 24 days after inoculation and the proportion of plants having fully-formed heads increased by 80% when the plot inoculated with *Nosema* was compared to plot B (infested but not treated) and plot C (non-infested) control plots. The damage on sorghum stems was reflected in the size of the plants (Table 7). A high number of larvae were also recovered from the infested control plants (365) and many fewer (82) from *Nosema*-treated and non-infested control (75). *Nosema* therefore gave good protection of the sorghum from an artificial infestation of *C. partellus*. Further tests on the factors pertinent to the full utilisation of this pathogen for the control of *C. partellus* are in progress.

Table 7. The size of plants after foliar application of *Nosema* sp. to control *Chilo partellus*

Days after plant emergence	Size of plants (CM±SD)		
	NT*	NIC**	IC***
40	124.6±16.7	116.0±23.2	102.9± 3
51	124.3±20.6	116.6±19.1	65.8±21.2
90	139.1±21.2	134.6±30.0	56.4±23.7

*Nosema treatment
**Non-infested control
***Infested non-inoculated control

Population Ecology of the Cowpea Pod Borer *Maruca testulalis* in Relation to its Natural Enemies

I.B. Okeyo-Owuor

Studies on the dynamics of *M. testulalis* were continued in the field and the laboratory during 1984. The studies in the field were aimed at further understanding the population fluctuations of the pest and identifying natural enemies both at the MPFS farm and at the farmers' field in the Lambwe Valley. The results showed that, although there was considerable overlap in the life stages, only 1 generation was completed on the crop at both sites. This finding confirms the observations made during 1983 and reported earlier. During the period, several larval and pupal parasites were also recovered at MPFS. These included *Hyperchalcidia* sp. and *Bracon sp.* on pupae and *Apanteles sp.* on larvae. In the Lambwe farmers' field, only larval parasites were recovered, especially *Apanteles sp.* and *Chelonus sp.* At both the sites, the impact of these parasites on *M. testulalis* population was insignificant. The pathogens recovered were similar to those of 1983 and *Nosema sp.* was the most prevalent. A partial ecological-life table of *M. testulalis* has been constructed from the data.

Further studies were conducted in the field at MPFS and Lambwe to determine the role of alternative wild plants on 1) survivorship of *M. testulalis*, and 2) perpetuation of the population of the pest and its natural enemies during the dry periods of 1984. Five wild hosts were discovered to harbour *M. testulalis*, namely *Sesbania sesban* (L.) Merr.; *Vigna vexillata* (L.) Rach; *Crotalaria deserticola* Bak.f. *Rhynchosia malacophyll* (Spreng.) Boj.; and *Rhynchosia* sp. nr. minima (L.) D.C. all being Papilionaceae (Leguminosae). *S. sesban* and *V. vexillata* carried the highest number of *M. testulalis* larvae on the flowers. At one site, Rusinga Island, the larvae recovered from *S. sesban* were parasitised by Braconid parasites.

Laboratory investigations were conducted to elucidate the role of one pathogen, *Nosema sp.*, in determining mortality and survival of *M. testulalis*. *Nosema sp.* was found to be an important pathogen of *M. testulalis* during

the population studies of the pest in the field. In the laboratory studies to determine the pathogenicity of *Nosema sp.* by artificial inoculation, it was found that the major stages of *M. testulalis* which were vulnerable were the larvae and pupae. The highest mortality was caused during the first (100%) and second (86.7%) larval instars and the larva died within 2-6 days of infection. The least susceptible instars were fourth (56.7%) and fifth (73.3%) which all died within 6.8 days.

Both field and laboratory observations showed that *Nosema sp.* is an important pathogen of *M. testulalis* and holds great potential in biological control of the pest as compared to the parasites and other pathogens.

The effect of temperature regimes on survival and development of *M. testulalis* was studied in the controlled temperature chambers. The range of temperatures tested was 15-35° C, comparable to that obtained in the field at MPES and Lambwe study sites. The results indicated that the temperature suitable for survival and development of *M. testulalis* was between 23 and 29 °C. Temperatures outside this range affected the survival and development adversely, especially in the case of larvae and pupae. It was found that temperature regimes significantly reduced the pupal weights at temperatures below 21 °C and above 29 °C. The highest pupal weight was attained at 23 °C. This finding was important as it provided a partial explanation for the low population of *M. testulalis* during dry and hot seasons both in the cowpea and wild-host plants.

INSECT MASS REARING TECHNOLOGY

R.S. Ochieng

During 1985, our efforts were focused in 2 directions. First, it was our desire to find a diet for *Chilo partellus* that did not require use of certain specific ingredients. Second, intense work was undertaken to establish a viable colony of *Glossina pallidipes* in the laboratory.

Chilo partellus

One factor that impedes the rearing of this insect is the inclusion of the leaf factor in the diet. This means that plants have to be grown throughout the year to make available the leaf component. It has also been observed that the leaf, although dried at 100 °C, still is capable of carrying fungi with it from the field to the laboratory. Yeast is particularly noted for this kind of infection.

A diet (Table 8) has now been developed in which the leaf factor and Vitamin E have been excluded. The results (Table 9) show that *C. partellus* survives and develops on this diet better than on the diet containing the leaf factor and Vitamin E. The developmental period on the new diet is shortened and the development is more uniform than on the previous diet. The advantages of this diet are that one possible source of diet infection has been eliminated and that the cost of production is reduced. Also, this diet is simple to prepare as there is no need to powder the beans before use; the beans are blended directly in the blender after soaking.

Table 8: Composition of the DIET D 85-1

Beans	1059.075 g
Brewers yeast	158.90 g
Ascorbic acid	15.89 g
Methyl paraben	9.95 g
Sorbic acid	4.95 g
Formaldehyde 40%	9.95 ml
Agar	63.54 g
Water (Distilled)	3177.77 ml

Glossina pallidipes

Experimental rearing of *G. pallidipes* was initiated in 1983 in a grass-thatched house at MPES. The colony was started from wild flies collected from the field in Lambwe Valley. A 3-room improved version of the grass-thatched house has been erected. The colony has been maintained in this laboratory structure for the last 2 years.

In order to rationalise handling, reduce recording and facilitate analysis of colony performance, the following modifications were introduced by the end of May 1985:

(i) All the female flies emerging daily during each of the 3 10-day periods of the month (1-10, 11-20, 21-30) or 31) are pooled together into 3 separate production units (i.e., 3 record-sheets per month).

(ii) Cages (oval PVC cages with 10 or 15 males and 10 or 15 females) and larviposition trays which are holding trays are labelled to designate the month and the corresponding 10-day period when the unit was formed.

Females are mated on day 8 or 9 following emergence, and the males are separated 1 week later.

(iii) During the first 20 days following initiation of the production unit, dead flies are removed then the sexes are separated and recorded to assess early mortality and the net in-put into the colony. During the remaining 70 days, mortality is checked and recorded at a 7-day interval every Thursday to reduce disturbance of the flies.

Puparia are collected daily and weighed in 5 weight classes A, B, C, D, E and the percentage of A class puparia is calculated to determine the quality of puparia the colony is producing.

For the last 6 months there has been a steady increase in the number of mated females. The colony is now viable, and self-sustaining. The performance for the last 6 months is shown in Table 10.

Membrane Feeding Technique

In vitro feeding technique of the tsetse was introduced in early August 1985, and has been confined to wild flies collected from the field. Use of this technique requires that the blood be completely sterile to eliminate contaminating bacteria. The sterility is best achieved by use of irradiation source. Since it is not yet possible to irradiate the blood used in our laboratory, it is felt that the technique should be confined to field-collected flies. The clean flies bred in the laboratory are fed on live animals.

Table 9. Evaluation of the performance of *Chilo partellus* on pure bean diet

		n	Mean	Minimum	Maximum	St. deviation
Larval period	0	35	26.600	23	29	3.397
(days)	0	45	27.900	23	39	3.679
Pupal weights	0	37	56.189	31	82	6.000
(mg)	0	45	81.311	53	121	18.741
Pupal periods	0	30	8.000	6	10	0.793
(days)	0	40	7.543	6	9	0.0836
Fecundity/		8	155.900	36	585	179.25
Longevity	0	10	4.000	2	7	1.414
(days)	0	10	3.900	2	5	0.994

INSECT MASS-REARING TECHNOLOGY UNIT (IMRT) NAIROBI BRANCH

IMRT - Nairobi Branch is a services and supply unit. During 1985, it mass-reared and supplied target insects to the programmes and the units as shown in Table 11. The unit maintained colonies of rabbits, rats, mice and hamsters for research purposes as well as rearing haematophagous insects.

This year we introduced in vitro methods for feeding tsetse flies using freeze dried blood. We also initiated new tsetse-recording systems adopted from IAEA laboratories.

SOCIO-ECONOMIC ASPECTS OF PEST MANAGEMENT

Evaluation of Traditional Intercropping Systems in Relation to Pest Control: Mbita Division

W. F. Conelly, A. Dissemond

Intercropping and related cultural practices comprise the integrated pest management package being developed by the Crop Pests Research Programme (CPRP). Before this IPM package can be integrated into local farming systems, however, additional information is needed concerning the traditional intercropping practices employed by small-scale farmers in the region. As a first step in this direction, the Bionomics and Applied Ecology Section conducted research in 2 farming communities located in Mbita Division, a semi-arid area of South Nyanza District in Western Kenya. Sociological and agronomic data related to intercropping and pest management were collected from a sample of 8 representative farm households. Sample plots chosen from 14 fields operated by these farmers were monitored throughout the season.

The research identified 3 key features of the local intercrop system that will influence the design of an improved pest management package. These features are: time of planting, the pattern of intercropping and relative population densities of the component crops.

Time of planting. It is generally recommended that early and simultaneous planting of sorghum and maize should be practised to reduce the crop damage caused by insect pests such as the sorghum shootfly and stem borers. The plantings in this study ranged from January through mid-April. However, most of the plantings were done within March, which coincides with the recommended planting time in the Lake basin. The Ministry of Agriculture recommends that by March 15th the planting should be completed. There are a number of constraints to such a recommendation. Among those to be investigated are: erratic onset of the rains; difficulty of access to plough and plough animals for timely land preparation; localised soil conditions making early ploughing difficult; the cost and availability of seed; the risk of early armyworms infestation in some areas; competing demands on farms' time; different work schedules of wives in polygamous households.

Intercropping pattern. Row intercropping is the experimental design most frequently utilised in the ICIPE farm trials. Though a few Mbita farmers already use this method, the majority employ mixed intercropping in their fields, a practice which they feel requires less labour. Experimental evidence has shown that cereal-legume intercrops have significantly lower insect-pest populations than monocropped maize or sorghum. In the sample farmers' fields the data on the incidence of insect pests of sorghum show virtually no advantage to intercropping, simply because there were not enough legume plants in their respective cropping patterns to make the difference between

Table 10. *Glossina pallidipes* colony dynamics and performance, March through August 1985

	March	April	May	June	July	August
Total females (End of month)	915	1026	1331	1600	2164	2057
No. females emerged	447	579	762	834	1142	1006
Females removed from colony		100	85	67	221	413
No. males emerged	472	646	792	864	1029	1078
Average No. producing females per month	715	795	1119	1156	1196	1330
No. of puparia produced	1701	1686	2177	2696	2514	2207
Fecundity (PPF)	2.27	1.47	1.95	2.31	2.08	1.65
Daily mortality %	0.44	1.08	0.84	0.84	0.55	0.90

Table 1 Primary users of insects and animals reared by the Insect Mass Rearing Technology Section (Nairobi Branch)

Insects and Animals	Livestock Ticks	Medical Vectors	Tsetse	Chemistry Bioassay	Histology and Fine Structure	Sensory Physiology	Biological Control	Other Institutions
Tsetse (*G. morsitans*)			X	X	X	X		X
Stem borers (*Chilo partellus*)				X		X		X
House fly (*Musca domestica*)	X							
Mosquito (*Aedes aegypti*)			X					X
Cotton stainers (*Dysdercus fasciatus*)			X					X
Eldana saccharina			X		X			
Armyworm (*Spodoptera exempta*)			X		X			
Rabbits	X	X	X	X	X	X	X	X
Rats		X	X					
Mice	X	X	X					
Hamsters		X						

intercropping and monoculture.

Density of component crops. A key reason for the poor performance of farmers' intercropping as a pest-control measure appears to be the relative population of the component crops found in most fields. In the ICIPE experimental plots, the ratio of sorghum:legume is usually 1:1. In contrast, because cereal production is their main priority, farmers typically plant a very high population of sorghum and a relatively low proportion of legume. The proportion of sorghum to legume in 4 sample fields in Ufira, for example, averaged about 5:1. The low proportion of legumes in these intercropping systems failed to benefit the farmers in terms of insect-pest control.

Discussion and conclusions. Research has identified several basic differences between the intercropping systems being developed by the CPRP and the current practices of small-scale farmers in the Mbita Division. Due to a variety of constraints, local farmers tend to stagger their planting over the season rather than planting early and simultaneously. They typically utilise mixed rather than row intercropping, and, because of their emphasis on cereal production, plant very low populations of cowpea or green gram in their cereal-legume mixtures.

In developing intercropping systems for improved pest control, the priorities and constraints of small-scale farmers must be identified and incorporated into the design of new technologies in order to facilitate adoption by these farmers. In the Mbita area, for example, research suggests that it would be useful to evaluate the pest-control potential of mixed intercropping and the planting of lower, but still adequate, proportions of legume in the intercrop system in order to develop a package that is both effective as a means of pest control and acceptable to farmers. A similar sociological and agronomic evaluation of intercropping systems in other agro-ecological zones in the region is recommended.

Livestock Ticks

Livestock Ticks Research Programme

The recent developments in tick-borne disease control make possible the use of other methods of controlling ticks besides the frequent application of acaricides. The main alternatives to saturation dipping or spraying of acaricides are greatly increased intervals between applications of acaricides and the stimulation of both naturally acquired and artificially induced host resistance to the tick.

The ICIPE Livestock Ticks Programme has concentrated its efforts on the host-immune response following initially successful demonstrations of immunity in rabbits and cattle in controlled-paddock experiments against Rhipicephalus appendiculatus. *It is becoming evident from further work in mixed tick challenge situations that the reinforcement of naturally acquired host resistance to one species does not stimulate sufficient immunity to prevent losses in productivity. A considerable build up in the tick population can occur and cross resistance between species is not sufficient to provide broad-spectrum control of all species.*

The programme has thus increased its efforts to isolate antigens which are not normally presented to the host during feeding, but will induce host antibodies that significantly interfere with the tick life cycle when injected parenterally into the host.

ASSESSMENT OF HOST RESISTANCE BY INTRADERMAL TEST

P.B. Capstick, J.J. de Castro, M. Nyindo

Binta and Cunningham described the use of *Rhipicephalus appendiculatus* larval extract to demonstrate an intradermal response in cattle which had been exposed to tick feeding. The response was of the immediate hypersensitivity type. It was proposed that this reaction could form the basis of a field test that could be used to select tick-naive animals for further immunisation experiments, or assess the immune status of herds of cattle.

A comparison was made of 3 antigens in both tick-naive and tick-exposed cattle. *R. appendiculatus* larval and tick salivary gland extracts were prepared by grinding in phosphate buffer saline (PBS) and centrifuging at 3000 rpm for 10 minutes to remove gross debris. Tissue culture extract was prepared by sonicating cells from cultures of embryonic *R. appendiculatus*. Protein levels in each antigen were estimated by the method of Lowry and were between 8.0 and 16.0 mg/ml.

Tenfold dilutions of each antigen were prepared in PBS and 0.1 ml of each dilution inoculated intradermally into both sides of the neck of 3 susceptible and 3 "exposed" cattle. The skin thickness was measured before inoculation and at 30, 60, 90 minutes and 24 and 48 hours after inoculation.

Results

Time of maximum response. All 3 antigens induced responses as rapidly as 10 minutes after injection. This reaction was an oedematous swelling which increased in size for up to 60 minutes and was reduced by 90 minutes. By 24 hours the response had disappeared.

Level of response. The reactions to the antigens were taken as positive if there was an increase in skin thickness of 4 mm over the original measurement. Using this as a basis the responses to the dilution of antigen are presented in Table 1.

Neither larval nor tissue-culture extract reacted at the undiluted level in the naive animals, but both extracts reacted to a dilution of approximately 1 in 500 in tick-exposed animals. Salivary gland antigen reacted in tick-naive animals to a similar dilution level as in tick-exposed animals, and was judged to be unsuitable for use in a discriminatory test between naive and exposed animals.

Table 1 Responses of six cattle to the intradermal inoculation of dilutions of three *R. appendiculatus* antigens

Cattle history	Antigen type inoculated	Reaction to dilutions inoculated					
		1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Tick naive	Salivary gland	6/6*	5/6	3/6	0/6	0/6	0/6
	Larval extract	0/6	0/6	0/6	0/6	0/6	0/6
	Tissue culture	0/6	0/6	0/6	0/6	0/6	0/6
Tick exposed	Salivary gland	ND	6/6	5/6	1/6	0/6	0/6
	Larval extract	ND	2/2	6/6	3/6	0/6	0/6
	Tissue culture	2/2	3/4	5/6	1/6	0/6	0/6

* No. reacting/No. sites injected

Both tissue-culture extract and larval-tissue extract have potential as a source of antigen for a discriminatory test to detect previous exposure of cattle to *R. appendiculatus* ticks.

IMMUNISATION OF RABBITS WITH SOLUBLE ANTIGEN FROM EMBRYONIC CELL LINES OF *RHIPICEPHALUS APPENDICULATUS*

M. Nyindo

A soluble antigen was prepared from cell lines of embryonic cells of *Rhipicephalus appendiculatus* grown in culture for 400 days. Cells were detached from the tissue culture flasks, and spun down into a pellet. The medium was discarded and the pellet was washed 3 times in 50 ml volumes of phosphate buffered saline (PBS) pH 7.4. After the last wash cells were resuspended in 20 ml PBS. Cells were disintegrated by sonication. Particulate matter was removed by centrifugation and the soluble fraction was used as the antigen.

A group of 8 New Zealand White rabbits, aged 6 months and weighing approximately 3 kg each, were selected for the immunisation experiment. Rabbits numbered 18, 19 and 20 were placed in the experimental group, rabbits numbered 24, 25 and 26 served as control and rabbits numbered 30 and 31 were injected with adjuvant and were designated as adjuvant control. Each of the experimental animals was inoculated with 15 mg soluble protein in PBS emulsified in an equal volume of Freund's Complete Adjuvan (FCA). Two injections were administered, 1 to each hind leg as a deep intramuscular injection. Ten days later similar injections of the protein in Incomplete Freund's Adjuvant (IFA) were given. A period of another 10 days was allowed to elapse and then a second booster of the antigen in IFA was given.

Animals in the adjuvant control group were injected with FCA and IFA without the protein materials; animals in the control group were not injected. All animals were challenged on day 30 with 100 larvae of *R. appendiculatus* on 1 ear and 25 male and 20 female *R. appendiculatus* on the other ear. Mean engorgement weight of the larvae and adults, percent egg hatchability and moulting of larvae were determined.

Table 2 summarises the results of the experiment. Inoculation of the rabbits with the soluble protein from the cell lines did not provide protection to tick challenge with either the adult ticks or the larvae. Experiments are in progress to determine whether intact cells from primary cultures would be a better source of antigen.

PRELIMINARY FINDINGS OF A THEILERIOSIS IMMUNISATION TRIAL AT OL PEJETA

J.J. de Castro, T.T. Dolan

Four hundred Boran (*Bos indicus*) heifers, after immunisation against theileriosis, were allocated to 5 groups which were subjected for 12 months to the following tick regimes:

Group 1 — Spraying in coumaphos twice each week.
Group 2 — Spraying once a week.
Group 3 — Spraying once every 14 days.
Group 4 — Application of ear tags (lindane/pyrethrum).
This group is not considered in the analysis.
Group 5 — Ten cattle with no tick control.

All animals were weighed every 2 months. Ten cattle were selected from each group to have all the ticks on one half of the body collected and examined for species and numbers present. Rainfall had a profound influence on tick population and this may be seen in the marked rise in total

Table 2 Results of challenge exposure of rabbits inoculated with soluble antigen from cell lines of *R. appendiculatus*

Rabbit No.	Treatment	Mean engorgement wt (mg) Adult	Larvae	% Egg hatchability	% Larval moult
18	Vaccinated	355.8 ± 85.8	0.48	98	94
19	"	369.1 ± 78.6	0.45	98	95
20	"	308.0 ± 98.1	0.48	96	89
24	Control	269.9 ± 106.7	0.47	98	92
25	"	371.5 ± 64.5	0.53	95	94
26	"	334.7 ± 78.8	0.52	98	99
30	Adjuvant control	239.5 ± 122.5	0.51	95	100
31	"	292.5 ± 73.9	0.52	98	99

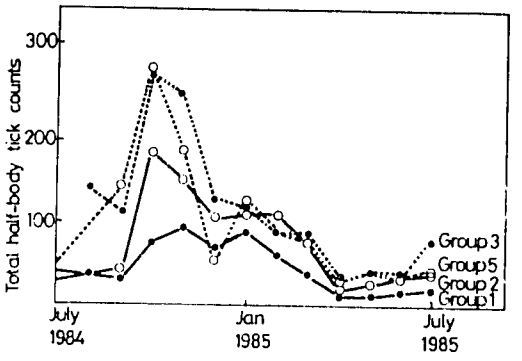


Figure 1. Mean total tick counts per month on 4 groups of experimental cattle at Ol Pejeta ranch

tick burdens on each cattle group following the onset of the rains in late September (Figure 1). *Rhipicephalus appendiculatus* was the predominant tick species collected from the cattle. Low numbers of *Rhipicephalus evertsi* and *Boophilus decoloratus* were also recorded.

The number of standard *R. appendiculatus* females recorded suggests that tick control was very efficient with twice-a-week spraying, except in the period of the second tick rise from December 1984 to February 1985. The tick infestation on Group 5 varied little from that of cattle on a 14-day spraying interval for the first 5 months, but subsequently they appeared to control their tick burdens at a level very similar to cattle sprayed once each week.

Weight gains of the different groups of cattle are presented in Figure 2. No significant differences among groups were recorded at the end of the trial. However, what was of considerable interest was the performance of Group 5. These cattle weights fell to the lowest level in September and March, but ultimately compensated for the loss and

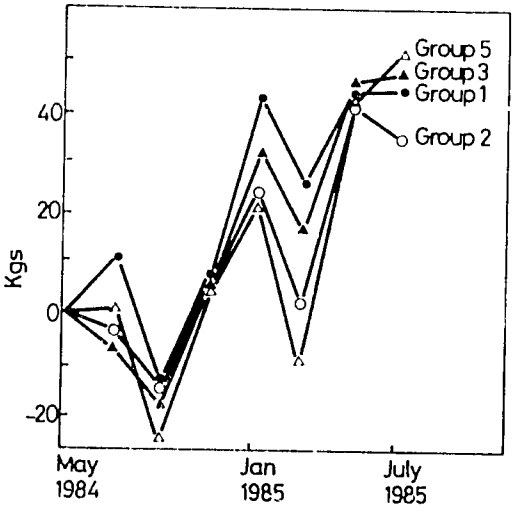


Figure 2. Mean cumulative weight gains for 4 groups of experimental cattle at Ol Pejeta ranch

showed a capacity to control their tick burdens while suffering very few disease problems.

The trial was part of a KARI/the ICIPE collaborative programme, coordinated by Dr. T.T. Dolan of VRD, KARI.

FIELD STUDIES ON NATURAL RESISTANCE TO *RHIPICEPHALUS APPENDICULATUS* IN CATTLE

J.J. de Castro

Following the successful development and use of reliable methods of immunisation against theileriosis, it has been possible to assess the potential use of host tick-resistance as an important component of an integrated approach to the control of ticks and tick-borne diseases.

This report presents our study of the development and establishment of natural tick resistance to *Rhipicephalus appendiculatus* in cattle undergoing a mixed field tick challenge of varied lengths of time. An electrified paddock, with the purpose of excluding alternative wild tick hosts from the cattle pastures, has been erected and the effects of tick-resistant and tick-naive cattle on the natural tick population present in the paddock are being monitored.

The area

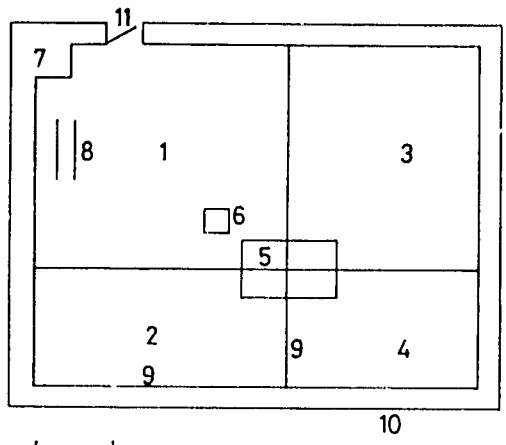
The work is being carried out at Intona Ranch in the Trans-Mara Division of Narok District, Kenya. The area's average altitude is 1800 m and its rainfall, approximately 1300 mm per annum, making it ideally suited to the survival and development of ticks. Several species are present, namely *Amblyomma cohaerens, Amblyomma variegatum, Boophilus decoloratus, R. appendiculatus* and *Rhipicephalus evertsi* which are vectors of anaplasmosis, babesiosis, heartwater and theileriosis. A lethal challenge of 100% due to theileriosis is known to occur.

The trial

A paddock of approximately 32 hectares, conventionally fenced and reinforced by 3 strands of electrified wire, was built and sub-divided into 4 separate sub-paddocks as illustrated in Figure 3.

Boran (*Bos indicus*) cattle from northern Kenya were obtained and shown to be tick-naive on arrival at the farm in May 1984. Ten animals were selected and 3 monthly applications of 500 irradiated adult *R. appendiculatus* were used to reinforce the natural development of tick resistance. A moderate level of resistance was detected in October 1984. The experiment was started by placing 10 resistant cattle in paddocks 1 and 3 and 10 susceptible (naive) animals in paddocks 2 and 4 (Figure 3). The latter are replaced at 3 month intervals to maintain a susceptible animal population in these paddocks. Four groups of 10 susceptible cattle have passed through the paddocks whilst the 10 resistant animals have remained unchanged, except for replacement because of deaths.

A third group of 6 animals has been maintained outside the paddocks under a twice weekly dipping regimen. All animals are weighed every 2 weeks. Tick numbers on the animals are being monitored by half-body counts every 2



Legend

1 and 3 resistant paddocks
2 and 4 susceptible paddocks
5 stockade
6 tent
7 ecology arena
8 crush
9 inner fence and sub-divisions
10 outer fence
11 gate

Figure 3. Layout of experimental paddocks at Intona ranch

weeks and collections every 6 months, and in the pastures by blanket draggings at monthly intervals. Monthly mean half-body counts on the different experimental groups are presented in Figure 4.

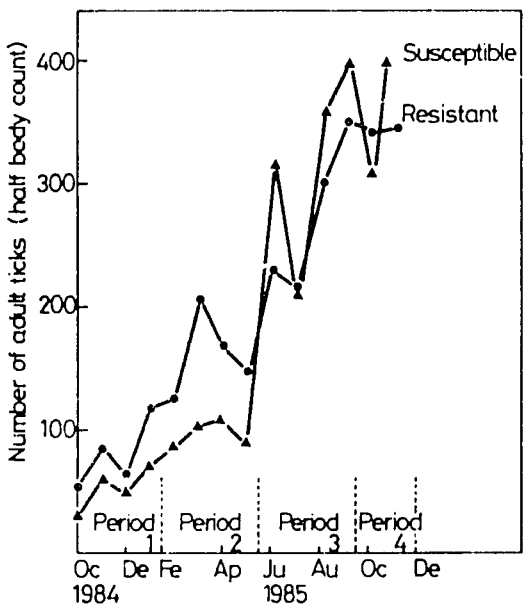


Figure 4. Total tick counts on susceptible and resistant cattle, periods 1 to 4 at Intona ranch.

Because the animals are immunised against theileriosis prior to the introduction in the paddocks, deaths due to this disease have been rare (1 case). However, 13 tick-naive and 2 tick-resistant cattle have succumbed, primarily to tick toxicosis with occasional concurrent heartwater.

The development of natural tick resistance in the animals has been assessed repeatedly by the application of 100 nymphs of *R. appendiculatus* in the ears of the cattle (de Castro, Twelfth Annual Report, 1984). In this way, the tick-resistance status of the animals under continuous challenge has been monitored since May 1984. The mean daily weight gain for the 3 groups of animals is shown in Figure 5. It should be noted that when total tick counts (Figure 4) in the resistant group of animals rose above 300 ticks per animal, daily weight gains fell to 0 and subsequently the animals lost weight daily as the tick counts rose to over 600 per animal. Susceptible animals lost weight steadily throughout the experiment.

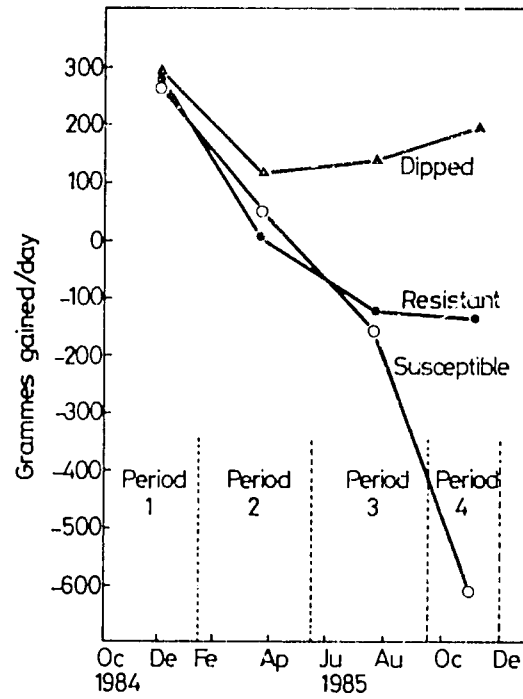


Figure 5. Mean daily weight gains during periods 1 to 4 for three groups of experimental cattle at Intona ranch.

The population of *R. appendiculatus*, *A. cohaerens*, and *A. variegatum*, has risen steadily throughout the entire experiment. *B. decoloratus* have decreased to negligible numbers on the cattle, particularly on the resistant groups.

Conclusion

The experiment is still in progress and it is too early to draw firm conclusions. However, it does appear that under the conditions outlined resistance to *R. appendiculatus*, induced by irradiated ticks and reinforced by natural exposure, is inadequate to control productivity losses from a heavy field tick challenge.

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

J.W. Chiera

This work is a continuation of the investigation reported in the Twelfth Annual Report, 1984, and forms part of a M.Sc. thesis of the University of Nairobi. A second field-derived strain obtained from Ol Pejeta farm in Nanyuki was included in the study. Results obtained with larvae and nymphs of the Ol Pejeta strain were similar to those of the Narok strain. When rabbits were successively infested with adult ticks, it was observed that there was no significant difference in the engorged weight of the laboratory and field strains when fed on susceptible rabbits (first infestation, Table 3). However, during the second and third infestations, the engorged weight of the field-strain females were double those of laboratory-strain females.

Table 3 Mean weights ± S.E. (mg) of females engorging when rabbits were successively infested with 8 adults each of Ol Pejeta strain and Narok strain

Infestation	Lab strain	Ol Pejeta strain
First	363 ± 24	393 ± 33
Second	120 ± 19	273 ± 21
Third	113 ± 24	195 ± 33

Infestation	Lab strain	Narok strain
First	394 ± 23	404 ± 28
Second	100 ± 25	208 ± 36
Third	74 ± 31	160 ± 32

The rabbits infested with adults were then challenged with 100 larvae, 50 nymphs and 10 adults (5 females and 5 males) of the laboratory and field strains. The results of the challenge were similar to those obtained earlier with larvae and nymphs. Ticks fed on heterologous hosts had higher engorged weights than those fed on homologous hosts (Table 4). The engorged weights indicated that the homologous hosts affected the laboratory strain more than they affected the field strains. The weight of the eggs from the females that laid showed twice as much weight was produced on heterologous compared to homologous hosts (Table 5).

These results indicate that the field strains reproduced better than the laboratory strain on previously exposed hosts, and that cross-resistance may be an important factor in immunisation.

RABBIT EAR CANKER AND TICK RESISTANCE

R.M. Newson, F.M. Thuo

All the rabbits used in our studies of host resistance to tick infestation are reared free of ticks, but occasionally during feeding tests to assess resistance a tick-naive control animal gives results indicative of resistance. We have also observed in our commercially-bred rabbits occasional cases of ear canker (caused by infestation with the mite *Psoroptes cuniculi*). We wished, therefore, to check whether undetected, or already cured, *P. cuniculi* infestations could cause cross-resistance to ticks, which are classified in the same order, Acari.

A rabbit with severe canker, and mites readily visible in the scabby material flaking off the ears, was isolated and an unsuccessful attempt was made to transmit the infestation to 2 additional rabbits. The infested animal was then treated by applying medicinal paraffin to the ears and left for 2 weeks for the lesions to heal.

A test feed was done using 20 ♂♂ and 20 ♀♀ of the tick *Rhipicephalus appendiculatus* on one ear and 100 nymphs simultaneously on the other. Identical feeds were done on 2 tick-naive rabbits with no known history or clinical symptoms of canker. The ticks were contained in cotton sleeves which were opened daily so that engorged detached ticks could be removed. All ticks were weighed individually to 0.1 mg and the females were allowed to lay eggs in individual tubes. The egg production of each female was removed and weighed on the tenth day and any additional production on the twentieth. Egg conversion factors (i.e.,

Table 4 Mean weight and number of challenge ticks engorging when applied after 3 infestation of 8 adults each

Challenge ticks	Rabbits infested with Lab strain ticks		Rabbits infested with Ol Pejeta strain ticks	
	No. engorged	wt (mg)	No. engorged	wt (mg)
Lab. larvae	27^{a*}	0.38^{a}	83^{c}	0.47^{b}
Ol Pejeta larvae	65^{bc}	0.51^{c}	58^{b}	0.46^{b}
Lab. nymphs	40^{a}	4.0^{a}	48^{b}	6.7^{b}
Ol Pejeta nymphs	48^{b}	8.9^{c}	43^{a}	6.7^{b}
Lab. females	3.6^{a}	74^{a}	4.0^{a}	193^{bc}
Ol Pejeta females	4.4^{a}	230^{c}	3.8^{a}	144^{b}

* Weights or numbers within each instar not having a common letter are significantly different ($P<0.05$).

Table 5 Mean weight of eggs of challenge females engorging when applied after 3 infestations of 8 adults each

Challenge ticks	Rabbits infested with Lab strain ticks		Rabbits infested with Ol Pejeta strain ticks	
	Number	Wt (mg)	Number	Wt (mg)
Lab females	10	47^{a*}	19	104^{b}
Ol Pejeta females	16	121^{b}	15	67^{a}

* Weights within each strain not having a common letter are significantly different

Table 6 Mean results (± S.D.) of feeding *R. appendiculatus* ticks on rabbits with (+) and without (−) previous ear canker, using 100 nymphs on the left ear and 20 males and 20 females on the right at the same time

Rabbit no.	Ear canker	Ticks used	Mean weight (mg)	Mean egg coversion (%)	Percent engorging	Mean du. ation (days)
1	+	adult	319.10 ± 82.8	56.5 ± 5.1	85	6.7 ± 0.7
		nymphs	7.12 ± 2.14	–	91	4.2 ± 0.3
2	–	adults	346.90 ± 69.8	52.9 ± 7.5	100	7.1 ± 1.1
		nymphs	7.08 ± 1.63	–	98	5.0 ± 0.5
3	–	adults	441.60 ± 101.8	51.4 ± 13.0	100	8.1 ± 1.7
		nymphs	7.62 ± 1.93	–	92	5.6 ± 0.9

the percentage of the weight at detachment converted into eggs) were calculated.

It can be seen from the results given in Table 6 that the rabbit which had ear canker did not differ significantly from the controls in any of the variables considered. Routine preventive treatment of all rabbits on arrival has now eliminated *P. cuniculi* infestations, so it has not been possible to repeat these observations.

PRELIMINARY RESULTS OF A TICK AND LIVESTOCK PRODUCTIVITY SURVEY ON RUSINGA ISLAND

D.K. Punyua

Introduction

Rusinga Island is situated on the eastern edge of Lake Victoria and is separated from Mbita Point by a causeway of some 50 m. The island is at the lake altitude of 1,128 m and rises to a peak of 1,433 m. In 1979 it had a population of 9,905 inhabitants. The mean annual rainfall is 1060 mm per annum. The island soil is principally loam with a gravel or stone surface and tends to be dry. Most of the cultivation and settlement is situated in 1 km strip around the shoreline.

The island has been selected as a suitable site for demonstrating the effects of tick borne disease control and consequent interventions in the tick control practised on the island. Before any intervention in the current situation takes place, a preliminary survey of some 45 farms for tick types and numbers will be carried out. These farms will become the base for a detailed disease survey and a full productivity assessment in 1986. The base-line data thus obtained will be used to assess interventions planned for the future.

Livestock

The livestock population in March 1985 was 6,228 cattle, 1,498 sheep, and 1,377 goats, all of indigenous unimproved stock. The general husbandry practice is that before the harvest of crops from the farms (mainly sorghum and maize) most of the animals are tethered along the roadside or in any of the open fields away from the planted crops. Some large herds, however, are communally herded and grazed. After the harvest the animals are allowed to graze freely.

Materials and Methods

Groups of approximately 5 farmers have been selected randomly from each of the following areas for sampling and questioning: Kakrigu, Siega, Luanda, Kamasengre, Wahemba, Ufira, Kaswanga, Wanyama, Nyamuga, Utajo and Wayagi — these total 45 farms. From each farm 5 adult cattle, 5 calves (below 4 years), 10 sheep and 10 goats have been sampled by whole body collection of ticks which are counted and identified in the laboratory. Each species is recorded as adults, nymphs and larvae.

At the time of tick sampling, further information is obtained by questionnaire on the total number of livestock owned by the farmer, productivity (i.e., milk), reproduction

Table 7 Ticks collected on Rusinga Island

Tick species	Tick stage	Mean no. per host with range		
		Adult cattle (n = 154)	Calves (n = 125)	Goats (n = 200)
R. appendiculatus	Adult	411.8(129–871)	186.2(61–499)	113.0(1–710)
	Nymph	71.2(0–363)	28.5(0–185)	99.3(0–821)
	Larva	31.1(0–209)	7.6(0–65)	11.4(0–77)
R. evertsi	Adult	34.3(6–91)	19.2(2–45)	45.0(4–243)
	Nymph	0.5(0–4)	0.5(0–3)	132.0 (0–9)
	Larva	1.0(0–9)	0.2(0–2)	482.0 (0–4506)
A. variegatum	Adult	147.2(29–385)	82.3(26–230)	4.1(0–23)
	Nymph	154.6(11–385)	60.5(10–141)	47.5(0–126)
	Larva	87.0(0–292)	39.6(0–22)	142.1(0–1118)
B. decoloratus	Adult	16.2(0–82)	4.6(0–20)	0.4(0–4)
	Nymph	5.8(0–76)	3.7(0–23)	0.2(0–2)
	Larva	1.8(0–20)	1.1(0–27)	NIL
H. rufipes	Adult	1.0	1.0	–
R. pulchellus	Adult	3.0	1.0	1.0
A. gemma	Adult	–	1.0	–

rate and market value. The number of dependants supported and the amount of land owned by each farmer is also recorded.

Survey Results

To date, of the identified 45 farms, 40 have been sampled. Thirty-two of the farms have had their tick collections identified and counted. The results are presented in Table 8. In order of prevalence *R. appendiculatus, Amblyomma variegatum, R. evertsi* and *Boophilus decoloratus* are the species of importance. A few isolated specimens of *R. pulchellus, A. gemma* and *Hyalomma rufipes* have been collected.

Apart from a few bushbucks, dik diks, hares, Sykes monkeys and hippos, the island is free from any other wild mammals. The ticks, therefore, appear to feed exclusively on the domestic hosts, since none of the above wild animals could be regarded as important hosts for any of the main tick species. The break down of the animals of each farm is shown in Table 8. It can be seen that there are only small farms on the island and in general these are resource poor.

Table 8. Domestic animal population on Rusinga Island

Species	Sex	Total No. of animals on farms	Mean No. per farm	No. of animals samples	%
Bovine	Male	128	3.2	56	44
	Female	266	6.6	144	54
	Calf	204	5.1	155	76
Sheep	Male	67	1.7	27	40.5
	Female	260	6.5	77	30
	Lamb	124	3.1	39	31
Goat	Male	58	1.5	27	46
	Female	260	6.5	143	55
	Kid	133	3.3	63	47

Preliminary productivity data is given in Table 8 and must be assessed with caution, as it implies an average productive life span of greater than 21 years for female bovines. This data will become more accurate as further detailed animal records accumulate.

Of the 25 families interviewed, there is an average of 12.8 dependants to each family milking an average of 2.0 cows at a time giving approximately 1,500 ml per day. The heifers reach maturity at an average age of 5 years, when they have their first calf. Sheep and goats become pregnant within the first year.

The tick-borne disease survey is planned for early 1986. A full monthly tick survey of 10 selected farms per year will also begin in 1986.

Table 9. Preliminary productivity data - Rusinga Island

Species	Milk yield (ml)	Progeny per animal	Breeding frequency (yrs)	Time to maturity (yrs)
Cattle	718	8.1±2.5	2	4.8±1.2
Sheep	N/A	9.6±1.6	1.6	1.8±0.9
Goats	N/A	9.5±2.3	1.8	1.5±0.6

RESISTANCE TO *R. APPENDICULATUS* INFESTATION IN CATTLE AND TRANSMISSION OF *THEILERIA PARVA* INFECTION

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

This was a joint project with colleagues in the Veterinary Research Department, Kenya Agricultural Research Institute, and this completes the report initially presented in the Twelfth Annual Report, 1984. Our objective was to induce type I resistance to *Rhipicephalus appendiculatus* in Boran (*Bos indicus* type) cattle and then see if the anticipated interference in tick feeding would also reduce the transmission of *Theileria parva* to cattle. The experiment unfortunately had to be terminated in the later stages.

Ten Boran cattle, from an area where both the tick and East Coast Fever (ECF) were absent, were confirmed as serologically negative for *T. parva*. They were then divided at random into 2 equal groups and housed in the laboratory. Group 1 was subjected to test feeds with 100 nymphs of *R. appendiculatus* and did not differ significantly ($P > 0.05$) in percentage fed or in mean engorged weight from the 2 tick-naive rabbits (Table 10). Based on this result, we assumed that the cattle in group 2 were also tick-susceptible.

The group 1 cattle then had feeds of radiation-sterilised ticks on them of 1000 adults and 500 adults (see our 1983 and 1984 Annual Reports), followed by 1000 non-irradiated adults. Test feeds with 100 nymphs were also performed 4 additional times, with the last feed following the third feed of adults (Table 10).

Table 10. Mean results (±S.D.) of 100 nymphal tests on cattle compared with tick-naive control rabbits.

	1st feed (day 0)		5th feed (day 179)	
Hosts	% fed	Wt (mg)	% fed	Wt (mg)
5 cattle	90.2±3.1	8.9±0.4	—*	5.5±0.5
2 rabbits	85.0±1.4	9.4±0.4	89.5±10.6	9.2±0.7

*Ear bags displaced; many ticks lost

Both groups of cattle were then left until nymphs, infected with *T. parva*, that would administer the ECF challenge, were ready for use (100 per bovine). During the intervening period, 3 of the resistant and 2 of the susceptible cattle were allowed to die of various causes (not ECF). Nevertheless, we decided to complete the experiment with the remaining cattle.

Seventy-six days after the last assessment, the cattle were infested with *T. parva* - infected ticks and were monitored for the development of ECF (Table 11). Thirteen days after this feed began a further 2000 nymphs were put to feed on the other ear of each animal in order to ingest any *T. parva* piroplasms present in the blood which would allow transmission of ECF to another host after the adult moult.

In view of the inconclusive results for the tick-susceptible cattle we did not examine the ticks fed on them after they

Table 11. Results of feeding 100 *T. parva*-infected nymphs on resistant and susceptible cattle and tick-naive control rabbits

Animal No.	State	% fed	Mean wt (mg)	Clinical results	% fed 2000 nymphs
229	resistant	30	6.3	no infection; no sero-conversion	10
243	resistant	15	6.7	no infection; no sero-conversion	1
230	susceptible	16	9.0	transient infection; no sero-conversion	75
245	susceptible	47	8.8	no infection; no sero-conversion	*
256	susceptible	43	9.3	died of ECF day 23	95
Control rabbit		61	7.7	—	—
Control rabbit		64	7.3	—	—

*Ear bags displaced; many ticks lost

had moulted, to pick up *T. parva*. The failure of 2/3 of the susceptibles to become infected also cast serious doubts on the infectivity of the ticks, and thus detracted from the apparently favourable outcome with the tick-resistant cattle. There is, however, no doubt that we succeeded in rendering the group 1 cattle resistant, which confirms our finding in the field that *B. indicus* develop resistance more slowly and less markedly (to judge by our test) than do *B. taurus* cattle.

QUANTIFICATION AND CHARACTERISATION OF ANTIGENS FROM SALIVARY GLANDS, REPRODUCTIVE SYSTEM, GUT AND HAEMOLYMPH OF PARTIALLY FED FEMALE TICKS OF *RHIPICEPHALUS APPENDICULATUS*

A.O. Mongi, M.P. Cunningham

The studies undertaken involved immunisation of animals with crude antigen extracts derived from unfed, partially fed or fully fed ticks. Initial information on protein components of *R. appendiculatus* organs producing the antigens responsible for the resistance response against ticks is described in this summary.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) Figure 6) resolved at least 52 protein bands detected by Coomassie Blue staining in the salivary glands, 42 in the reproductive system, 40 in the gut and 41 in the haemolymph. In each of these organs there were several unenumerated indistinct bands. There also appears to be a number of protein bands shared by all 4 tick tissue organs. Although these protein bands may be of similar molecular weight, they need not necessarily have the same primary structure and function.

Immune-precipitation studies were performed using antiserum from a rabbit previously immunised with unfed tick homogenate and with lyophilised homogenate of fully fed female ticks. (Ticks feeding on this rabbit showed reduced reproductive potential). The tick antigen extract used was radiolabelled with ^{35}S methionine and ^{35}S cystein. The selected anti-tick serum immune-precipitated (in the presence of *Staphylococcus aureus*) 9-10 radiolabelled proteins (Figure 7) as analysed by SDS-PAGE. The molecular weights of these proteins were 180,000; 160,000; 140,000; 130,000; 98,000; 92,000; 88,000; 85,000; and 82,000 daltons. The pattern of the specifically immune precipitated proteins was remarkably similar between the tick tissue antigen extracts (Figure 7).

It is hypothesised from these results that the antigen causing the reduced reproductive response in ticks may be one of the recognized antigens. Detailed studies are being carried out, characterising and isolating sufficient quantities of native or subunit proteins for further immunisation trials, using the gut as the selected organ.

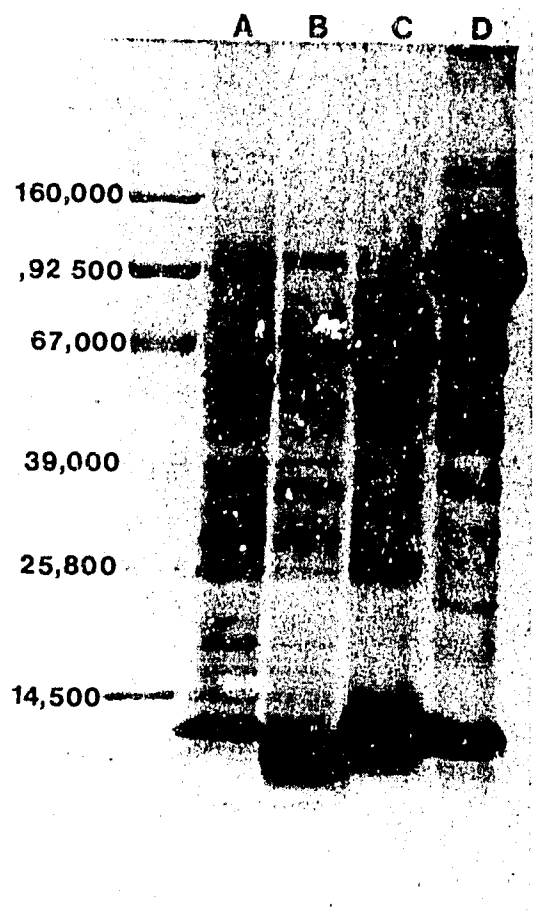


Figure 6. Electrophoretic analysis of tick proteins derived from salivary glands (A), reproductive system (B), gut (C), and haemolymph (D) of 5-day fed female *R. appendiculatus*. Molecular weight marker proteins in daltons are indicated in the margin on the left of the gel: RNA plymerase, 160,000 and 39,000; phosphorylase 'a' 92,000; bovine serum albumin, 67,000; chymotrypsionogen, 25,800 and lysozyme 14,500.

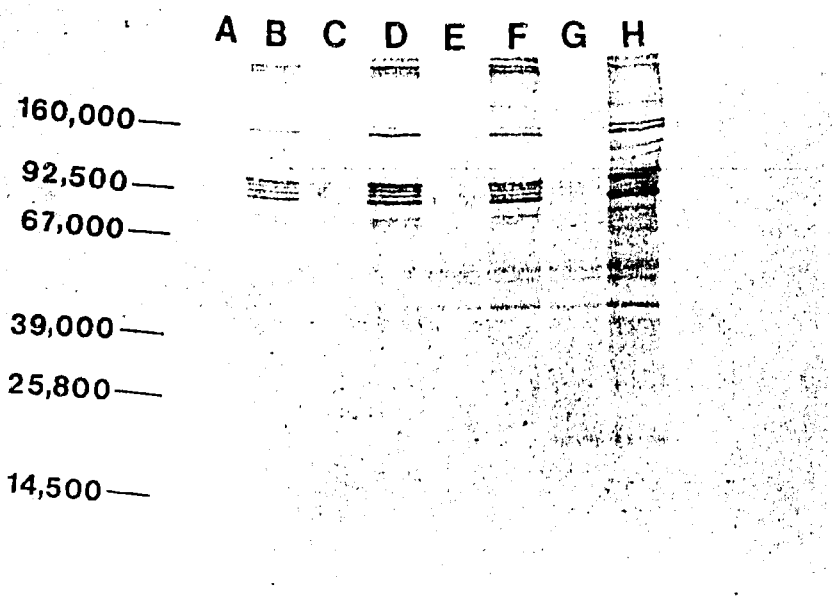


Figure 7. An autoradiograph of immuno-precipitated radiolabelled tick proteins obtained from salivary glands (B), reproductive system (D), gut (F) and haemolymph (H) of 5-day fed female *R. appendiculatus* (see Figure 6). Lanes A, C, E and G are control precipitations while Lanes B, D, F, H, are the specifically immune precipatated polypetides from the corresponding tissues (see Figure 6). Molecular weight markers as in Figure 6

Medical Vectors

Medical Vectors Research Programme

During 1985 the Medical Vectors Research Programme focussed its activities on the epidemiology of leishmaniases in the following endemic areas of the disease:

- *In Baringo focus of both cutaneous (*L. major*) and visceral leishmaniases, epidemiological investigations were extended.*
- *In Mount Elgon a focus of* L. aethiopica, *a high altitude form of cutaneous leishmaniasis, taxonomic investigations on complex species of Phlebotomine sandfly vectors of the disease continued. Vector-parasite investigations on* P. pedifer, *the known vector of* L. aethiopica, *were performed.*
- *In Machakos and Kitui, investigations were carried out on animal reservoirs of the disease and vector bionomics.*
- *In West Pokot District, an endemic focus of visceral leishmaniasis, an initial survey of collaborative work with the Ministry of Health was made to define areas for intensive investigation.*
- *The investigations on host-parasite relationships, parasite identification through isoenzyme technique and vector studies through biochemical and isoenzyme techniques were extended.*
- *A Ph.D. student initiated his work on the ecology of* Anopheles *mosquitoes in Baringo.*
- *The Programme continued its collaboration with CBRU on a natural plant extract which showed potential in the treatment of leishmaniasis.*

Significant achievements made in some of these areas are highlighted in this report.

EPIDEMIOLOGICAL INVESTIGATIONS OF LEISHMANIASES

M.J. Mutinga, C. Matero, C. Kanyo, M. Kyai
D.M. Omogo, J. Mwandawiro, J. Ndambuki

Breeding Sites of Phlebotomine Sandflies

Investigations of the breeding sites of sandflies continued, with special emphasis placed on the vectors of leishmaniasis species in the Baringo focus. Previous investigations had not revealed the breeding sites of *P. duboscqi*, a species which was found restricted mainly to the inside of animal burrows. During these investigations, the breeding sites were elucidated and found to be deep inside the animal burrows, in soil samples associated with rodent nests. The occupants of the burrows were discovered to be gerbils, various species of rats, elephant shrew, mice and porcupines. Table 1 provides a break-down of the various species of phlebotomine sandflies which emerged from soil incubated from rodent burrows in which *P. duboscqi* immature forms were found to thrive.

Soil samples were carefully removed from various animal burrows and placed in rectangular containers with an area of about 660 cm^2. The soil was vigorously agitated and searched for adult flies. The soil was then wetted and covered with fine mesh of cotton netting held tightly by an elastic rubber band to prevent any escape by emerging adult sandflies during incubation. The temperature of the soil ranged from 22°C during the night to 30°C during the day.

The breeding sites for *P. duboscqi* were found to be deep inside the animal burrows, mainly in soil samples which

Table 1. Emergence of *P. duboscqi* and other sandfly species from soil samples excavated from animal burrows

Species	Number	Male	Female	Percent prevalence
P. duboscqi	27	15	12	6.75
P. rodhaini	2	0	2	0.50
P. martini	1	1	0	0.25
S. ingrami	250	134	116	62.50
S. antennatus	111	38	73	27.75
S. schwetzi	3	2	1	0.75
S. bedfordi	3	3	0	0.75
S. africanus	2	0	2	0.50
S. grangeri	1	0	1	0.25

were associated with nesting areas of the occupiers of the animal burrows.

Parasite Isolation and Infectivity

The work of parasite isolation and infectivity of these parasites to various susceptible laboratory animals continued. Parasite isolates from Baringo, Kitui and West Pokot, which were preserved in liquid nitrogen, were revived and multiplied in NNN medium and then injected into hamsters, lizards and Balb-c mice to test their infectivity in these hosts. Their clinical route would give a clue to their tentative identity pending isoenzyme characterisation.

Isolates from *S. garnhami, S. ingrami, P. duboscqi* and *P. martini* caused lesions resembling those of *L. major*. These findings are of tremendous significance; *Phlebotomus duboscqi, P. martini* and *S. ingrami* now confirmed to share niches i.e., animal burrows, and to breed in these burrows were shown by our investigations to be anthropophilic. The potential exists therefore for these species to extensively spread the disease, because of the wide distribution of *P. martini* and *S. ingrami* in arid and semi-arid Eastern Africa.

Mark Release and Recapture Studies

The flight range of various phlebotomine sandflies in the Eastern Africa region has not been studied. Although resting and breeding sites of certain species of sandflies have been established, in the final analysis the vector potential largely depends on how far the flies travel in search of hosts or are attracted by hosts. This investigation was focused to obtain this type of information in Marigat, Baringo District.

Various animals were used as baits including avians, bovides, rodents and canids. They were placed inside cages with wire mesh to allow odour emanating from them to flow freely. Sticky polythene sheets coated with castor oil "THE ICIPE STICKY TRAP" were placed near the animals in the direction of the release sight. As control traps, sticky traps were placed at various distances from the sandfly release sites without animal bait. Other traps were set near human dwellings to assess if the dwellings had influence on the flight direction.

The animals were placed at varying distances from the release site of the phlebotomine sandflies. Flies were captured from natural resting sites, brought into the laboratory, marked with a fluorescent dye and released in the evening near resting sites. Wind direction and temperatures were recorded.

A significant amount of data has been collected and is being analysed. We trust this information will be useful in assessing the vector potential of phlebotomine sandflies of Marigat.

INVESTIGATIONS ON LIZARD LEISHMANIASES

W.S. Yorawi, M.J. Mutinga, J.B. Kaddu, El Wasila

The work on lizard *Leishmaniae* centred on comparative studies of behaviour of lizard isolates, both in mammalian and lizard hosts, and the pathological picture of those animals that took up infection. Investigations were also conducted on natural infectivity of the same species of phlebotomine sandflies reared in the colony at the ICIPE

Materials and Methods

Groups of lizards of the species *Mabuya striata* were interperitoneally inoculated with 3 isolates: The ICIPE L 140 (lizard isolate), *L. major* (human isolate) and *L. adleri* (lizard isolate). The dose given to the lizards was $1 \times 10^7 - 2 \times 10^7$ promastigotes.

The isolates L 140 and *L. adleri* were also inoculated interperitoneally and in the nose and tail of white mice. Each mouse received a dose of approximately 10^7 promastigotes.

The lizards and mice were sacrificed at different definite time intervals. Geimsa-stained smears were made of the various tissues as well as histological sections stained with Haematoxylin and Fiosin (J & E).

Four species of laboratory-bred sandflies were fed on lizard infected with the above isolates, namely *Sergentomyia ingrami, S. schwetzi, S. clydei* and *S. bedfordi*.

Results

The results indicate that:

- L 140, a lizard isolate from Kenya which was characterised at the ICIPE and found to be biochemically identical to *L. major*, acted similarly to *L. major* in the lizard *M. striata*.

- Both L 140 and *L. major* acted differently from *L. adleri*, which is a typical lizard *Leishmania*.

Table 2. Inoculation of mice with *L. adleri* promastigotes

Weeks Post inoculation	Number of mice			% of Mice
	Inoculated	With amastigotes	Dead	With amastigotes
1	10	0	0	0
2	10	3	1	30
3	10	4	3	40
4	10	3	2	30
5	10	0	0	0
6	10	0	0	0
7	10	0	0	0
8	10	0	0	0

Table 3 Inoculation of mice with L. 140 promastigotes

Weeks post-inoculation	NI				IP			
	Inoculated	with amastigotes	Dead	%	Inoculated amastigotes	with	Dead	%
1	5	0	0	0	5	0	0	0
2	5	0	0	0	5	0	0	0
3	5	0	0	0	5	0	0	0
4	5	0	0	0	5	0	0	0
5	5	0	0	0	5	0	0	0
6	5	0	0	0	5	0	0	0
7	5	0	0	0	5	0	0	0
8	5	0	2	5	0	0	0	0
9	5	3	2	60	5	4	2	80
10	5	4	2	80	5	4	3	80
11	5	2	1	40	5	4	1	80
12	5	3	2	60	5	4	1	80

- Infection of L. 140 and *L. major* is a transient nature, while that of *L. adleri* is natural to the lizards.

- *L. adleri* had a transient infectivity to the mice, while L. 140 had more persisting infectivity.
- Promastigotes were seen in the anterior midgut of 2 of the sandflies fed on lizards inoculated with *L. adleri*. One of the flies was *S. schwetzi* and the other was *S. ingrami*.

TAXONOMIC STUDIES BASED ON PHLEBOTOMINE (DIPTERA: PHLEBOTOMINE) SANDFLIES IN LEISHMANIASIS ENDEMIC AREAS OF KENYA

J.M. Rogo, M. Okuki

Studies on the identification of the morphologically indistinguishable female species of *Phlebotomus pedifer* complex (*P. pedifer* and *P. aculeatus*) and also *P. martini* complex (*P. martini, P. celiae* and *P. vansomeranae*) by biochemical and morphological methods were concluded. During the year, the emphasis of our work was on isoelectric focusing of adult specimen and morphological identification of the larvae. These 2 methods complemented previous investigations of the same species by the use of thin layer starch gel electrophoresis to study adults and scanning electron microscope to study their eggs.

Isoelectric Focusing Technique

Investigations using this method were done only on *P. pedifer* complex. Such studies could not be done on the *P. martini* complex because *P. celiae* and *P. vansomeranae* were found in very low numbers in the wild, and eggs laid by *P. martini s.l* females did not give *P. celiae* and *P. vansomeranae* offspring. These 2 species could not therefore be reared in the laboratory. Of the 14 enzymes assayed, 5 (GPI, HK, MDH, ME and PGM) could be resolved in individual sandflies. Only GPI differentiated *P. pedifer* from *P. aculeatus*. *P. pedifer* had only 4 distinct bands and *P. aculeatus* had 6 distinct bands of GPI. The other 4 enzymes which could be resolved (PGM, HK, MDH, and ME) could not differentiate the 2 species.

The Fourth Instar Larval Morphology

The larval morphology of *P. pedifer* and *P. aculeatus* revealed that the larvae of the 2 species are similar except for some minor differences observed in the head. On the ventral side of the head, apparent differences were observed in the position of all the setae. On the dorsal side of *P. aculeatus*, abdominal segments 4-7 lack a lateral seta; these seta are present in the same segments of *P. pedifer*. Four rounded dots were present on abdominal segments of *P. aculeatus* and absent in that of *P. pedifer*. Although the morphology of larvae of *P. martini* was examined, comparisons with *P. vansomeranae* and *P. celiae* could not be done because of the inability of these 2 species to be reared in the laboratory. When the 2 biochemical techniques used to differentiate the vector sibling species were compared, thin layer starch gel electrophoresis separated the 2 species by 4 enzymes (GPI, HK, ICD, and PGM), while isoelectric focusing differentiated the 2 species by only 1 enzyme (GPI). It was further observed that isoelectric focusing technique revealed more isoenzyme bands than in thin layer starch gel technique for PGM and GPI. However, thin layer starch gel electrophoresis was better because it was more sensitive utilizing less enzyme (5μl) than in isoelectric focusing (10μl).

A literature review indicated that these investigations were the first to utilise isoelectric focusing technique in the study of sandfly enzyme. The technique proved to be an additional biochemical method that could be used in the taxonomy of sandflies.

To compare the 4 biochemical methods that have been used in sandfly taxonomy, namely thin layer starch gel electrophoresis, isoelectric focusing (in the present investigations), thick layer starch gel electrophoresis and cellulose acetate thick layer starch gel electrophoresis would perhaps be the best technique because it permits 4 enzymes to be studied in a single specimen. Thus, the thick layer starch gel electrophoresis would be invaluable where sandfly specimens are scarce, as was the case with the *P. martini* complex.

The findings of this investigation demonstrate that biochemical techniques are the most diagnostic and reliable when trying to differentiate between closely related species of sandfly groups. Biochemical techniques make it possible to accurately identify females that are otherwise morphologically indistinguishable and yet are the most important sex in disease transmission.

It was unfortunate that members of *P. martini* were not

conclusively studied, because of the unavailability of an adequate number of specimens. These studies have touched on a small lead: perhaps only *P. vansomeranae* is different, and *P. martini* and *P. celiae* are biochemically similar. And since it was found that *P. vansomeranae* was the rarest in nature of the 3 species, its importance in the transmission of visceral leishmaniasis may be negligible.

With the revelation that *P. aculeatus* is different from *P. pedifer*, it is important to investigate its possible involvement in the transmission of cutaneous leishmaniasis. Thus, this work should be valuable in epidemiological studies to pinpoint the vector of cutaneous leishmaniasis due to *L. aethiopica*.

SCREENING PLANTS NATURAL PRODUCTS AGAINST LEISHMANIA PARASITES

B.N. Odero

During 1985 components of the crude active extract were isolated chromatographically by TLC and HPLC and 3 anti-microbial components were detected and purified. These components are being structurally characterized NMR, UV, IR and MS, after which we intend to study them pharmacologically in animals.

EXPERIMENTAL INFECTION OF SANDFLIES WITH LEISHMANIA

J.B. Kaddu, M.P. Nyamori, R. Muruki

Further investigations into the infectivity of human-infective leishmania to Kenyan sandflies were undertaken, with a view toward pinpointing the vector species, using laboratory-reared sandflies (*Sergentomyia schwetzi, S. antennatus* and *S. ingrami*). Human-derived *Leishmania donovani* and biochemically confirmed *Leishmania aethiopica* isolated from a natural vector *Phlebotomus pedifer* were used.

S. schwetzi, S. antennatus and *S. ingrami* were fed on either hamsters infected with *L. donovani* or defibrinated heat-inactivated hamster blood containing amastigotes of *L. donovani*, a one-to-two-day old cockerel skin membrane. Both the sandflies fed on hamsters and those fed through membranes were maintained in an oven of 22 ± 1°C with the relative humidity fluctuating between 60% and 80%, and offered unlimited supply of sugar (fresh apples or sucrose). They were dissected at various periods ranging from 1 to 12 days post-feeding and examined under a light microscope for presence of leishmania parasites. The results are summarised in Table 4.

Sergentomyia schwetzi and *Leishmania donovani*

Two of the 202 *S. schwetzi* fed on hamsters infected with *L. donovani* and promastigotes in the gut. In one of the flies 5 very actively moving promastigotes per microscopic field (X40 objective lens) were found in the thoracic midgut on day 6 post-feeding. In the second sandfly, very scanty parasitaemia was found in the hindgut on day 7 post-feeding.

Actively moving promastigotes were found in one of 6 *S. schwetzi* fed on blood infected with promastigotes of *L. donovani* through a membrane and dissected on day 6 post-feeding.

Sergentomyia schwetzi and *L. aethiopica*

Of the 30 *S. schwetzi* which fed on blood infected with promastigotes of *L. aethiopica* through a membrane, promastigotes were found in the posterior midgut of 2 sandflies dissected on day 3 post-feeding.

Sergentomyia ingrami and *L. donovani*

Heavy infection with promastigotes was found in the midgut of 2 of the 5 *S. ingrami* on day 4 after feeding on blood infected with *L. aethiopica* through a membrane.

These results establish that Kenyan *L. donovani* can transform from amastigotes into promastigotes in the gut of *S. schwetzi*, and that *L. aethiopica* naturally transmitted by *P. pedifer* can survive in the gut of *S. ingrami*.

FACTORS AFFECTING THE INFECTIVITY OF LEISHMANIA TO SANDFLIES: THE SANDFLY GUT

J.B. Kaddu, M.P. Nyamori, R. Muruki

Pilot studies were performed on the physiological factors of the sandfly which are likely to influence the infectivity of Leishmania in the sandfly gut.

Freshly dissected whole gut or gut parts were incorporated in NNN culture medium containing promastigotes of *Leishmania donovani*. The cultures

Table 4. Artificial infection of sandflies with *Leishmania*

Sandfly Species	Route of infection	*Leishmania* species	Form	Total	Sandflies investigated Unfed	Fed	Dissected -	Dissected +
S. schwetzi	AF	L.d	a	536	321	302	176	2
	MF	L.d	a	25	19	6	5	1
	MF	L.ae	p	175	145	30	25	2
S. antennatus	AF	L.d	a	150	87	63	59	0
S. ingrami	AF	L.d	a	518	229	201	197	0
	MF	L.d	a	89	83	11	11	0
	MF	L.ae	p	38	28	10	8	2

AF = Fed on hamster; MF = Fed through membrane; p = Promastigote; a = Amastigote; L.d = *Leishmania donovani*; L.ae = *Leishmania aethiopica* + = Parasitaemic; - = Aparasitaemic

containing the guts were examined over a period of 20 days for parasitaemia. Preliminary results indicate that:

- *Sergentomyia ingrami* whole gut was without inhibitory effect on the growth of *L. donovani* for 16 days in 3 of 16 cultures.
- Cultures containing *P. guggisbergi* hindgut and also those containing whole guts lost parasitaemia in 3 days after the incorporation.
- Cultures containing *P. guggisbergi* thoracic midgut together with the posterior midgut retained parasitaemia up to day 16 post-incorporation.

These preliminary results indicate that the hindgut of *P. guggisbergi* has anti-*L. donovani* activity.

THE QUANTITY OF BLOOD SUCKED BY SANDFLIES

J.B. Kaddu, M.P. Nyamori, R. Muyodi

Among the factors which influence the infectivity of blood parasites to blood-sucking insects and the subsequent transmission of the parasites is the quantity of the blood sucked.

The quantity of blood sucked by 3 species of laboratory-reared sandflies was determined by weighing the flies before and after feeding the flies on hamsters infected with *Leishmania donovani*. Because large quantities of laboratory-reared sandflies are unavailable in one lot, the work was carried out over a period of time. The information gained from this work is intended to be used in the assessment of the vectorial potential of various species of Kenyan sandflies. The results are summarised in Table 5.

Table 5. The quantity of blood sucked by sandflies

Species	Weight (mg) ± standard error	
	Unfed sandflies	Blood sucked
S. ingrami (584)	0.182±0.034	0.192±0.050
S. antennatus (111)	0.3 2±0.047	0.211±0.034
S. schwetzi (12)	0.185±0.0187	0.247±0.0538

The figures in brackets indicate the total quantities of sandflies investigated.

The results indicate that *S. ingrami* and *S. antennatus* are able to suck more than half their own weight and that *S. schwetzi* is able to suck its own weight in blood.

LEISHMANIA AETHIOPICA IN THE OESOPHAGUS AND PHARYNX OF *PHLEBOTOMUS PEDIFER*

J.B. Kaddu, B.M. Okot-Kotber, M. Chumakwa, M.P. Nyamori, R. Muyodi

Investigations into the infectivity and localisation of leishmania in laboratory-reared sandflies, as a guide to the identification of vectors and potential vectors, requires comparison with known natural leishmania infection. Studies were undertaken to establish the locality of *Leishmania aethiopica* in the mouth parts of *Phlebotomus pedifer* and to relate the infection in the mouth parts to that in the gut.

Sandflies were captured from their natural resting sites at the slopes of Mount Elgon, Western Kenya, using suction tubes. They were dissected and examined under a light microscope for the presence of parasites. Promastigotes isolated from the oesophagus using NNN culture medium were confirmed by one of us to be biochemically indistinguishable from a reference isolate of *L. aethiopica*. The oesophagus and mouth parts were processed and examined under the electron microscope using standard technique. Two of 71 *P. pedifer* which were dissected had parasitaemia. The infection with motile physiologically active promastigotes of *L. aethiopica* was simultaneously spread to the cibarium 'pharynx' oesophagus and midgut. In the pharynx, promastigotes attached to the lining by their flagella (Figure 1) with the body suspended in the

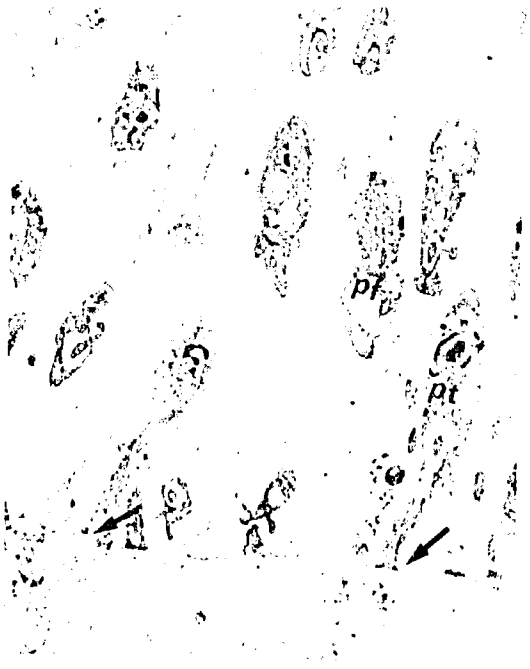


Figure 1 Electron micrograph showing promastigotes of *Leishmania aethiopica* free (pf) in the lumen, and others attached (pt) by their flagella to the lining (arrow) of the pharynx of *Phlebotomus pedifer* x 5775.

lumen. This phenomenon seems to make *P. pedifer* with leishmanial infection in the mouth parts remain infected for life. In turn, the life-long infection in the mouth parts confers a high degree of vectorial capacity to *P. pedifer*.

CHARACTERISATION OF LEISHMANIAL PARASITES BY ISOENZYME TECHNIQUES

B.M. Okot-Kotber, R. Ndauru

The leishmanial parasites which were utilised in the experiments were treated in the following manner:

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 phenyl-methysulphonyl-fluoride, a proteinase inhibitor, to protect proteins from breaking down during processing. This fraction was used for a 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)
Phosphoglucommutase (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 10 selected isoenzyme systems consistently differentiated among strains.

Results

Six lizard strains were found to be unknown profiles needing further investigation. Eleven isolates from phlebotomine sandflies were similarly identified as isolates of unknown profiles needing further investigation. These isolates will now be subjected to further scrutiny i.e., animal infectivity trials and DNA probes, for identification of their specific types.

Tsetse

Tsetse Research Programme

During the last 2 years, the Tsetse Research Programme has focussed attention on the ecology of Glossina pallidipes *in relation to the role it plays as a vector of African trypanosomiasis. The field studies have been centred in 2 study sites (Lambwe Valley in Western Kenya, and Nguruman in the Rift Valley, Kenya). The research has been aimed at examining ways to reduce or eliminate the problem of trypanosomiasis using environmentally-acceptable methods, that can be integrated with other control measures. The approaches employed both in the field and laboratory have been:*

- *Fully involving local community participation in tsetse control and utilising the new low-cost trapping technology.*
- *To obtain sufficient data to develop a tsetse-population model in which costs and benefits of control can be included. A predictive-population model should enable us to simulate the efficacy of various control measures and thus devise optimal control strategies for particular situations.*
- *To study tsetse-population dynamics and the epidemiology of the disease after a tsetse spraying campaign in order to estimate population-growth rates and the effect this has on the disease (trypanosomiasis) situation.*
- *To assess endemicity of African trypanosomiasis, in livestock and in* G. pallidipes *population, under normal conditions and when subjected to chemical insecticide pressure.*
- *To examine the possibility of using tsetse tissue in immunising tsetse-host animals.*

This report highlights some of our major areas of activity during 1985.

TSETSE AND TRYPANOSOMIASIS STUDIES AT NGURUMAN

The intention of the Nguruman tsetse and trypanosomiasis project is to reduce or eliminate the problem of trypanosomiasis in the Nguruman area by developing a participatory community tsetse control programme. Together with the strategic use of chemoprophylaxis and proper resource management, this should increase the general level of livestock productivity. It is intended that the knowledge and expertise gained from this study should then be applied to tsetse - infested areas in other parts of Kenya and neighbouring countries.

The first approach has been to gain a better understanding of the tsetse and trypanosomiasis situation, and also to obtain sufficient data to develop a tsetse - population model. A predictive - population model will enable us to simulate on the computer the efficacy of various control measures and thus devise optimal control strategies for particular situations. As our collaboration with the veterinary research institutes in Kenya increases, the population model will be incorporated into a full epidemiological model in which cost and benefits of control can be included.

The second approach has been to put the emphasis on participatory community control programmes with minimal external import. By fully involving the local community and utilising the new low-cost trapping technology, we believe these problems can be overcome.

TSETSE POPULATION DYNAMICS

Population data analysis

R.D. Dransfield, R. Brightwell

A map of the study area at Nguruman is shown in Figure 1 with the sampling transects and different vegetation types indicated. Cattle, sheep and goats are grazed on the open plains when grass is available, but are moved into the more wooded areas and occasionally up the escarpment during drought conditions.

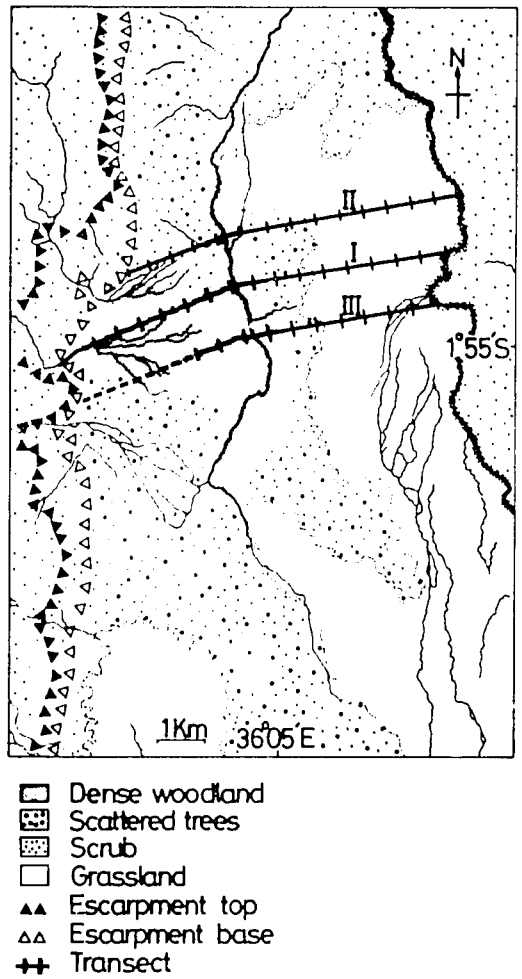


Figure 1. Map of Nguruman study area.

The relative density and population structure of *G. pallidipes* have been monitored since May 1983, and some of the more interesting results of analysis of data collected through September 1985 are presented here. Changes in the relative density of *G. pallidipes* over the period, as indicated by 2 month running means of biconical trap catches, are shown in Figure 2A, together with adult-mortality rates estimated from the age distribution. There is a significant negative correlation (Figure 2B) indicating that adult-survival rate plays a major role in determining population

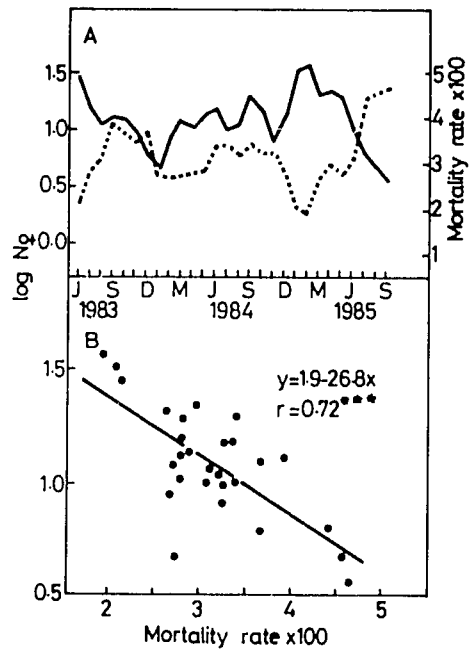


Figure 2 A. Two month running mean catches of female *G. pallidipes* (closed symbols) and mortality rates estimated from two month running totals of numbers in age categories 1-7 (open symbols) over the study period. B. Relationship between log number of female *G. pallidipes* and mortality rate.

change. Deviations from this relationship may indicate when pupal mortality and abortions become significant.

There is evidence that variations in the relations in the relative density result partly from changes in nutritional stress as reflected by the wing-vein length. This changes significantly over time (Figure 3A), and up to April 1985 there was a significant though weak correlation between relative density and wing-vein length. From May 1985 other factors must be responsible for the high adult-mortality rate and consequent low catches.

Analysis over time of the variability within the age categories showed that the numbers in ovarian age category 1 were the most stable. While the OA/OB ratio is positively correlated with adult-mortality rate, there is a significant negative correlation between OA/OB ratio and the OB/1 ratio. These results suggest that density-dependent mortality may be acting to regulate population size at the OB stage.

Monthly changes in the activity of the tsetse were assessed by plotting mean-wing fray against ovarian age (categories OA-3). The slope of the regression lines is an index of activity during the previous month. Such estimates of activity will enable the apparent densities, shown by the biconical traps, to be corrected for changes in activity, an essential step in refinement of a population model.

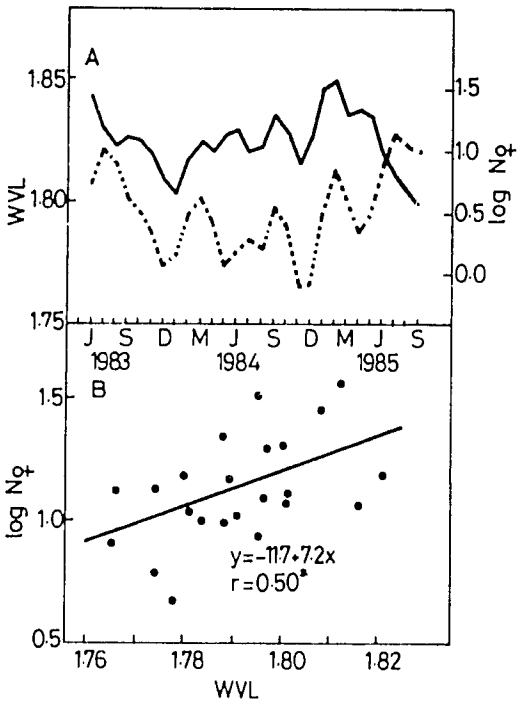


Figure 3 A. Two month running mean catches of female *G. pallidipes* (closed symbols) and two month running means of the wing vein length of female OA and OB flies (open symbols), over the study period. B. Relationship between log number of female *G. pallidipes* and mean wing vein length.

Reproductive biology of *G. pallidipes*

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

For the Nguruman Tsetse Project a multidisciplinary-team approach has been adopted for simultaneous studies on vector biology, population dynamics and trypanosome infection rates. The study reported here contributes to the understanding of the population regulation of *G. pallidipes* at Nguruman by identifying factors and reproductive mechanisms which may be playing a role in regulating the population. Sampling was carried out monthly, initially over 1 transect, and later over 2 additional transects from the Ewaso Ngiro River to the Nguruman escarpment, using biconical traps emptied at intervals of 1½ hrs, 3 hrs, and 24 hrs. Female *G. pallidipes* and *G. longipennis* caught during 1½ hrs, and 3 hrs duration were examined to determine age, insemination rate, fertilization and embryonic development, rate of larval development and occurrence of any reproductive abnormalities. Laboratory experiments with *G. pallidipes* were also conducted to answer questions emerging from the field data.

Project results. Teneral females of *G. pallidipes* (calendar age 1-5 days) examined were uninseminated. More than 90% of the non-teneral nulliparous females (age 6-10 days) were inseminated. Findings from the laboratory studies indicated that there is a direct correlation between insemination and development of the first oocyte. Most of *G. pallidipes* females possessing a developing oocyte measuring 1.5 mm are successfully inseminated both in the laboratory and in the field. Females with smaller oocyte usually resist mating in the laboratory. This phenomenon is peculiar to this species of *Glossina*.

Comparative studies on 4 successive pregnancy cycles of the field-collected *G. pallidipes* showed that the egg from the first cycle was relatively small in about 50% of the females examined. In the laboratory, more than 60% of the females failed to produce a viable progeny during the first pregnancy cycle. In the field we have observed a high rate of abortion during the first cycle. A small egg could be the contributory factor for the missing first cycle in the laboratory-reared females reported by other workers.

The abortion phenomenon could be an important mortality factor affecting the tsetse population. The rate of abortion has been determined for the entire sampling period. The abortion rate was highest in the 2 months preceeding those showing the 2 lowest mean wing vein length, indicating the importance of nutritional stress. The percentage of empty uteri in relation to age of the fly indicates an initial decrease with age, but also suggests that in very old flies the rate may increase again. Since the abortion rate was generally low (<5%), it was thought that abortions might represent only a small fraction of the reproductive loss occurring in the Nguruman population.

At times of stress the small third instar larvae were probably giving rise to small pupae from which non-viable flies would emerge. The mean lengths of the uterine stages (egg to third instar larva) have therefore been measured each month and have been found to follow similar trends to those shown by mean wing vein length over the same period.

Population model

R. Brightwell, R.D. Dransfield

An empirical-cohort model with age structure has been developed. The initial age structure is set by runs of the model using known average survival rates over a period sufficient to give a stable age distribution. The model runs in steps of 1 day when separate survival rates are applied to pupae, tenerals, males and females. Density dependence is introduced by making feeding success and hence survival rate dependent on adult density. Results are calculated at a chosen interval and consist of numbers of each stage as well as the female-age structure. Further model development is underway to incorporate relationships demonstrated by analysis of the field data and to make developmental times dependent on temperature.

Even in its present relatively simple form, runs of the model have demonstrated how sensitive the population is to slight changes in adult female survival rate compared to other survival rates. It is also focusing attention upon areas where data are lacking for the development of a fully predictive population model.

COMMUNITY PARTICIPATION CONTROL PROGRAMME

Odour attractant

R.D. Dransfield, R. Brightwell

Following the successful use by Owaga of buffalo urine to increase catch size, further work has been carried out on the use of cow urine in conjuction with acetone, given the ready availability of cow urine at Nguruman.

Cow urine was dispensed from glass jars with a 12.3 cm diameter aperture, while 3 different dose rates of acetone were used with jar apertures of 0.2 cm, 0.6 cm and 2.2 cm. Very considerable increases over unbaited traps were obtained (Table 1), with even the low dose rate giving a 15.1x increase for males and an 18.7x increase for females. Subsequent experiments have indicated that this index of increase is apparently dependent on temperature, yet even during the cool rainy season an index of 13x was obtained for females. The attractants are not effective if mixed together in the same jar.

Trap development

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

The objective here is to develop a cheaper trap for both *G. pallidipes* and *G. longipennis*, that is easy to construct and can be made locally in the manyattas at Nguruman. Several modifications of the biconical trap and the Zimbabwe F3 trap have been tested, and the latest version (the NG2 trap) has shown promising results. The NG2 trap consists of a modified triangular version of the F3 trap with materials costing about Ksh. 85; it can be constructed using a stapler in about 1½ hours. It was tested baited with cow urine and the medium dose of acetone, against similarly baited biconical traps and an ealier version of the new trap, as well as against an unbaited biconical trap. The results are presented in Table 1B, and show that in the first trial the new trap provided about twice the male catch and 4-5 times the female catch as the baited biconical.

TRYPANOSOME VECTOR INTERACTIONS AND DISEASE EPIZOOTIOLOGY

S.A. Tarimo, P.K. Golder

The role of *G. pallidipes* in the epizootiology of animal trypanosomiasis in Nguruman

Baseline data for the past 2 years on the prevalence of trypanosome infection in *G. pallidipes* have been collected at Nguruman (see the Twelfth Annual Report, 1984). Since June 1984 similar observations on cattle trypanosomiasis have been collected. These parameters, namely, trypanosome infection in the tsetse and in cattle are crucial in attempting to quantify tsetse challenge and trypanosomiasis risk to cattle. Figure 4 summarises trypanosome infections both in tsetse and cattle during the period 1984-1985. Infections in tsetse have been plotted as n + 1 months in order to correspond with infections observed in cattle. Infections due to *T. vivax* and *T. congolense* were observed in both tsetse and cattle. Trypanosome infection rates in flies did not rise beyond 7% during this period; although trypanosome infection rates in cattle were very high (up to 45%).

Table 1 Detransformed mean catches of *G. pallidipes* and indices of attraction for (a) comparison of unbaited biconical trap with biconical traps baited with acetone and cow urine and (b) comparison of baited and unbaited biconical traps with baited NG1 and NG2 traps.

(a) Treatment	Dose rate acetone (mg/h)	Males Mean catch	Index of increase	F	Females Mean catch	Index of increase	F
Control	-	7.9a			12.9a		
CU + low dose acet	150	119.3b	x15.1	277***	241.1b	x18.7	341**
CU + med dose acet	500	136.2b	x17.2		303.6bc	x23.5	
CU + high	2300	146.5b	x18.5		330.3c	x25.6	
(b) Control	-	3.8a			1.7		
Baited	500	47.2b	x12.6		22.8b	x13.4	
Baited NG1	500	71.7c	x19.1		66.4c	x39.1	
Baited NG2A	500	92.3d	x24.6		107.5d	x63.2	
Baited NG2B	500	89.3d	x23.8		105.9d	x62.3	

N.B. NG2A & NG2B are slight modifications of the same trap. Means within one sex are not significantly different if followed by the same letter.

*** P<0.001

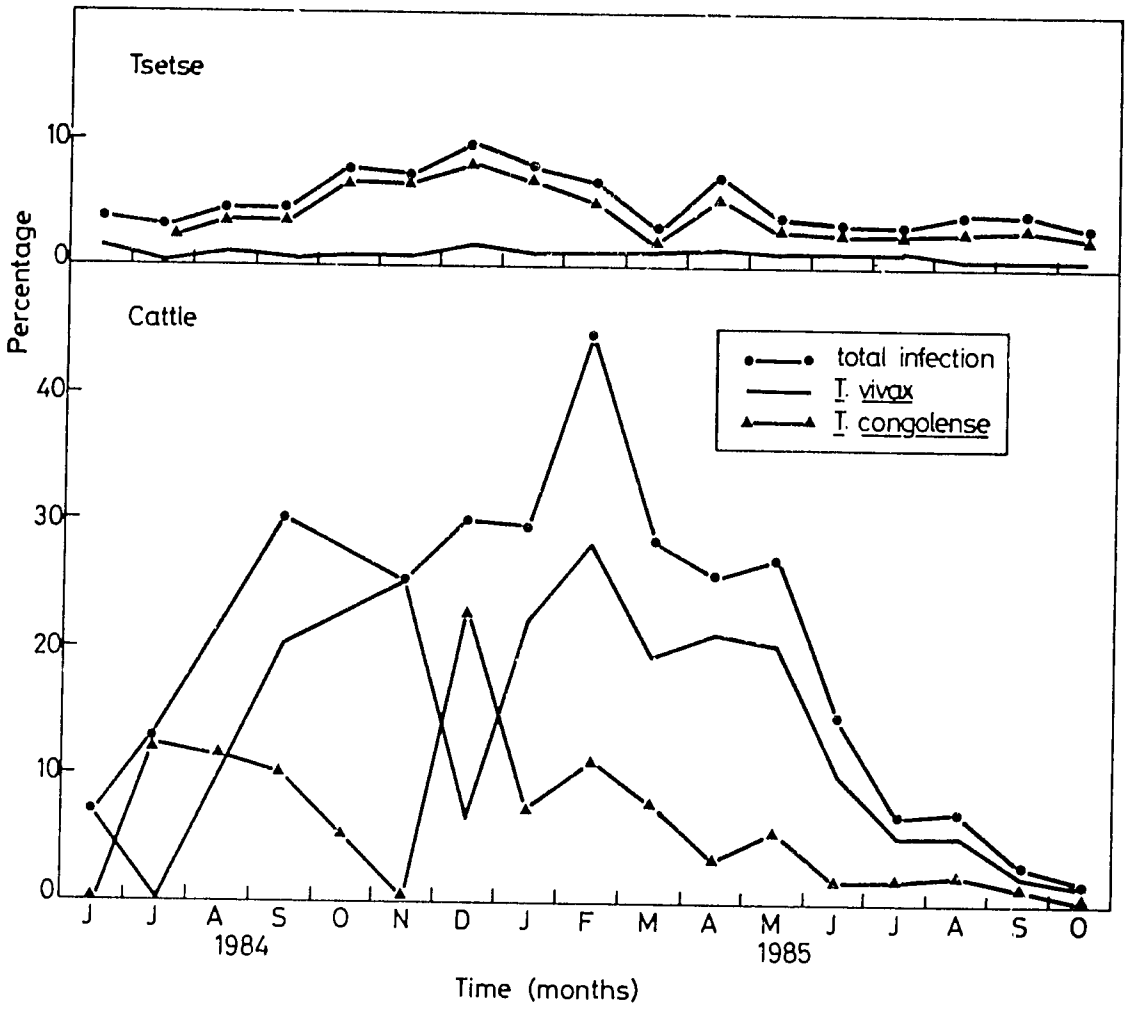


Figure 4. Trypanosome in infected *Glossina palpalis* and cattle at Nguruman

Relative challenge in the different habitats, (river, plains, Acacia woodland, lower woodland, upper woodland and valley) in the study area have been marked out. Although the highest infection rate in tsetse was observed in the plains, the highest relative challenge was observed in the lower woodland because of the high tsetse density.

Trypanosome infection rate and fly activity period

We have also noticed variations in trypanosome-infection rates at different times of the day. Figure 5 shows the total trypanosome infection rate in both males and females captured during the following 3 hourly intervals: 6:30-9:30 a.m (A); 9:30-12:30 (B); 12:30-3:30 (C); and 3:30-6:30 p.m (D). The data show that infection rates were significantly lower in flies caught between 12:30-3:30 p.m. This cannot be explained by diurnal changes in the age distribution, and may reflect feeding behavioural differences between infected and uninfected flies.

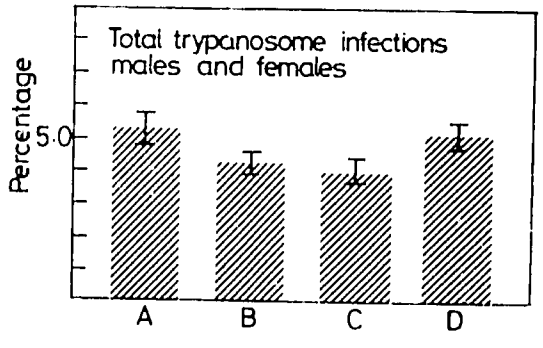


Figure 5. Activity pattern of *G. palpalis* at Nguruman.

THE COINCIDENCE OF VIRUS-LIKE PARTICLE INDUCED SALIVARY GLAND HYPERTROPHY AND *TRYPANOSOMA* INFECTION IN *GLOSSINNA PALLIDIPES* AUSTEN

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

The virus-like particles (VLPs) which cause salivary gland hypertrophy (SGH) in *Glossina pallidipes* have been considered as possible biological-control agents, because of the high incidence of sterility in infected males. It has been suggested that flies with SGH might be particularly well suited for trypanosome infections. Therefore, we have collected a large sample (12,330) of *G. pallidipes* from our study site at Nguruman to elucidate the relationship between VLP and trypanosome-infection rates. Flies were dissected for trypanosome infection, SGH and age determination. The overall trypanosome-infection rate (*T. vivax, T. congolense,* and *T. brucei*) in flies with normal salivary glands (both sexes) was 4.19% (n = 12,174). In flies with SHG, the overall trypanosome-infection rate was 13.46% (n = 156). The difference in trypanosome-infection rate is significant ($X^2 = 27.44$, $P < 0.001$, df = 1) and confirms a previous observation that VLP-infected flies have a higher trypanosome-infection rate than flies with normal salivary glands. If VLP-infested flies are more susceptible to trypanosome infection, they would hardly be suitable biological-control agents. However, the frequency of SHG increases with the age of the fly (Figure 6) as does the trypanosome-infection rate. Thus, the data reflect a coincidence based on age rather than an increased susceptibility to trypanosome infection of VLP-infected flies.

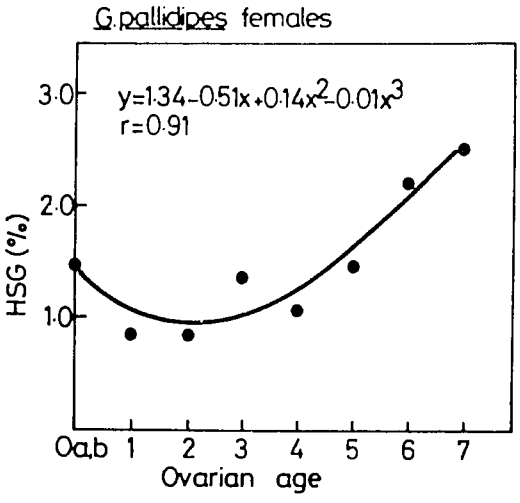


Figure 6. Percent hypetrophied salivary glands (HSG) *Glossina pallidipes* of various ovarian age groups.

TSETSE ECOLOGY IN THE LAMBWE VALLEY

D.A. Turner

Spraying operations were carried out in the Lambwe Valley from November 1984 to April 1985. Field work resumed in July 1985, when the tsetse population began to show signs of recovery, and continues to date. Completed studies, and those which have been resumed or newly initiated, are itemised below.

An analytical approach to determining the effectiveness of the sequential aerial spraying method for tsetse control

From the data obtained in the Lambwe Valley before, during and after the 1981 sequential aerial spraying operation, a predictive model has been developed which attempts to explain survivors of spraying in terms of the number of flies (adults and puparia) present initially and the probable effectiveness of the spray. The model estimated that the aerial spraying was approximately 90% effective in killing adults per application, and that several thousand flies probably remained after spraying. This adequately explained the failure to achieve eradication, even after 9 spray cycles of comparatively heavy dosages of insecticide. Some confirmation that the spray was only 90% effective per application was found in the similarity between observed and predicted times to recovery of the population following spraying.

Studies on pupal ecology

From visual inspection of puparial remains collected from the field, it was estimated that predation accounted for less than 1% (5/540) of the possible causes of mortality of *G. pallidipes* puparia. No evidence was found to indicate parasitism as a cause of death. Further studies are underway to investigate the nature and extent of predation and parasitism of puparia, involving the burial and later exhumation of laboratory-bred puparia in typical breeding sites and at varying densities. Under experimental conditions, very high rates (around 50%) of predation have been recorded with indications that the rates are density-dependent.

Calibration of *G. pallidipes* female ovarian age-graded data with calendar age (in collaboration with the Tsetse Research Laboratory, Langford)

In order to assign accurate calendar ages to female flies age-graded by the ovarian dissection method, it is usually necessary to apply mark-recapture techniques to newly emergent (teneral) flies. This has not been possible in Lambwe for 2 reasons: the bias against tenerals in biconical trap samples, and the lack of opportunity to carry out such studies due to the intervention of spraying operations. A promising alternative method being developed is to compare ovarian age data with estimations of pteridine levels in the eyes of tsetse, which appear to be linearly correlated with age. Toward this purpose, 200 *G. pallidipes* have been age-graded and their heads sent for pteridine analysis at the Tsetse Research Laboratory, Langford. Analyses showed discrepancies in the slopes of regressions of pteridine fluorescence regarding age between field and laboratory-reared flies. Before this method becomes a practical tool for age determination the cause of discrepancies must be found.

INSECTICIDE SUSCEPTIBILITY STUDIES

D.A. Turner, I.K. Golder

The Lambwe valley *G. pallidipes* have been subjected fairly regularly to insecticides over a period of 15 years, including 3 attempts at eradication by spraying. The theoretical expectations for the development of resistance may therefore have been met in this isolated population. We are investigating this possibility by comparing the susceptibility to topical application of dieldrin and endosulfan of wild-caught, non-teneral flies from the Lambwe Valley with flies of the same species from Nguruman which have never been sprayed. Data so far indicate some significant differences with both insecticides in dosage-mortality regression lines and LD50 values between the Lambwe Valley and Nguruman males and females.

G. PALLIDIPES POPULATION DYNAMICS IN RELATION TO THE EPIDEMIOLOGY OF AFRICAN TRYPANOSOMIASIS

I.H. Otieno

The aim of this study was to examine and compare *G. pallidipes* population dynamics and the incidence of both human and animal trypanosomiasis in 2 study areas within and around the Ruma National Park, the Lambwe Valley, South Nyanza, Kenya. It had been suspected that the fly population might spill over and inhabit the surrounding areas of the Park. It was discovered during the course of these studies that the fly population had indeed spilt over and established itself in completely different (ecological conditions) vegetation types, about 5 km from the edge of the Park. A third sampling area was therefore included in this investigation. This area was within the human settlement area with many human activities e.g., cattle grazing, cultivation and water source.

The results of fly-population studies are summarised in Table 2. Fly population has continued to be higher in the Ruma study site (in the Park) than at Riamkanga which is on the edge of the Park. In the third study site (God Jope) within the settled area, it appears that only a small population of flies have managed to establish themselves in the area.

Beginning in November 1984, an insecticide ground-spraying campaign of first cypermethrin and later dieldrin was undertaken by the Kenya Government Ministry of Agriculture and Livestock Development. The spraying operation was interrupted in April 1985 because of heavy rains. The fly population had not been completely suppressed when the operations were suspended. Table 2 summarises the observations made before, during and after the spraying campaign. Of particular interest is the observation that as soon as the spraying campaign was launched *Trypanosoma brucei* virtually disappeared (judging from the flies sampled). It is interesting to note however, that other trypanosome species (*T. congolense* and *T. vivax*) were present throughout this period. We are anxious to see when *T. brucei* will reappear in this population.

CHARACTERISATION OF *T. BRUCEI* STOCKS FROM THE LAMBWE VALLEY BY HUMAN PLASMA RESISTANCE AND ISOENZYME ELECTROPHORESIS

N. Darji, I.H. Otieno

The distribution of genetically different trypanosome populations may affect the prospective control measures, such as immunoprophylaxis and chemotheraphy. Analysis of isoenzyme distribution of *T. brucei* complex has been one of our major objectives in the study of the epidemiology of the African trypanosomiasis in the Lambwe Valley. Since 1983 extensive field studies have been carried out in our programme. Monthly visits to the field sites in the Lambwe Valley, namely Riamakanga and the Ruma Thicket, to collect various geological data for ecology and epidemiology of *G. pallidipes* have been carried out. Infected salivary glands of *G. pallidipes* obtained from the above studies were macerated with buffer and inoculated into mice. Fifty stocks of *T. brucei* were thus collected from June 1983 through June 1984. Out of the 50 stocks tested for

Table 2. Monthly sampling of *G. pallidipes* from study areas - Ruma and Riamakanga and later God Jope in the Lambwe Valley.

	RUMA					RIAMAKANGA					GOD JOPE				
	No. Exam	*T. brucei*	*T. congolense*	*T. vivax*	% infected	No. exam	*T. brucei*	*T. congolense*	*T. vivax*	% infected	No. exam	*T. brucei*	*T. congolense*	*T. vivax*	% infected
Jun. 1984	899	2	20	146	18.7	622	2	7	94	16.6					
Aug. "															
Sept. "	789	5	19	130	19.6	620	2	7	110	15.1					
Oct. "	743	1	24	102	17.1	746	7	22	147	24.6					
Nov. "	123	2	2	23	22.0	1309	11	34	261	24.4					
Dec. "	805	0	16	59	9.4	769	4	8	63	9.8					
Jan. 1985	143	0	3	5	7.1*	18	0	0	1	5.5*	13	0	0	1	7.7
Feb. "	81	0	3	7	13.6*	116	0	0	9	7.8	106	0	1	2	3.8
March "	31	0	2	3	16.1	2	0	0	0	0	58	0	1	3	6.9
April "	STUDY AREAS INACCESSIBLE										16	0	0	2	12.5
May "	19	0	1	7	42.1	15	0	1	2	20.0	4	0	0	0	0
June "	94	0	5	24	30.9	47	0	1	19	42.6	10	0	1	1	20.0
July "	85	0	2	17	22.3	18	0	1	7	44.4	2	0	0	0	0

* Includes 1 fly with gut infection only

Ground spraying of cypermethrin from November 1984 to April 1985 (Ruma and Riamakanga areas only)

Note the disappearance of *T. brucei* following insecticide application

(BIIT), 14 stocks produced positive results, 9 of these stocks were from Riamakanga and 5 were from the Ruma thicket.

Isoenzyme analysis of 30 stocks were separated into 11 zymodemes, with the variations observed in the electrophoretic patterns in 6 out of the 11 examined (Table 3). Zymodemes 1-7 had been previously described in our study (L.H. Otieno and N. Darji, *Trop. Med. Parasitol.* 36, 123-126). Zymodemes 8,9,10 and 11 were new and had the same enzyme profiles as described previously by other workers. Zymodeme 8 was described as ZY 35 by Gibson 1980; 9 was described as ZY 68 by Gibson and Gashumba 1983; 10 was described as ZY 69 by Gibson and Gashumba 1983.

Zymodemes 1,2,3 and 6 are closely related, since they were distinguishable only by different combination of 2 alternative electrophoretic patterns for each of the 2 enzymes (ME and PEP). Zymodeme 4 was previously described as "*T. gambiense*-like"; this stock was also human-plasma resistant. Stocks belonging to zymodemes 1,9 and 10 can be considered to be potentially pathogenic to humans, and may therefore cause *T. rhodesiense* sleeping sickness based on the isoenzyme profiles and the BIIT results.

Conclusions

- Human pathogens are being transmitted by a high proportion of infected flies in the Lambwe Valley.
- Zymodemes 9 and 10 have the same enzyme profiles as the human isolates in Uganda. These parasites are now found in *G. pallidipes* in Western Kenya, showing that there may be an active exchange of strains in these foci, probably by fishermen and others moving within these areas.
- It has recently been suggested, on the basis of indirect evidence, that the genetic exchange is possible in *T. brucei*. If this is true, then zymodemes 1,2,3,6 could simply be hybrids of a parent zymodeme, and that would explain why so many new zymodemes are being isolated in one area.

TSETSE TRAPPING STUDIES

M.L.A. Owaga

Field studies started in mid-1984 to elucidate the effectiveness of buffalo urine as a tsetse olfactory attractant continued. Having confirmed its potency, the emphasis was turned to the identification of the potent components of the urine; necessitating collaboration with the Chemistry and Bioassay Unit.

Our objective was to fractionate urine samples, test the fractions in the field, and on the basis of the field results decide what fraction to analyse further. The intention was to identify the chemical composition of the active components with a view to synthesising it for field use. The test samples were utilised as bait near a biconical trap. The tests were done on a comparative basis using a multiple Latin square design so that the results could be directly comparable. The field studies were done in the Nguruman escarpment. The first 4 fractions tested were LC-I, LC-II, LC-III and LC-IV. The chemical nature of these fractions is explained on page 66 of this report. The fractions were tested against a control (unbaited trap). The results are presented in Table 4. LC-II was analysed further chemically and tested in the field. The field studies were done using non-phenolic and phenolic compounds from this fraction. The results are presented in Table 5.

Table 4 Captures of *G. pallidipes* in biconical traps baited with various fractions of buffalo urine

	Control		LC I		LC-II		LC-III		LC-IV	
	F	M	F	M	F	M	F	M	F	M
	112	91	120	59	899	691	127	108	145	68
	204	81	148	124	984	898	173	247	340	334
Total	316	172	268	182	1893	1589	300	355	485	402
Grand Total	188		450		3482		655		887	

Table 3 Isoenzyme electrophoretic patterns of 8 enzymes (GPI, NAL, MDH, IDH, PEP2) which were invariant

Zymodeme	Stocks	AcAT	ASAT	PGM	ICD	ME	PEP1
1[a]	347	I	I	III	II	I	II
2[a]	137, 103, 341, 876, 37	I	I	III	II	?	I
3[a]	492, 674, 338, 144, 415, 382, 606, 591	I	I	III	II	I	VII
4[a]	922	I	IV	I	II	VIII	VII
6[a]	862, 580	I	V	I	III	VI	II
7[a]	926, 222, 173, 709	I	I	I	II	X	VII
8[b]	171, 448	I	I	II	II	I	VII
9[c]	518, 237	II	I	I	III	I	II
10[d]	13	I	VII	I	II	II	I
11	132, 261	II	V	I	III	II	III

a Zymodemes number same as described previously by Gibson *et al* 1980
b Previously numbered Z35 (Gibson *et al* 1980)
c Previously numbered as Z68 (Gibson and Gashumba 1983)
d Previously numbered as Z69 (Gibson and Gashumba 1983)

Table 5 Captures of *G. palpalis* in traps baited with phenolic and non-phenolic compounds

	Control trap		Baited with LC-II		Baited with LC-IIa, non phenolic		Baited with LC-IIb phenolic	
	F	M	F	M	F	M	F	M
	49	125	312	343	42	108	136	269
	27	44	190	328	42	82	99	138
	10	22	101	92	11	22	82	166
	21	25	165	206	11	16	157	193
Total	107	216	768	969	106	228	474	766
Grand Total	323		1737		334		1240	

Traps baited with LC-II produced catches of *G. palpalis* that were 7 times that of the control, this being 75% of the activity shown by the crude-urine sample. It is evident, that the most active component of buffalo urine is composed of phenolic compounds. Seven of these have been identified so far (see page 66). The ongoing work will determine the relative importance of the various phenols, in contributing to the high activity in the urine. It will also determine the proportions in which they occur in "a typical urine" and the blend effect of each one of them.

PURIFICATION OF TRYPSIN FROM THE POSTERIOR MIDGUT OF *G. M. MORSITANS*

R. Rosenberg, R.M. Vandia, L.H. Otieno*

Otieno et al. (1984) showed that when rabbits were immunised with crude extracts of *G. m. morsitans* midgut proteases, the rabbits produced antibodies which when ingested by tsetse resulted in the failure of the flies to digest their blood meal. Such flies died eventually from indigestion, but mated females which managed to digest the blood meal larviposited premature larvae.

It was the intention of this experiment to find out which particular enzyme, or group of enzymes, was causing the observed effect. We started by examining the role of trypsin, one of the proteases suspected of causing the effect. Isolation and purification of trypsin from the tsetse midgut is described.

Protocol

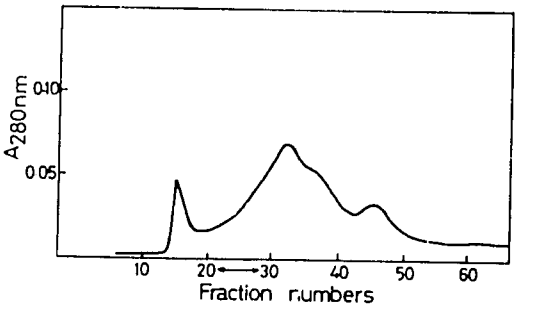
1. The posterior midgut of approximately 250 *G. morsitans* Westwood were homogenised in a minimum volume (2-4 ml) of chilled 0.1 M Tris-acetate buffer, pH 6.0, 0.05 M. NaCl and then sonicated for 2 minutes in ice, using a Headland electronic H60-Z. The homogenate is centrifuged at 20,000 RPM (47,800 RCF) for 15 minutes in the sorvall RC5C centrifuge at 4°C. The equivalent sedimentation effect, as indicated by w^2dt is 3.6×10^9. (This centrifugation does a better job of removing cellular debris than that using the Heraeus, which has lower speed limit). The supernatant is assayed for absorption at 280 nm and for activity towards TAME.

2. The supernatant from the centrifugation is brought to 50% saturation with $(NH_4)_2SO_4$ (299 mg/ml) by shaking until all the $(NH_4)_2SO_4$ is dissolved. The dissolution of $(NH_4)_2SO_4$ is much faster if the salt is finely ground before addition. After standing in ice for 30 minutes, the suspension is centrifuged at 20,000 RPM for 15 minutes at 4°C, the precipitate is discarded, and the supernatant is assayed for A280 and TAME activity.

3. The supernatant from 50% saturated $(NH_4)_2SO_4$ is brought to 80% saturation by the addition of an additional 179 mg $(NH_4)_2SO_4$ per ml of supernatant, with shaking to dissolve all the $(NH_4)_2SO_4$. (It is difficult to see the remaining undissolved $(NH_4)_2SO_4$ because the solution is so opaque). After standing in ice for 30 minutes, the suspension is centrifuged at 20,000 RPM, 15 minutes, at 4°C. The precipitate now contains the trypsin activity, and the supernatant is assayed as a check before it is discarded.

4. The 80% $(NH_4)_2SO_4$ precipitate is dissolved in the minimum volume (about 1 ml) of 0.1 M Tris-acetate buffer, .05M NaCl, pH 6.0, and dialyzed against 200 ml of the same buffer for 4 hours. (An alternative to dialysis is ultrafiltration with addition of the replacement buffer until the total volume of buffer is 200 ml and the final volume of solution is 1 ml). The resulting solution is centrifuged at 20,000 RPM, 15 minutes 4°C to clarify before chromatography.

5. The clarified 1 ml of solution is applied to a Sepharose CL-6B column that has been equilibrated with 0.1 M Tris-acetate buffer, .05 NaCl, pH 6.0 and eluted at 30 ml per hour. The curve of absorption at 280 nm is shown in Figure 7. Fractions 22-33 were found to have activity to TAME (but each experiment should be checked for the location of this activity by assay). The "trypsin" fractions are pooled and concentrated to a volume of 1 ml by ultrafiltration against a buffer, 0.30 M Tris-HCl, pH 7.0, as used by Gooding and Rolseth for DEAE-cellulose chromatography.



6. The 1 ml concentrate of the "trypsin" fraction is applied to a DE-52 column and eluted with a gradient from 0 to 0.30 M HCl in .03 M Tris-HCl, pH 7.0. The peaks of absorption at 280 nm should be assayed for activity

against TAME. The individual fractions with "trypsin" activity can be concentrated and used to produce antibodies.

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SOME ASPECTS OF THE PHYSIOLOGY OF TRYPANOSOME-INFECTED *G.M. MORSITANS*

Y. Chigusa•, L.J. Otieno

Golder et al. reported in 1984 that trypanosome-infected *G. m. morsitans* were more sensitive to endosulfan and pyrethrum. This observation suggested that *G. m. morsitans* with mature salivary gland infection were not as healthy as clean non-infected flies. The studies have now been extended to see if trypanosome infection affects the general physiology of the tsetse.

We have examined the effect of trypanosome infection on the longevity of the fly, fecundity, feeding frequency and the size of a blood meal. The studies are still in progress, but preliminary observations indicate that trypanosome infection (*T. brucei*) has a statistically significant effect on the longevity of infected flies. Whereas clean males lived under laboratory conditions for 76.73 ± 18.01; the infected flies lived for 65.61 ± 14.28. In the case of females there appears to be no apparent difference in the longevity of infected and clean flies. Infected males mated with infected females, produced smaller (24.8 ± 4.7 mg) pupae than clean females mated with infected males (28.4 ± 4.0).

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BARRIERS TO *T. CONGOLENSE* DEVELOPMENT IN *G.M. MORSITANS*

M. Mwangelwa•, L.H. Otieno

In 1982 Distelmans et al. demonstrated that *G. p. palpalis* could not be infected with *T. congolense* in the proventiculus and proboscis after 32 hours of emergence, but older flies (2-25 days old) could be infected in the gut. On the other hand, Gingrich et al. (1982) reported that starvation influenced the transmission rate of *T. b. rhodesiense*; showing that *G. m. morsitans* 21-25 days old could be infected with *T. b. rhodesiense* as readily as in teneral flies.

In our attempts to examine some of the barriers to *T. congolense* infection, we have observed the effect of age and sex of the fly as well as starvation on the transmission rate of *T. congolense*. We have also looked at the effect of ingesting a mixed *T. congolense, T. brucei* blood meal on the resulting *T. congolense* infection.

The following groups of *G. m. morsitans* were fed on rats (at peak parasitaemia) infected with *T. congolense* Maruam strain: 7-day old flies, starved during the last 48 hours; 7-day old flies fed daily; 2-day old flies not fed previously; 2-day old flies fed once prior to infection; and 16 hr old flies not fed previously. After the infected blood meal the flies were maintained on separate rabbits for 30 days after which they were dissected and examined for trypanosome infection. The results showed that mature infections in both males and females were highest among the 16-hr old and 2-day old starved flies (23.8-25.1%) for males and (13.4-16.7%) for females. Seven-day old starved group had the next highest infection rates, 14.1% for males and 8.0% for females.

The number of males and females infected with trypanosomes was observed to change with the course of infection from the gut to the hypopharynx. Females in all the age groups were normally more infected in the gut than males. The pattern of infection between the sexes however, changed in mature infections. Overall infections showed that more males were infected ($X^2 = 14$; $p < 0.001$) than females. It would appear that a larger proportion of trypanosomes in the female gut and labrum failed to reach the hypopharynx.

Mixed infections were carried-out by mixing infected blood from 2 rats, 1 infected with *T. congolense* and 1 with *T. brucei*. Teneral flies were fed in vitro on the mixture; control groups were fed on *T. congolense* and *T. brucei* infected blood separately. Just before killing the rats, clean flies had been fed on them as a further control in assessing the transmission rates. It was later discovered that hypopharyngeal infections in females fed on mixed *T. congolense/ T. brucei* were significantly lower than females which were fed on a meal infected with *T. congolense* alone. Since most of hypopharyngeal infections are attributed to *T. congolense*, it is suggested that the dramatic decline in infection observed in flies fed on mixed infection is due to competition between the 2 trypanosome species.

• *Collaborating Scientist, Department of Zoology, University of Nairobi, Kenya*

G. PALLIDIPES GENETICS

S.A. Tarimo, L.H. Otieno, R.H. Gooding•

G. pallidipes samples were collected from the Lambwe Valley, Western Kenya and Nguruman in the Rift Valley, Kenya and frozen in liquid nitrogen. The frozen samples were analysed by polyacrylamide gel electrophoresis for electrophoretic variation in testicular esterase (Est.t), midgut alkaline phosphatase (Alkph) and 10 thoracic enzymes: glucose-6-phosphate dehydrogenase (G5pd) arginine phosphokinase (Apk); X-glycerophosphate dehydrogenase (XGpd); Xanthine oxidase (X0) octanol dehydrogenase (Odh); aldehyde oxidase (AO); esterase (Est.1); malic dehydrogenase (Mdh); malic enzyme (Me); and tetrazolinm oxidase (To).

G. pallidipes from the ICIPE Mbita Point Field Station colony, Nguruman and the Lambwe Valley samples were examined electrophoretically by comparing allele frequencies and heterozygosity at 12 loci. The banding pattern indicated that the polymorphic loci *AO, AO, Odh* and Est.1 were located on autosomes, Me was probably on the X chromosome. The populations were usually in Hardy-Weinberg Equilibrium. In general *G. pallidipes* from Nguruman were clearly different from those in the Lambwe Valley and MPFS samples with respect to the allele frequencies at Odh, Est.1, XO, and AO. The Nguruman population also showed less genetic variation than was

shown by either of the other populations. Surprisingly, the MPFS colony (which originated from the Lambwe Valley) had a slightly greater mean heterozygosity than the Lambwe Valley population; the 2 populations also differ in the allele frequencies at Est.1.

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INDUCIBLE HUMORAL IMMUNITY IN TSETSE FLIES, *GLOSSINA MORSITANS MORSITANS*

G.P. Kaaya

Tsetse flies, the natural vectors of human and animal trypanosomiasis, have been shown to have unusually low infection rates by trypanosomes both in the field and in the laboratory. This low infection rate is of importance in the epidemiology of African trypanosomiasis and reflects the presence of an efficient defence mechanism within the fly. In order to acquire a better understanding of tsetse defence mechanisms, we decided to start our investigations using bacteria as inducing agents.

Injection of live bacteria, e.g., *Escherichia coli* or *Enterobacter cloanae* induced a strong antibacterial activity in the tsetse starting from 6 hrs and reaching peak 24-48 hrs post-injection, as demonstrated by zone-inhibition assay in soft agar seeded with bacteria. Higher doses induced production of higher titres of antibacterial activity; the activity declined only when the amount of bacteria had dropped to low levels. Injection of heat-killed bacteria failed to induce production of the antibacterial activity. Simultaneous electrophoresis of Cecropia and tsetse-immune haemolymph in acidic gels, which were subsequently overlaid with agar seeded with bacteria, revealed that tsetse, like Cecropia and other lepidopterans, produces 2 families of antibacterial proteins, namely attacins and cecropins.

Injection of cycloheximide, a known inhibitor of protein synthesis in eukaryotic cells inhibited production of the antibacterial activity completely when injected at the time of bacterial inoculation, thus proving *de novo* synthesis of the antibacterial activity. Lysozyme, a bacterial enzyme, was also produced in the haemolymph following bacterial injections, as demonstrated with *Micrococcus luteus* plates. Peak activity, however, was reached 1-2 hrs post-bacterial injection and was not inhibited by cycloheximide, suggesting release from storage sources. Primary and secondary injections of *E. coli* into tsetse induced 2 peaks of antibacterial activity and lysozyme, not characteristic of anamnaestic (memory) response.

Different mutants of *Bacillus thuringiensis* were among the few species of bacteria found to be resistant to tsetse immune haemolymph. Purified inhibitor-A of *B. thuringiensis*, which is known to inactivate both attacins and cecropins of *Hyalophora cecropia* also inactivated tsetse immune haemolymph. When In-A diluti[illegible].0 was incubated 1:1 with whole haemolymph at 37°C, complete inactivation occurred within 5 minutes.

SOME PROPERTIES OF A NON-OCCLUDED DNA VIRUS ISOLATED FROM THE TSETSE FLY *GLOSSINA PALLIDIPES*

M.O. Odindo, C.C. Payne N.E. Crook*, P. Jarret*

Enlarged salivary glands from *Glossina pallidipes* were triturated with TK buffer and purified through differential centrifugation, and in continuous sucrose gradients. Virus preparations were examined in a JEOL JEM 100S electron microscope after negative staining with saturated aqueous uranyl acetate or 2% potassium phosphotungstate. Particle sizes were measured in comparison with the crystalline lattice of catalase photographed at the same magnification.

The virus particles were rod-shaped, 57 nm wide by 700 to 1300 nm long. Concentration of protein was measured by the Folin test using bovine serum albumin as a standard and polypeptide mol. wts. determined by comparison with the electrophoretic mobilities of marker proteins on SDS-polyacryamide gel slabs. Virions contained at least 12 polypeptides, the major component having a mol. wt. of 39,000.

Presence of the nucleic acid was tested by Diphenylamine (DNA) or Orcinol (RNA). The viral DNA was analysed by ethidium bromide -- caesium chloride gradient centrifugation. The electrophoretic mobility of viral DNA was compared with the mobilities of covalently -- closed and linear DNA from an acrystalliferous mutant of the HD1 strain of *Bacillus thuringiensis*. For electron microscopy studies, the viral DNA in NaCl - EDTA buffer was spread on ANALAR water, rotary shadowed with gold-pallidium and the grids examined in a JEOL JEM 100S microscope. The size of the viral DNA was compared with that of relaxed circular molecules of PM2 DNA. The virus contained double-stranded DNA. The molecules appeared to be linear and heterogenous in size.

The tsetse virus therefore superficially resembles the baculoviruses of insects. However, they lack the proteinaceous sheath and apparently do not have any viral envelope. Furthermore, the virus particles are much longer than baculovirus nucleaocapsids. These results suggest that the *G. pallidipes* viruses cannot be placed in any of the existing taxonomic grouping of insect DNA viruses.

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CATION EXCHANGE CHROMATOGRAPHY OF BLOODSTREAM *T. BRUCEI* INFECTIVITY TO *GLOSSINA MORSITANS* AND VARIANT ANTIGEN TYPE

*T.K. Golder, N. Darji, M. Honigberg**

Bloodstream froms of *T. brucei*, at peak parasitemia, display a high degree of polymorphism. The parasites vary from long slender forms (LSF) to short stumpy forms (SSF) and often a substantial population of intermediate short stumpy forms (ISS). When such a mixture of forms is passed through cation exchange resin (carboxymethyl cellulose) at an appropriate pH and ionic strength, some of the parasites elute rapidly (peak 1) while the rest remain bound to the column. The bound parasites elute (peak 2) when the pH and ionic strength of the elution buffer are

raised (Eleventh Annual Report, 1983). The population of peak 2 is enriched for SSF, which are thought to be preadapted for survival in the tsetse. Thus, we predicted that peak 2 parasites would be more infective for tsetse flies than peak 1 parasites. We tested this hypothesis by separating these forms, resuspending them in defibrinated blood and feeding them via silicone membrane to newly emerged male *G. morsitans morsitans*. Infected flies were dissected 3 to 4 weeks post-feeding and examined for the presence of trypanosomes. The data are summarized below.

	No flies	Gut	Proboscis	Salivary Gland	S G / Gut Ratio
Peak 1	169	39.1%	7.1%	5.3%	13.6%(9) 66
Peak 2	196	42.4% NS	20.4%***	18.4%***	43.7%, 36** NS

These results show that there is a significant difference in infectivity of the parasites in peak 2 in both the salivary gland and proboscis infections. Yates Chi squared analysis of the differences are salivary gland, $X^2 = 13.099$ (df = 1), $P < 0.001$; proboscis, $X^2 = 12.09$ (df = 1), $P < 0.001$; and gut, $X^2 = 0.28$ (df = 1), $P < 0.5$, N.S. It is interesting to note that peaks 1 and 2 exhibit no difference in the ability to establish a gut infection in the fly. This would argue against the idea that the experimental procedure had somehow damaged the parasites in peak 1. Also interesting is the data on salivary gland infection and gut infection ratio. In flies fed on peak 1 parasites, 13.6% of the gut-infected flies show mature salivary gland infections. This compares with 43.7% in flies fed on peak 2 parasites. The difference is significant, $X^2 = 8.6$ (df = 1), $P < 0.01$. These data suggest that there may be a trypanosome factor involved in establishing mature infections in *G. morsitans* and that the major barrier to infection may not be the gut.

The results presented above reveal that our method of cation-exchange chromatography yields 2 populations of parasites, 1 of which is highly infective for tsetse flies. Since trypanosomes of different variant antigen types (VATs) display, among other attributes, differences in growth rates, surface sugar residues and infectivity of laboratory rodents, it was of interest to define the relationship between the VATs and our other previous experimental results.

The primary aim of the following experiments was to ascertain whether there existed any relationship between the 2 fractions of bloodstream-trypanosomes and VAT. This aim necessitated the employment of a homogenous (homoVAT) population. We therefore obtained for 3 experiments a fast growing, highly pleomorphic, clone homoVAT strain of *T. brucei* (ILTat 3). An anti-ILTat 3 serum was then produced in New Zealand white rabbits. Each of 2 rabbits received an i.v. injection of 10^7 live bloodstream forms from a parasitemia less than 72-hrs old. The rabbits were bled terminally from the heart on the sixth day post-inoculation. This inoculation route and time of bleeding commonly used for the indirect fluorescent antibody (IFAT) as suggested by Van Meirenne et al. (1985), *Ann. Soc. Belg. Med. Trop. 55*, 1. This method was employed in all IFATs performed in the course of this study. These tests were designed to analyse the antigenic heterogeneity of the trypanosome fractions obtained from the CM-52 column at various days post-inoculation.

The results of the experimental series are shown in Tables 6 and 7. White Swiss mice, immunosupressed by exposure to 600 rads, were used in the first experimental series while nonirradiated mice of the same outbred "strain" were employed in the second experimental series. Parasites obtained at the intervals shown were separated into peak 1 and peak 2 populations by cation-exchange chromatography. They were then smeared on slides for Giemsa staining and prepared for IFAT.

Inspection of the data in Tables 6 and 7 show that the peaks obtained by cation-exchange chromatography are not the result of the appearance of new VATs. The fluorescence data illustrate this lack of relationship very clearly. Note that on day 5 of parasitemia there are approximately equal ratios of original VAT and new VAT(s) in both peak 1 and 2.

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Table 6. Results of experimental series No. 1*

Day of parasitaemia	FLUORESCENCE								GIEMSA-STAINED FILMS							
	PEAK 1				PEAK 2				BEFORE SEPARATION				PEAK 1			
	LS[a]		NLS[b]		LS		NLS		LS	ISS[c]	SS[d]	LS	ISS	SS	LS	ISS	SS
	+		+		+		-									
3	ALL PARASITES HOMOVATS				ALL PARASITES HOMOVATS				968 94.1%	54 5.2%	7 0.7%	957 93.3%	35 3.4%	34 3.3%	771 76.8%	165 16.4%
5	275 26.4%	285 27.3%	473 45.3%	10 1%	30 7.1%	0 0	390 92.9%	0 0	643 55.4%	215 18.5%	302 26.1%	403 37%	343 32.1%	322 30.2%	88 15.4%	64 11.2%
9	ALL PARASITES HETEROVATS				0	5 1%	0	495 99%	285 25%	386 34.6%	455 40.4%	613 59%	337 32.5%	88 8.5%	60 5.4%	472 42.8%

Tables 6 and 7

* Infectivity rates to tsetse flies, which was to constitute an integral part of both experimental series, failed to yield meaningful results because of the paucity of flies and their low viability.

[a] LS refers to long slender trypanosomes,

[b] NLS refers to forms which were not long slender,

[c] ISS refers to intermediate short stumpy forms and

[d] SS refers to short stumpy forms

+ means positive fluorescence and denotes the presence of the original VAT.

- refers to those parasites which did not fluoresce and indicates the presence of a new VAT.

Table 7 Results of experimental series No 1*

Day of parasitaemia		FLUORESCENCE						GIEMSA-STAINED FILMS								
		PEAK 1			PEAK 2			BEFORE SEPARATION			PEAK 1			PEAK 2		
		LS**	NLS*		LS	NLS		LS	ISS**	SS*	LS	ISS	SS	LS	ISS	SS
	+		+	+		+										
3	86	87	226	97	TOO FEW TO COUNT		607	247	179	421	427	209	TOO FEW TO COUNT			
		17%	19.2%	46.6%	19.2%			58.8%	23.9%	17.3%	39.8%	40.4%	19.8%			
5	146	66	151	199	25	26	171	175	270	300	468	400	239	425	108	310	196
	26%	11.7%	26.8%	35.4%	6.3%	6.5%	43.1%	44.1%	26%	28.9%	45.1%	21.3%	32.3%	46.4%	17.6%	50.8%	31.9%
11	ALL PARASITES HETEROXENIS				ALL PARASITES HETEROXENIS			429	134	482	622	218	164	288	138	261
								42.3%	13.2%	44.4%	61.9%	21.7%	16.3%	36.1%	22.1%	41.8%

For foot notes see Table 6

CATION-EXCHANGE CHROMATOGRAPHY OF BLOOD-STREAM FORM OF *T. BRUCEI*

I. Irungu

Attempts to standardise the separation technique

Although we are able to separate *T. brucei* into 2 populations by cationic chromatography, there has been a great deal of variation in the size of the peaks, particularly peak 2 which in some cases is very small. This has led to a deficiency of peak 2 trypanosomes available for infecting experiments. We felt it necessary to investigate the sources of this variability in order to standardise the technique, enabling us to predict the size of peak 1 and peak 2.

All the elution profiles gave varying heights of peak 1 and peak 2 despite making the above alterations. Peak 1 parasites were predominantly S; while peak 2 were enriched for intermediate and short stumpy forms. It was concluded that the varying heights of the peaks were largely due to the manner and rate at which ionisable groups on the cell surface of the different trypanosomes populations were ionising with changes in pH and ionic strength. They do not all ionise simultaneous, giving rise to different populations of long slender, intermediate and short stumpy forms with either positive or negative charges, hence the variance in the peak size.

Separations using monomorphic strains of *T. brucei* and subsequent infection of *G. morsitans* on peak 1 and 2 trypanosomes

Two monomorphic strains of *T. brucei* were investigated, *T. brucei* 1416 and *T. brucei* 052. Rats were infected with strains and the blood passed through the DE52 and CM52 columns. In 6 separations *T. brucei* 1416 gave 2 peaks, peak 1 being larger than peak 2. Examination of the morphology of these 2 peaks showed that both consisted entirely of long forms. In 3 of these separations, *G. morsitans* flies were fed on peak 1 and peak 2 strains and on blood from the infected rats; this acted as a control. Of 188 flies fed on rat blood, none were infected. Similarly of the 87 flies fed on blood infected with peak 1 trypanosomes and 60 on blood infected with peak trypanosomes, none became infected.

Four separations of *T. brucei* 052 were carried out and in 2 of these separations, flies were infected with peak 1 and 2 trypanosomes including blood from the rat which acted as a control. As in the previous strain, 2 peaks were obtained and peak 1 and 2 trypanosomes were all found to be long slender forms. Eleven percent of the flies fed on rat blood had infections while 10% and 5% of flies fed on peak 1 and 2 infected blood had gut infections, none being found in the proboscis, or salivary glands. These results suggest that the separation of trypanosomes into 2 peaks on the CM 52 column is mainly due to differences in the ionisable groups on the cell surface of the trypanosome and not on the morphology.

Chemistry and Bioassay

Chemistry and Bioassay Research Unit

The principal activities of the Chemistry and Bioassay Unit have been directed towards the implementation of the current strategic plan. The installation of VG 12-250 quadrupole gas chromatograph-mass spectrometer during the last quarter of 1984 meant that chemical work involving volatiles could be more vigorously pursued. The identification of both tsetse attractants from buffalo urine, and a number of dinolophenolic compounds from the brown ear tick were made feasible by acquisition of this facility. Progress has been made as well in the identification of the sex pheromone components of the African biotype of Chilo partellus.

The major focus of our collaborative research with the Livestock Ticks Programme has shifted to gut antigens, and the disruption of the mid-gut wall and digestion of being the primary objective. An array of new instruments now available for protein work include a Pharmacia FPLC, a low temperature freezer, a Sorval high-speed centrifuge and a Beckman DU-50 spectrophotometer. Various smaller items of equipment specifically designed for biochemical and immunological work, are on order and 1986 will see a well equipped biochemical facility within the Unit.

The Unit's first ARPPIS student began laboratory work in October. His research, on sorghum allelochemics affecting feeding and oviposition of Chilo partellus, *represents a part of the growing collaborative activities between the Unit and the Crop Pests Programme. The arrival in late 1985 of an RI-detector for the HPLC instrument means that a number of non-chromophoric allelochemics encountered in cowpea and sorghum can be monitored chromatographically with greater ease. It is therefore anticipated that this area of the Unit's research will show faster progress during the coming year.*

A SYNOPSIS OF THE UNIT'S MAJOR ACCOMPLISHMENTS

Protein biochemistry

A semi-purified carboxyl proteinase isolated from partially engorged female *R. appendiculatus* ticks was shown to have a molecular weight of 25,000 and an isoelectric point of 6.5. Anti-serum to the enzyme inhibited the enzyme activity in vitro. Current in vivo tests may demonstrate the potential of the enzyme for disruption of tick digestion.

The reproductive potential of *R. appendiculatus* females, fed on rabbits immunised with purified *R. appendiculatus* vitellin, was found to be unaffected by the ingested immunoglobulins. Low permeability of the mid-gut to the globulins may be an important factor. Specific attention is being paid to the screening of gut proteins in the hope of identifying antigens that could be used to raise antibodies which may help in disrupting the gut wall and in changing its permeability.

Allelochemical research

Progress has been made in our work on the feeding deterrents from a *Maruca*-resistant variety of cowpea, TVu 946. A mildly active crystalline fraction was shown to be a mixture of phytosterols; isolation of the compounds from more active fractions is underway.

We have demonstrated that the oviposition of sorghum shootfly on a susceptible sorghum cultivar is the result of a mixture of polar and non-polar compounds. The polar fraction has been identified as a mixture of glucose and sucrose. Studies to identify the non-polar components are in progress.

Pheromone and kairomone research

In addition to 2,6-dichlorophenol, reported last year, 2-bromo-6-chlorophenol and 2,6-dibromophenol have been shown to be present in extracts of the tick *R. appendiculatus*. The role of these compounds in the sexual behaviour of the ticks remains to be elucidated.

A chromatographic fraction of dichloromethane extract of buffalo urine, demonstrated to be potent in increasing tsetse catches in the field, was found to be a blend of 7 simple phenols. Of these, 3-n-prophylphenol appears to be crucial fo. the activity of the blend. We anticipate that the relative importance of the other phenols and their proportions will be made clear from current field tests.

Anti-insect compounds from tropical plants

A potent mosquito larvicide from *Spilanthes mauritiana* was identified as dodeca-2,4,8,10-(E)-(E)-(E)-(Z)-tetraen N-isobutylamide. In addition to plumbagin, reported last year, a number of other naphthoquinones have shown mosquito larvicidal activity. Crude extracts of plants containing these larvicides may prove to have potential in small-scale mosquito-control programmes.

A novel tetranortriterpenoid isolated from *Harrisonia abyssinica* has been shown to be very effective in inhibiting feeding in *Eldana saccharina*. We are performing assays on other insects and details of this work will be presented in the 1986 Annual Report.

Lignocellulose project

Species of *Termitomyces* associated with *Macrotermes michaelseni* and *Odontotermes ssp.* have been successfully cultured in the laboratory and shown to produce significant quantities of laccase, one of the enzymes implicated in lignin degradation. During the coming year, the project will focus on screening *Termitomyces* and other fungi, isolated earlier from the fungus combs, for their ability to selectively breakdown lignin and demask cellulose from lignocellulosic materials.

DIGESTIVE ENZYMES OF THE BROWN EAR TICK, *RHIPICEPHALUS APPENDICULATUS* NEUMAN INHIBITION OF THE GUT CARBOXYL PROTEINASE BY ANTISERUM TO THE ENZYME

R.M.W. Vundla, V.L. Tabong'o

In our ongoing work on the digestive enzymes of *R. appendiculatus* we have partially purified a carboxyl proteinase on Sephadex G100. The enzyme had a molecular weight (M.Wt.) of 25,000 and an isoelectric point (pI) of 6.5. Anti-serum to the semi-purified enzyme inhibited the enzyme activity in vitro, but had no effect on the activity of commercial bovine cathepsin D.

Partial purification of the carboxyl proteinase

Midguts were dissected from partially engorged (day 5) female ticks in chilled (4°C) 1% NaCl solution, washed several times in the same solution, and homogenised in 2×10^{-2}M phosphate buffer pH 7.0, containing 2×10^{-1}M NaCl and 0.5% triton X-100. After autolysis for 2 hours at 4°C, the homogenate was centrifuged for 20 minutes at 6,200 g. The clear supernatant was chromatographed on a 90×1.6 cm sephadex G100 column equilibrated with 2×10^{-2}M phosphate buffer pH 7.8 containing 2×10^{-1}M NaCl. The

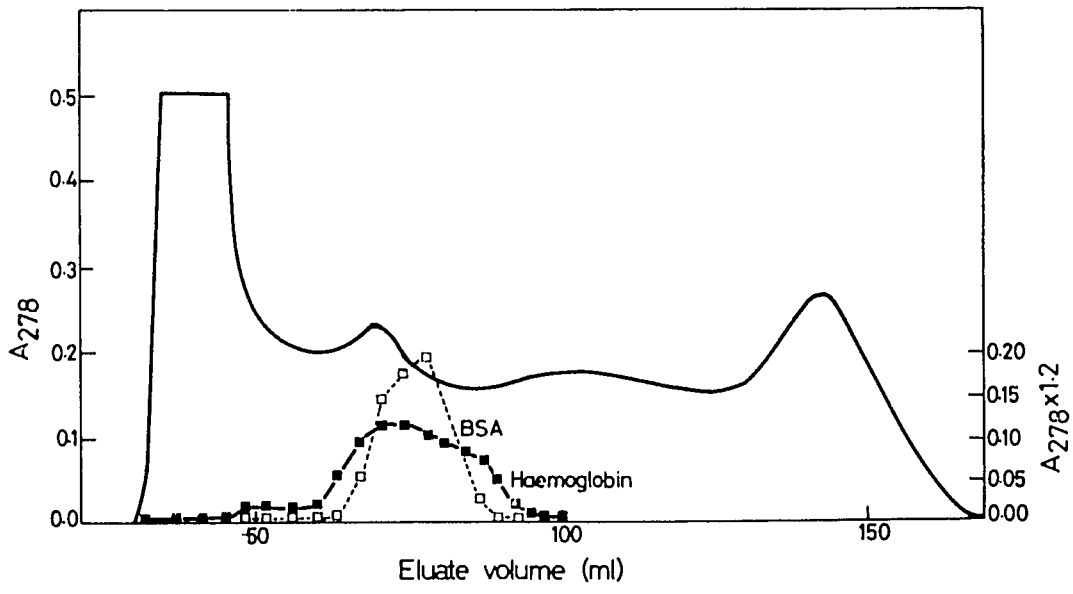


Figure 1. Gel filtration of *R. appendiculatus* on sephadex G-100. Pharmacia column K 15/ 90; bed height: 90 cm; eluent: 2 x 10^{-2}M phosphate buffer, pH 7.8; flow rate 30 ml, hr. $^{-1}$; fraction size: 3.8 ml.

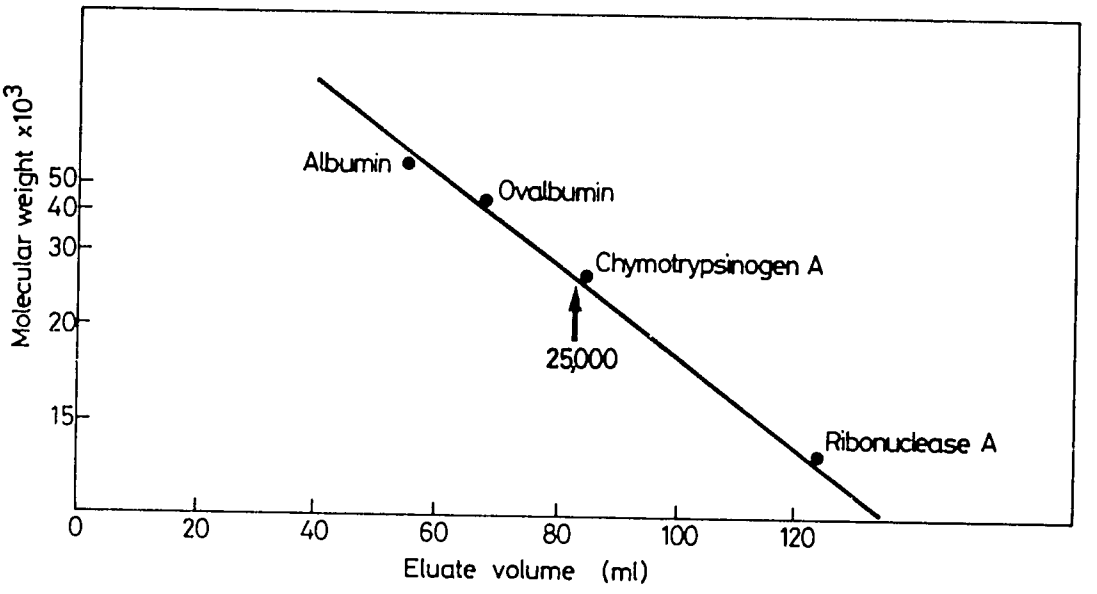


Figure 2. Determination of the molecular weight of the proteinase on sephadex G-100

column was calibrated with Blue Dextran 2000, albumin, ovalbumin, chymotrypsinogen A and ribonuclease A. The results of the gel-filtration experiment are shown on Figures 1 and 2.

Determination of the pI

Analytical isoelectrofocusing was done on Ampholine PAG plates, pH 5.5-8.5. Gel slices (0.5 cm) from the unstained half of the gel were assayed for pH and proteinase activity. The remaining half of the gel was stained for protein using Coomassie Brilliant Blue R250. From this experiment, the pI was found to be 6.5 as compared to 6.0 for the commercial bovine enzyme (Figure 3).

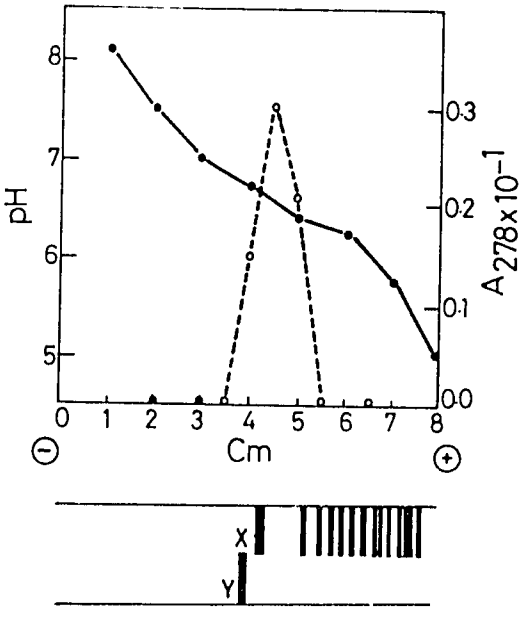


Figure 3. Isoelectrofocusing of *R. appendiculatus* semi-purified carboxyl proteinase (X) and commercial bovine cathepsin D (Y) on LKB PAG plate pH 5.5—8.5. Anode electrode solution was 0.4M hepes; cathode electrode solution was 0.1M NaOH.

In vitro inhibition of the enzyme by antiserum

460 μg of the semi-purified enzyme in saponin solution (50 μg saponin/rabbit) were injected subcutaneously into each experimental rabbit. Control rabbits were injected with 50 μg saponin/rabbit. The rabbits were boosted on day 9 and bled for sera on day 14. Each serum was concentrated 2.5 fold. The tick enzyme and commercial bovine cathepsin D were each assayed with i) experimental serum, ii) control serum, iii) no serum.

The experimental serum inhibited the tick carboxyl proteinase, but had no effect on bovine cathepsin D. There was also some inhibition of both enzymes by the control serum, but this was expected as sera are known to contain proteinase inhibitors (Figure 4).

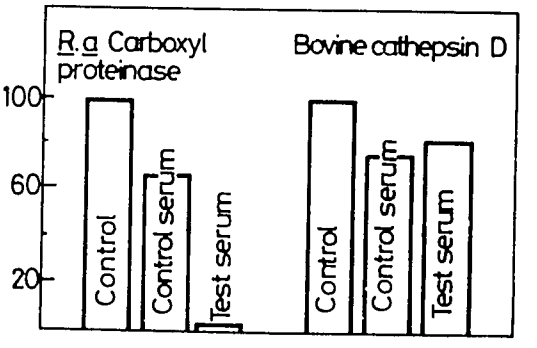


Figure 4. The inhibition of *R. appendiculatus* carboxyl proteinase by antiserum to the tick enzyme

It is an important finding that the anti-carboxyl proteinase inhibits the tick enzyme, but not bovine cathepsin D. The indication is that it may be possible to use such antibodies to inhibit tick digestion in vivo.

TEST OF *R. APPENDICULATUS* VITELLIN AS AN IMMUNOGEN FOR INDUCING TYPE II RESISTANCE IN RABBITS

T.S. Dhadialla

Purification of the major yolk protein, vitellin (Vn), from the eggs of *R. appendiculatus*, was reported in the Twelfth Annual Report, 1984. Results of its partial biochemical and immunological characterization were also described.

To test the potency of Vn as a potential tick antigen in type II resistance, rabbits in 3 groups (3 per group) were immunised with Vn in Freund's Complete Adjuvant (FCA; Group I), phosphate buffered saline (PBS) in FCA (Group II) or left untreated (Group III). Three months after the initial immunisation, and 10 days after the second booster injection, 20 adult ticks of each sex were put to attach and feed on 1 ear of each rabbit. As the engorged female ticks dropped, their weights were recorded. Twenty days after drop, an egg batch from each tick was also weighed as an indicator of its reproductive capacity.

One month after the ticks had dropped, the rabbits in Groups I and II were once again boosted as above, and 2 additional tick-naive rabbits included as fresh untreated controls. Ten days after the booster treatment, the rabbits were challenged with another set of adult ticks, in the same manner as the first challenge.

The results, expressed as mean engorged and egg batch weights for ticks from each of the 3 groups of animals after the 2 tick challenges, are shown in Figure 5. It is quite clear from the results that rabbit-anti-Vn immunoglobulins had no effect on the engorgement weight of ticks fed on immunised rabbits when compared to those which had dropped from the treated or untreated control group. Similarly, the weights of eggs oviposited by ticks which dropped from rabbits in any of the 3 groups were not significantly different.

However, for the second tick challenge both the engorgement and egg batch weights of ticks which dropped from rabbits in the 3 groups were significantly reduced. On the other hand, ticks from any 1 group were not significantly more affected than ticks from any other group. This suggests that the observed reduction in engorged tick weight and weight of egg batches is due to type I resistance effects induced after the first challenge.

Possible explanations for the lack of effect of rabbit-anti-Vn immunoglobulins to, at least, reduce the reproductive potential of ticks can be presented on the basis of the following 2 observations: (i) when haemolymph from ticks which had engorged fully on rabbits was tested, against goat-anti-rabbit IgG in an Ouchterlony's immunodiffusion test, it was found that only a very small fraction of ingested IgG crossed the mid-gut into the haemocoele, and (ii) vitellin, whose haemolymph precursor, vitellogenin, is presumably synthesized by the fat body, constitutes more than 80% of the total egg proteins. One of the main terminal

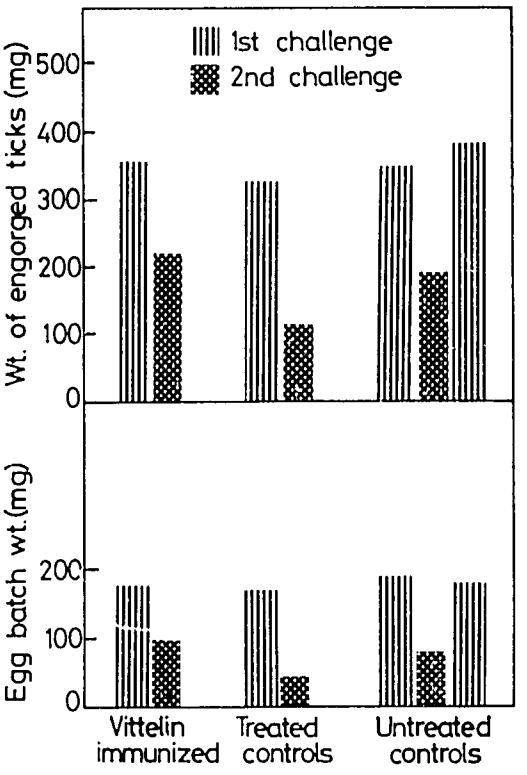


Figure 5. Histogram showing the effect on engorgement weights of adult female ticks and their egg productivity after feeding on Vn immunized, treated (adjuvant + PBS) and untreated control rabbits (open bars) The stippled bars represent results from a second challenge with adult ticks on the same rabbits 45 days after the first challenge and ten days after a booster injection for the immunized and treated control rabbits.

physiological and biochemical functions of a fully engorged adult female tick is to convert about 50% of its body weight into thousands of eggs. It seems, therefore, possible that with such a high production rate of Vg (which is immunologically identical to Vn) in the haemolymph and relatively insignificant quantities of IgG crossing the mid-gut, these immunoglobulins are very rapidly neutralised and hence rendered ineffective in distrupting egg production.

The above results demonstrate that Vn on its own is an ineffective immunogen for use to immunochemically disrupt egg production in ticks feeding on immunised host animals. However, if the mid-gut could be made more permeable to immunoglobulins to cross into the the haemolymph, then an antigen like Vn might be more useful for inducing type II resistance in host animals.

INDUCTION OF TYPE II RESISTANCE IN RABBITS WITH PROTEIN EXTRACTS FROM MID-GUTS OF PARTIALLY ENGORGED VIRGIN FEMALE *R. APPENDICULATUS*

T.S. Dhadialla, A.A. Latif

Since the reproductive potential of *R. appendiculatus*, females fed on rabbits immunised with purified *R.*

appendiculatus vitellin was not disrupted when compared to ticks fed on control rabbits, for reasons explained in the preceeding report, it seemed logical to first try and disrupt the mid-gut wall so as to make it more permeable to larger quantities of immunoglobulins. Moreover, since in ticks digestion of the blood meal and reproduction are so intimately tied, if the digestive functions of the mid-gut could be immunologically ablated, then one could also expect reproduction to be affected. The present report gives a summary of the effect of immunising rabbits with protein extracts from the mid-guts of partially fed virgin female ticks as part of a long-term project to identify, isolate and characterise the appropriate antigens from the tick mid-guts.

Adult female *R. appendiculatus* were allowed to feed on rabbit ears for 6 days before forcibly detatching them for dissection. Mid-guts of ticks were dissected out under cold phosphate buffered saline (PBS; pH 7.0), rinsed in PBS and then dropped into liquid N_2. Pooled mid-guts in liquid N_2 were then stored at -70°C until needed.

Frozen mid-guts were homogenized in PBS on ice using a Sorvall® homogenizer and the homogenate centrifuged at 10,000 xg for 15 min at 4°C. The clear supernatant was removed and kept for future use. The precipitate was again homogenized in PBS containing 1% Triton X-100/deoxycholate. The solublised proteins obtained after centrifugation (described above) were also used for immunisation of rabbits.

Five groups of rabbits (4 rabbits group) were immunised as follows:

Group I: Soluble mid-gut proteins: Freund's Complete Adjuvant (FCA)
Group II: Soluble mid-gut proteins: Freund's Incomplete Adjuvant (FIA)
Group III: Detergent soluble mid-gut proteins: FCA
Group IV: PBS: FCA
Group V: Untreated controls.

One month after initial immunisation treatment, the animals in Groups I – IV were boosted twice with protein extract or PBS in FIA at fortnightly intervals. Three weeks after the second booster treatment, animals were challenged with 100 larvae and 50 nymphae on 1 ear and 35 females and 40 males on the other ear. The fed weight, moulting, egg batch weight and any abnormalities were recorded as the experiment progressed. When all the ticks had engorged and dropped, blood was collected from all the rabbits by intra-cardiac puncture in order to have the post-tick challenge serum. Serum from all the rabbits had also been collected 2 weeks before the tick challenge.

The results obtained are summarised in Table 1. While there were virtually no differences in the mean weight of larvae and nymphae dropping from rabbits in the 5 groups (nymphae from group III did have significantly reduced weight) there was significant reduction in the engorged weight of females which dropped from immunised rabbits compared to those which dropped from treated and untreated control rabbits. Female ticks in Group I and II had fed weights which were about 75% of those in Groups IV and V. Ticks in Group III were the most affected and had engorgement weights which were about 60% of those from the 2 control groups. While the egg batch weight of ticks in Groups I, II and III were also reduced, the ability of these ticks to convert their blood meal into eggs was not affected. The egg conversion factor of ticks from Groups I, II, III, IV and V were not significantly different.

The above results indicate that while the feeding performance of ticks feeding on rabbits immunised with mid-gut antigens was affected, there were no obvious direct effects on the ability of these ticks to convert their body weight into egg mass. Experiments to explain the above effects in biochemical and immunochemical terms are in progress.

CHEMICAL ASPECTS OF COWPEA RESISTANCE TO THE POD BORER *MARUCA TESTULALIS* (GEYER)

D.A. Otieno, L. Moreka, E. Nyandat

In the Twelfth Annual Report, 1984, we reported the isolation of a mixture of phytosterols from organic extracts of the stems of the resistant cowpea cultivar TVu 946. The mixture displayed moderate feeding deterrent activity against *M. testulalis*, and its basic role in TVu 946 stem resistance to the pod borer was established as feeding inhibition. During the year under review we undertook further bioassay guided separation work on the mixture to establish the identity of its constituents and their role in cowpea - *Maruca* interaction.

Submission of the crude mixture of phytosterols to liquid chromatography (silica Gel G), eluting with a mixture of petroleum ether (b.p 40-60°C) and ethylacetate (4:1), produced a number of fractions, 1 of which gave colourless

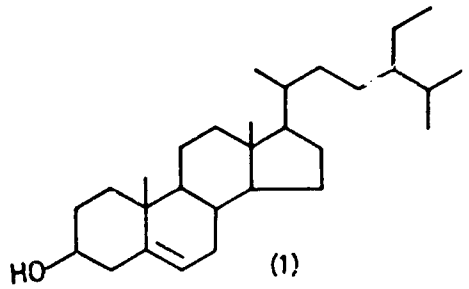
Table 1. Weight of larvae, nymphae and adult *R. appendiculatus* and of oviposited egg batches after engorgement on immunised and control treated and untreated rabbits.

Group	Mean ± S.E.M. weights (mg)				Egg conversion Factor* (X ± S.E.M.)
	Larvae	Nymphae	Adult female	Egg batch	
I	0.39 ± 0.03	8.95 ± 0.62	250.7 ± 18.9	124.1 ± 9.3	0.50 ± 0.01
II	0.41 ± 0.02	7.76 ± 1.33	241.4 ± 38.2	117.8 ± 19.1	0.49 ± 0.02
III**	0.41	6.66	194.7	90.8	0.46
IV	0.36 ± 0.01	8.42 ± 0.29	324.8 ± 24.8	164.0 ± 13.5	0.50 ± 0.01
V	0.41 ± 0.01	8.55 ± 0.30	329.7 ± 26.0	166.7 ± 13.4	0.51 ± 0.00

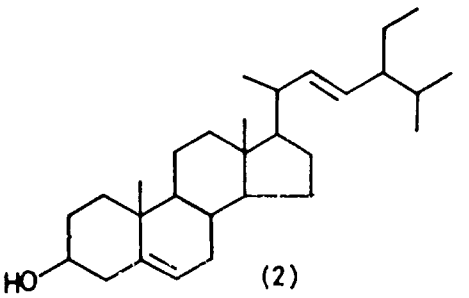
* Egg conversion Factor = Wt. of egg batch / Wt. of engorged female

** Two of the rabbits in this group died before tick challenge. Therefore, only the group means are presented

needles (m.p. 138.5°C) on standing. Absorption spectra data (^{1}H-nmr ^{13}C-nmr) and mass spectral pattern of this product were together consistent with β-sitosterol formulation (1). Subtraction of the mass fragmentation pattern of commercial β-sitosterol (Aldrich Co) from the pattern of the isolated product left a mass spectrum which corresponded to that of 3-hydroxy-23-ethylcholestan-5, (6), 21-diene (2).



(1)



(2)

Feeding deterrent activity of the isolated product against *M. testulalis, Spodoptera exempta* and *Eldana saccharina* is shown in Table 2. The level of feeding inhibition displayed by this product against *M. testulalis* is weak compared to the percentage inhibition (82.6%) of the crude extract from TVu 946 stems (Twelfth Annual Report, 1984). This shows the presence of other more active components in the extract. Purification and identification of these components continues.

Table 2 Feeding-deterrent activity of isolated β-sitosterol mixture against *Maruca testulalis, Spodoptera exempta* and *Eldana saccharina*

Dose μg disc	Feeding Inhibition (%) *M. testulalis*	*S. exempta*	*E. saccharina*
100	29.6	31.2	24.3

A NEW PHENOL IN THE TICK *RHIPICEPHALUS APPENDICULATUS*

P.G. McDowell, S.M. Waladde

Following the discovery that the tick *Rhipicephalus appendiculatus* (the East Coast Fever [ECF] vector) contained the ubiquitous tick phenol, 2,6-dichlorophenol (2,6-DCP), and the detection of the same compound in the larval stage of the tick, further structural elucidation work has lead to the identification of a new phenol, not previously reported in ticks. It has been shown that a second major phenolic component found in the adult unfed female tick (Annual Report, 1983) causes stimulation of the md3 sensillum of male *R. appendiculatus*; the same sensillum which is highly responsive to 2,6-DCP.

The new phenol has been identified as 2-bromo-6-chlorophenol (*1*) by gas chromatography (GC), combined gas chromatography-mass spectrometry (GC-MS) and by comparison with the synthetic standard.

Figure 6 shows a capillary gas chromatogram of the acids and phenols extracted from unfed, adult female *R. appendiculatus* on a 25 m FFAP column, indicating 3 main components sensitive to the electron capture detector employed for the analysis. The first of these was identified as 2,6-DCP on the basis of retention time, in comparison to the synthetic standard, and mass spectrometry. The second peak, which was in lesser amounts as indicated in Figure 7

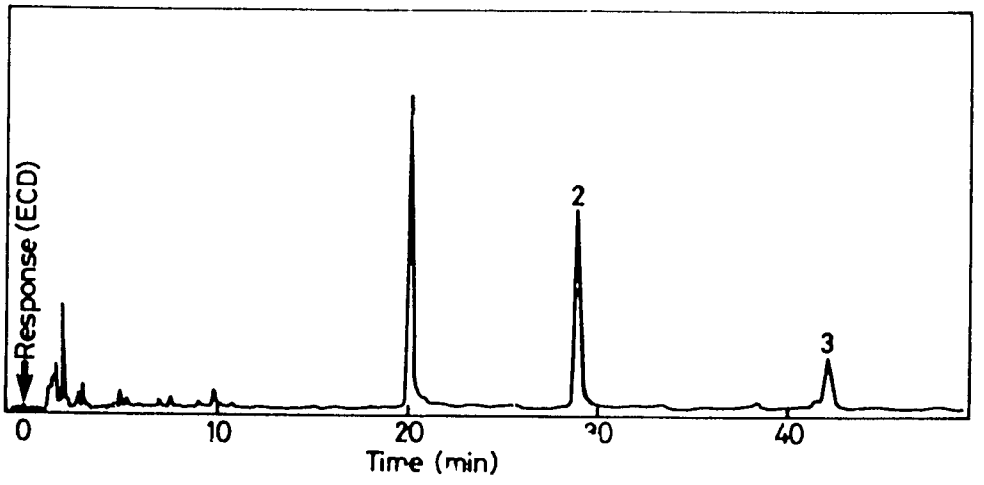


Figure 6. Hexane extract of *Rhipicephalus appendiculatus* unfed female adult — acids and phenols fraction capillary gas chromatogram (ECD) 25m x 0.22mm i.d. FFAP Fused Silica column - 150°C

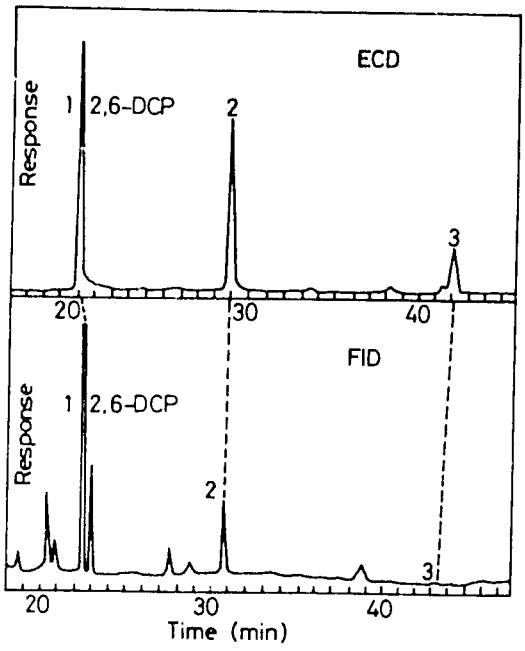


Figure 7. Acids and phenols fraction from extract of *Rhipicephalus appendiculatus* unfed female adults section of ECD and FID chromatograms

which shows a comparison between ECD and FID responses of the compounds, was identified as a bromochlorophenol from its mass spectrum (Figure 8). The specific isomer of bromochlorophenol could not be determined from the mass spectrum. However, the retention time differed significantly from both 2-bromo-4-chloro and 4-bromo-2-chlorophenols, which were available for comparison. It seemed possible that the natural isomer would be the 2,6-isomer, in keeping with the presence of the 2,6-dichlorophenol. This bromochlorophenol was not available commercially and was, therefore, synthesised in small amounts according to the synthetic scheme outlined below.

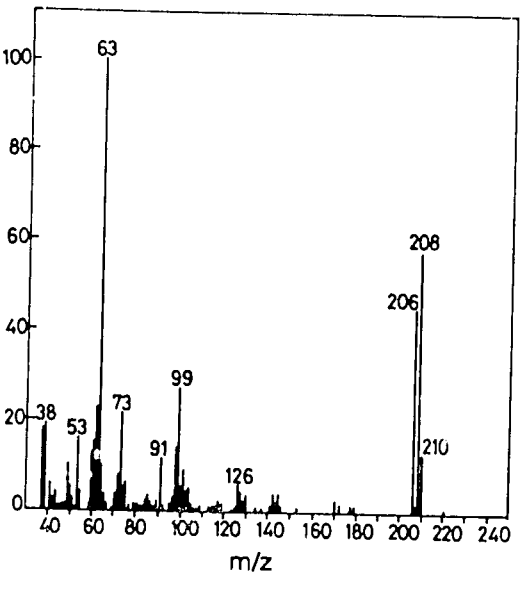
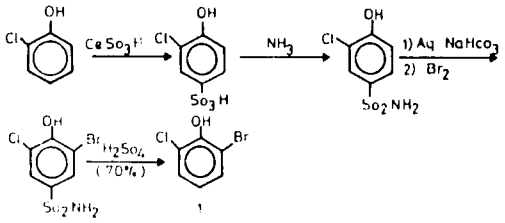


Figure 8. Mass spectrum of peak 2 (Figure 6) following background subtraction.



Spectral data for the synthetic 1 were in keeping with the 2,6-isomer (^{1}H NMR ($CDCl_3$) δ 5.86 1H s (phenolic H); 6.76, 1H tr (J = 8 Hz); 7.36, 2H 2xdd (J = 8, 1.4 Hz); MS (EI + 70eV) M + 210 (11%), 208 (46%), 206 (39%); 63 (100%) and the mass spectrum closely resembled that of the isolated compound. GC coinjection of the synthetic compound with the extracted component confirmed the assignment of the compound as 2-bromo-6-chlorophenol. The quantities present in the female were estimated to be of the order of 0.3-1 ng per female, less than the quantities of the dichloro compound (ca. 12 ng/female).

In single cell electrophysiological recordings from md3 sensilla of male *R. appendiculatus*, the synthetic bromochlorophenol was found to stimulate these sensilla in a manner very similar to that found with 2,6-dichlorophenol. Behavioural tests are currently underway.

The third ECD sensitive component (Figure 6) was only present in trace quantities. The mass spectrum did, however, indicate that it was also a phenol and based on GC coinjection with the authentic compound it appeared to be 2,6-dibromophenol.

IDENTIFICATION OF TSETSE ATTRACTANTS FROM EXCRETORY PRODUCTS OF A WILD HOST ANIMAL, *SYNCERUS CAFFER*

A. Hassanali, P.G. McDowell, M.L.A. Owaga, R.K. Saini

Buffalo urine was shown recently to be a potent attractant of tsetse flies in the field, giving trap catches almost 10-fold greater than control traps (Owaga, 1984; Owaga, 1985). In this report we outline the progress made in the identification of the active constituents.

The active compounds were found to be completely extractable with dichloromethane. Figure 9 depicts a gas-

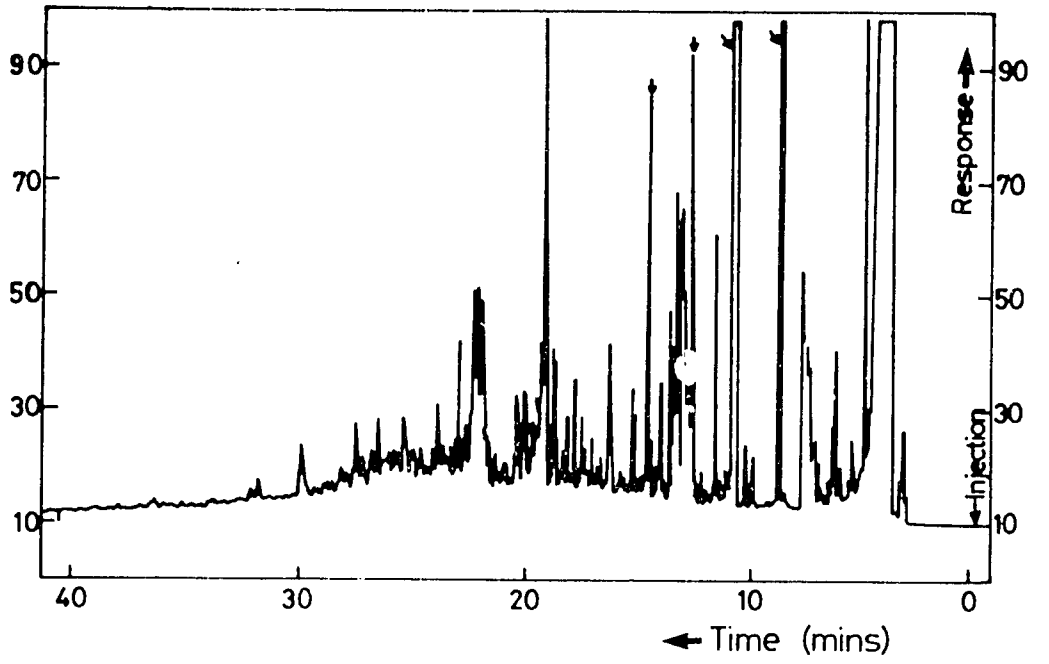


Figure 9. Gas chromatogram of dichloromethan extract of buffalo urine. Column: 50m CP Si/5 fused silica; temperature programme: 50°C (isothermal for 0.5 min) programmed to 210°C at 8° min.

Table 3. *G. palladipes* (males + females) captured in biconical traps, baited with four LC fractions of CH Cl extract of buffalo urine

Fraction	LC-I	LC-II	LC-III	LC-IV	Control
Locality A	179	1590	238	213	203
Locality B	272	1882	420	674	285
Total	451	3472*	658	887	488

* This represents a significantly higher total catch than the others ($P < 0.05$, Duncan's test)

chromatographic profile of the whole extract on a 50 m CP Sil 5 column. Reproducible fractionation of the dichloromethane extracts into 4 fractions (LC-I, II, III and IV) was achieved by flash-liquid chromatography; the urine dyes providing convenient internal markers in delineating the 4 fractions. These 4 fractions were tested as baits in 2 different localities (A and B) in the field, using biconical traps in a randomised multiple latin square design. The results of this test are summarised in Table 3. Fraction LC-II was significantly more effective than the others ($P < 0.05$, Duncan's test) in increasing the trap catches; the increase being of the order of 7-fold when compared to the controls.

Capillary gas chromatographic examination of LC-II on an FFAP capillary column gave 4 main peaks in the ratio of ca. 1.5:66:3:1.0 (Figure 10). Gas chromatography-mass spectrometric analysis on the same column gave mass spectra for the first 3 peaks, which resembled very closely those of phenol, 4-cresol and 4-ethylphenol respectively. Coinjection studies with the authentic samples confirmed that the 3 peaks corresponded to these phenols. The mass spectrum of the fourth peak suggested that it might be a propylphenol, and it was found to coincide with an

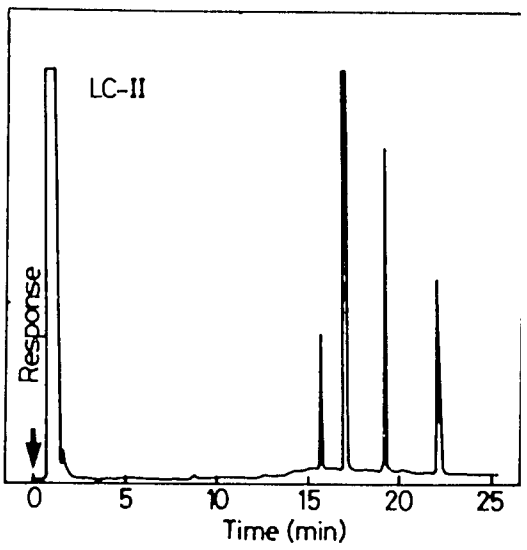


Figure 10. Gas chromatogram of buffalo urine fraction LC-II. Column: 20m FFAP fused silica; temperature programme: 50°C (isothermal for 5 min) programmed to 150°C at 10°C/min.

authentic sample of 4-n-propylphenol in coinjection experiments on the FFAP column. However, its mass spectrum showed 2 additional features, a more prominent peak at m/z 108 (M-28, 48%) and another at m/z 121 (M-15, 10%), which were not present in the spectrum of 4-n-propylphenol. Moreover, ions at lower mass range were

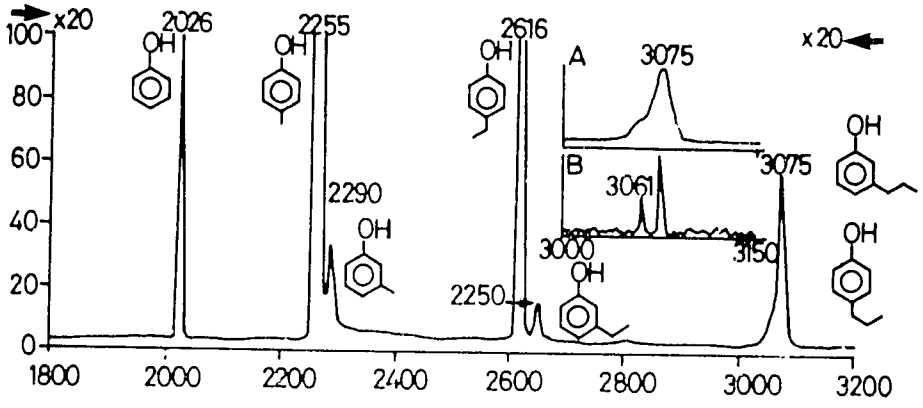


Figure 11. Reconstructed total ion chromatogram (scan numbers 1800-3200 of GC-MS analysis of buffalo urine fraction LC-II. GC Column: 50m CP Wax 51 fused silica; temperature programme: 50°C (isothermal for 5 min) programmed to 180°C at 5°C m. MS: Scan time, 0.5 sec; interscan delay, 0.1 sec; scan range, m/z 35-500; source temperature, 150°C; GC interface temperature 200°C. Inset A: scan numbers 3000-3150. Insect B: scan numbers 3000-3150 showing the results of a peak enhancement analysis of this region. The presence of two peaks is clearly indicated.

relatively more intense in the urine sample. These observations suggested that the peak might be made up of a mixture of closely related isomers, not resolved on the FFAP column

Analysis of fraction LC-II on a 50 m carbowax 20 M type column was more rewarding. Each of the 3 substituted phenols resolved into a pair. Figure 11 depicts a reconstructed total ion chromatogram of gas chromatography-mass spectrometric analysis of fraction LC-II, carried out on the carbowax type column. The small peaks corresponding to scan numbers 2290 and 2650 had mass spectra closely resembling those of 3-cresol and 3-ethylphenol. Coinjection studies with authentic samples confirmed that those were indeed the *meta* substituted isomers. Resolution of the propylphenol peak was incomplete. However, the presence of 2 components was confirmed by use of a peak enhancement programme in the scan region 3000-3150, which clearly indicated the presence of 2 peaks (Figure 11, Inset B)

The lesser component in the propylphenol region, corresponding to scan number 3061, was found by mass spectral comparison and coinjection studies to be 4-n-propylphenol. This was in contrast to the cresol and ethylphenol mixtures, where the *para* isomers were the predominant components. The major propylphenol was inferred to be the *meta* isomer. Unavailability of this isomer, needed for comparison from normal commercial sources, prompted us to synthesise the compound from 3-bromonisole. The synthetic sample gave a mass spectrum identical with that obtained from the urine compound. A sample of the natural phenol was isolated by reverse-phase HPLC on a micropreparative column and further purified by preparative gas chromatopgraphy. Cochromatography of this sample with the synthetic 3-n-propylphenol on the carbowax capillary column, as well as comparison of their ultraviolet (λ max [hexane], 271 and 277nm) and nmr (H-1) spectra, confirmed that the 2 were identical.

We concluded, therefore, that the most active buffalo urine fraction LC-II is composed of 7 phenolic compounds which include phenol, 3- and 4-cresols, 3- and 4-ethylphenols and 3- and 4-n-propylphenols. The relative importance of the various phenols in conferring the high activity to the blend will become clear from the current field studies utilising various compositions of the component phenols. It is interesting to note that traps baited with fraction LC-III which contained phenol, the cresols and the ethylphenols, but which lacked the propyl compounds gave rather low catches of flies in the field suggesting that one or both of the propyl homologues may be crucial to the potency of the blend.

Fraction LC III also contained some dimethyl sulphone which was the major component of fraction LC IV. Other components of LC IV which appeared to demonstrate some activity in the field, remain to be identified and the effect of their copresence with the phenolic blend in baited traps, remains to be determined.

A MOSQUITO LARVICIDE FROM *SPILANTHES MAURITIANA*

Isaac J.O. Jondiko

The methanol extract of wet vegetative aerial parts of *Spilanthes mauritiana* afforded, after repeated chromatographic separations and larvicidal bioassays, a potent mosquito larvicide (Twelfth Annual Report, 1984). The present report gives a summary of the evidence for the structure of the only compound that caused 100% mortality at 10^{-1} mg ml to third instar larvae of *Aedes aegypti*.

The low resolution mass spectrum of the oil gave a molecular ion observed at m/z 247, assignable to $C_{16}H_{25}NO$ and the analysis of the fragmentation pattern favoured the structure shown in Figure 12. The most labile bond was the doubly allylic bond, C_6-C_7 and its cleavage led to the base peak observed at m/z 81 and another major ion observed at m/z 167. The presence of N-H group was inferred from the i.r. signal at 3295 cm^{-1} and a broad resonance at δ 5.6 in the nmr spectrum which disappeared on addition of D_2O.

The u.v. absorption at 260 nm and the i.r. peaks at 1550 and 1650 cm^{-1} were attributed to the 2 double bonds conjugated to the amide group. Further evidence for the trans-conjugated amide group came from the ^{1}H-nmr and

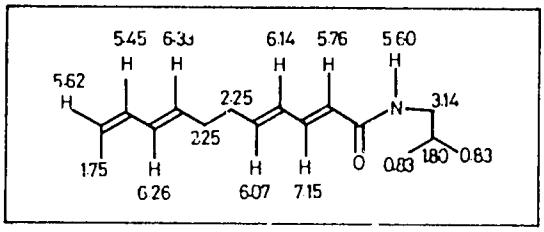


Figure 12. Proton nmr shifts in S values

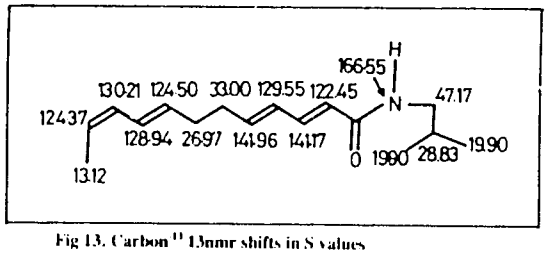


Fig 13. Carbon [13] 13nmr shifts in S values

[13]C-nmr data summarised in Figures 12 and 13. The 10-cis geometry was inferred from the observation that chemical shifts for H-10 and H-11 were at higher field than those expected for protons of trans-double bond. Moreover, it was observed that $J_{10\text{-}11}$ was found to be 10Hz instead of 15 Hz expected for a trans-double bond. The [13]C-nmr spectrum, further corroborated the presence of 10-cis geometry, due to a quartet observed at δ13.12 assigned to C-12. If this bond was *trans* then the methylcarbon shift would be expected at δ18. The evidence for the isobutyl group came from the [1]H-nmr and [13]C-nmr shifts assigned as shown in Figures 12 and 13. The mass spectral peaks observed at m/z 43 and 57 corresponding to ions $[C_3H_7]^+$ and $[C_4H_9]^+$ respectively resulted from cleavages involving the isobutyl group. Summation of this data, led to the conclusion that the active larvicide is dodeca-2,4,8,10-(E)-(E)-(E)-(Z)-tetraen N-isobutylamide.

PLUMBAGIN AND ITS ANALOGUES AS A MOSQUITO LARVICIDE

A. Chapya, G.V. Achieng', B.A. Etemesi, A. Hassanali

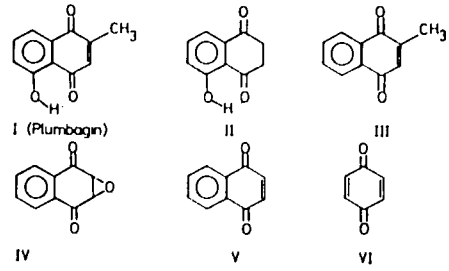
The larvicidal activity of extracts of *Plumbago zeylanica* has previously been shown to be due to the presence of the naphthoquinone, plumbagin (Twelfth Annual Report, 1984). As a continuation of that work, 5 purified synthetic naphthoquinones and one benzoquinone have been assayed against third instar larvae of *Aedes aegypti*. The results are summarised in Table 4.

The rather low activity of benzoquinone demonstrates the importance of the naphathalene skeleton for the larvicidal activity of the quinones. On the other hand, substitution differences in the naphthoquinone nucleus appear to have little effect on the activity of the compounds. Since naphthoquinones occur widely in tropical plants, their crude extracts may prove effectual in small-scale mosquito control programmes.

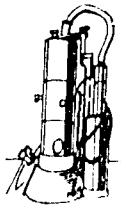
Table 4. Larvicidal activity of various quinones against 3rd instar *Aedes aegypti* larvae

Compound Dose (μmole x 10⁻² 20 ml)	Mean % Mortality after 24 hours[a]					
	I	II	III	IV	V	VI
7.4	100	100	100	100	100	15
3.7	100	100	100	93 ± 7	97 ± 4	0
1.75	92 ± 25	96 ± 6	36 ± 11	59 ± 7	93 ± 8	0
0.875	52 ± 10	63 ± 20	1 ± 2	25 ± 20	67 ± 14	0
0.438	13 ± 7.5	26 ± 13	0	6 ± 3	17 ± 13	0
Control	0	0	0	0	0	0

[a] average of 6 replicates involving 20 larvae each



Histology and Fine Structure



Histology and Fine Structure Research Unit

In 1985, apart from giving attention to research on insect gross morphology, aspects of insect biochemistry and immunology were studied. Another area of investigation was the application of the radio-isotope techniques to the synthesis and secretion of different components of tsetse male accessory reproductive glands and the transfer of these components into the spermatophore. Similar methods were applied to studies dealing with juvenile hormone biosynthesis in the corpus allatum-corpus cardiacum complex of Glossina morsitans.

Research work expanded into the field of experimental cell biology enhanced by the acquisition of a Flow Hood. This equipment will improve in vitro studies of the hormonal effect on cultivated tsetse and other target insect pests tissue organs.

Isoelectrofocused patterns of total proteins from fully differentiated male accessory reproductive glands (ARG) and spermatophore of tsetse, G. morsitans, *were found to be remarkably similar.*

With regard to immunological investigations, antibodies were raised against the male accessory reproductive gland (ARG) secretory proteins, spermatophore and testicular extracts. The ARG secretory proteins and spermatophore material were shown to share common immunological characteristics.

Teneral male tsetse flies were fed artificially with radioactivie Leucine (3H-G-Leucine) and C14 inulin. Time-course study showed radioactivity in the crop and gut 1 hr after the feed and 24 hr later, it was found in ARG and tsetse.

Collaborative research aimed at localising areas or cells within the midgut of the tick, which are responsible for the production of would-be target antigens, was instigated with scientists from the Livestock Ticks Programme.

Histological studies on resistant and susceptible sorghum cultivars, which exhibited difference in the length of tunnel and extent of damage, were undertaken with the Crop Pests Research Programme.

Assistance to the Sensory Physiology Research Unit was continued on crop borers, to determine the relationship between the degree of innervation of certain gustatory receptors and their electrophysiological responses to known stimuli.

Several ARPPIS students were supported in their research. In one line of research a histological and ultrastructural study was carried out on various tissues of mice infected by Leishmania *parasites.*

A collaborative study with the University of Nairobi on the morphology of the gas-exchange organs of some East African vertebrates (the naked mole rat, monitor lizard, pancake tortoise) showed differences in the design and organisation of their lungs.

In West Africa, cooperation was established with IITA in Nigeria and investigations were initiated on the leafhopper species (Cicadulina *spp.*) *which spreads maize streak virus disease. This study, aimed at species identification, examined the sensilla on male genitalia of* Cicadulina similis *and* C. arachidis. *The investigations will be extended to other leafhoppers collected from different climatic zones in West Africa.*

A COMPARATIVE MORPHOLOGICAL STUDY OF THE RESPIRATORY ORGANS OF SOME EAST AFRICAN VERTEBRATES

E. D. Kokwaro, J. N. Maina

Collaborative research with the Department of Veterinary Anatomy examined the morphology of the gills and accessory respiratory organs in the African catfish, *Clarias mossambicus*, the lungs of the naked mole rat, *Heterocephalus glaber*, the monitor lizard, *Varanus exanthematicus*, and the pancake tortoise *Malacochersus tornieri*. The investigation attempted to elucidate the organization of the gas-exchange organs and to relate this to the modes of life and the evolutionary levels of these vertebrates.

The African catfish was observed to exhibit a bimodal type of gas exchange, the accessory organs supplementing the oxygen exchanged by the gills. The gill system comprised four branchial arches, each with two hemibranches bearing gill filaments. The gill filaments gave rise to secondary lamellae through which gas exchange takes place (Figure 1). This adaptation enables the catfish to survive in the oxygen deficient waters which largely constitute its environment. The reptilian lungs were found to be less elaborate than their mammalian counterparts. The lungs of the monitor lizard and the pancake tortoise were observed to be simple and saccular components which conduct blood and air (Figure 2). The differences in the design and organisation of the mammalian and reptilian lungs were attributed to the fact that endotherms, like the naked mole rat, are very energetic and require large amounts of oxygen for their mode of life while ectotherms, like the monitor lizard and the pancake tortoise, are relatively less active and thus have a far much lower demand for oxygen.

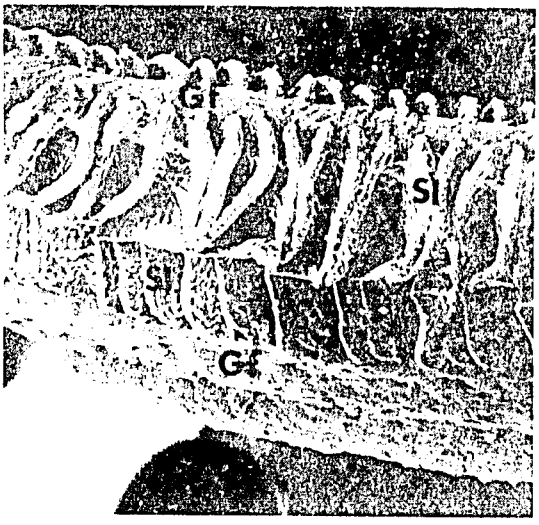


Figure 1. A gill filament (Gf) in the gill system of the catfish *(Clarias mossambicus)* showing the secondary lamellae (Sl) emanating from the filament. The gas exchange takes place between the water flowing in the interlamellar space and the blood in the marginal channels of the lamella. Mag. X 700.

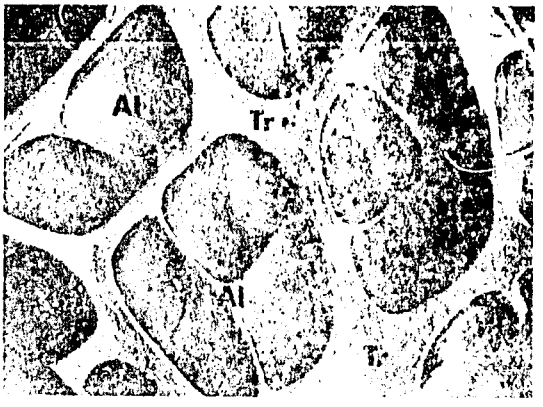


Figure 2. The gas exchange region of the lung of the monitor lizard showing the trabeculae (Tr) subdividing the exchange tissue into alveoli (Al). This compartmentation greatly increases the surface area available for gas exchange in the lung. Mag. X 50.

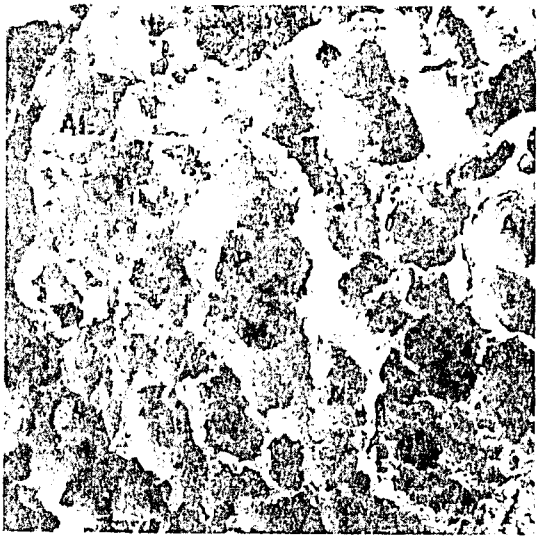


Figure 3. The parenchyma, the gas-exchange region of the lung of the naked mole rat comprise of the *alveoli* (Al) separated by the interalveolar septa. The mammalian lung exhibits an extreme case of the subdivision of the exchange tissue in an attempt to increase the alveolar suface area available for gas exchange. Mag. X 130.

IMMUNOLOGICAL AND BIOCHEMICAL EVIDENCE FOR THE ORIGIN OF THE SPERMATOPHORE MATERIAL IN *GLOSSINA MORSITANS*

E. D. Kokwaro, M. Okot-Kotber, J. Muruthi

Among the events taking place during mating in the tsetse, *G. morsitans*, is the deposition of a male spermatophore into the uterus of the female. Ealier studies at the ICIPE on the ARG and spermatophore, revealed that ARG secretions and spermatophore have a number of similar structural components. Recent investigations

were undertaken to provide further evidence of the origin of the spermatophore material in *G. morsitans*. Much of this work was done through immunochemical studies.

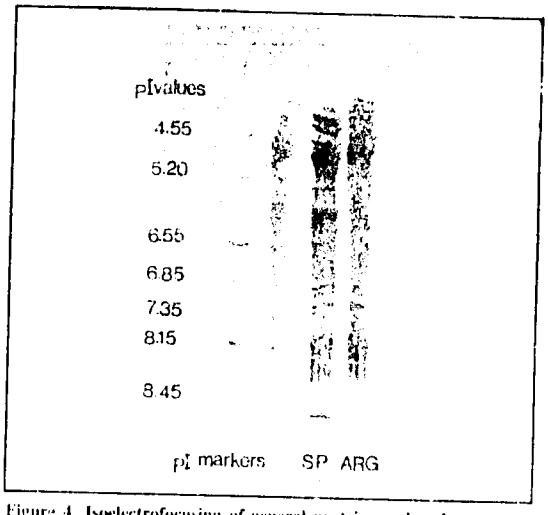


Figure 4. Isoelectrofocusing of general proteins and male accessory reproductive glands (ARG) and spermatophore (SP) materials from *Glossina morsitans*.

Running conditions:
Voltage = 1500V
Power = 40W
Current = Unlicited
Volthour = 1500VH

Isoelectrofocusing of material from ARG and spermatophore showed close similarity in their protein profiles. At least 27 protein bands were discernible (Figure 1), but of these, 13 were major protein bands.

Immunisation results showed that antibodies to ARG, spermatophore and testes were produced. The anti-serum raised against extractable materials from teste, could recognise materials from the testes and spermatophores, but hardly any material from ARG. Anti-testes antiserum also detected some additional material from the testes which was not found in the spermatophore. Anti-spermatophore antiserum recognised materials from ARG and testes, but the precipitation lines of complete identity between spematophore and testes were different from those formed between spermatophore and ARG (Figure 2).

Anti-ARG anti-serum was able to recognise exclusively materials from ARG and spermatophore which turned out to have complete identity. Immunoelectrophoresis results showed that anti-testes antiserum was able to recognise materials from testes and spermatophore but not from ARG. Anti-ARG antiserum recognised only materials from ARG and spermatophore but not from testes.

Materials obtained from spermatophore and ARG of *G. morsitans* behave in similar biochemical and immunological patterns. These results strongly support our earlier finding that spermatophore and ARG have a number of proteins with common physical properties.

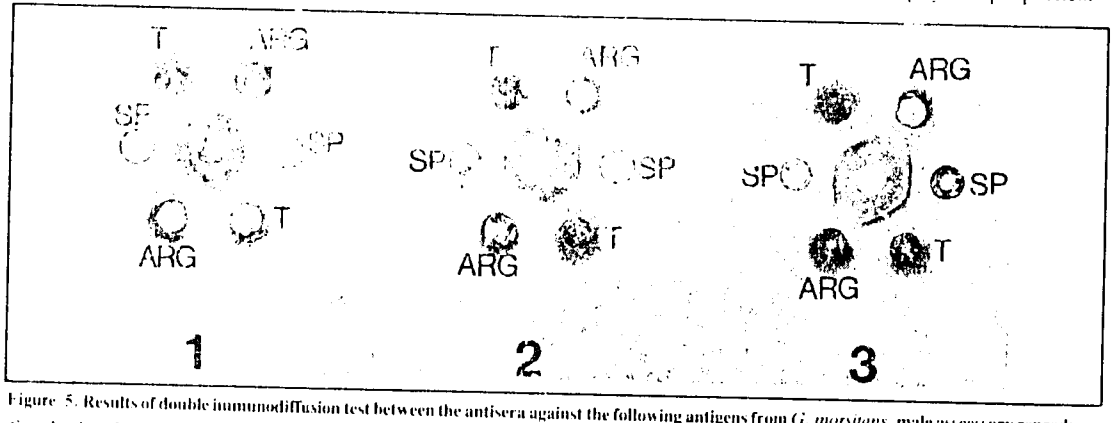


Figure 5. Results of double immunodiffusion test between the antisera against the following antigens from *G. morsitans*, male assessory reproductive glands (ARG), spermatophore (SP) and testes (T).

Sensory Physiology

Sensory Physiology Research Unit

The Sensory Physiology Research Unit continued to collaborate with the Crop Pest and Tsetse Research Programmes as well as with the Chemistry and Bioassay and Histology and Fine Structure units. Natural and synthetic compounds used in behavioural and electrophysiological biossay were provided by the Chemistry Unit. Functional morphological studies using electron microscopy techniques on insect sensilla continued.

Special effort was devoted to the development of suitable feeding biossay technique for the borers. A method for conducting electrophysiological tests using complex organic compounds was also perfected. The above 2 procedures will be effectively used in investigating the effects of host-plant material. The use of tsetse fly antennal responses will continue to be perfected and utilized as a routine method for screening natural and artificial compounds.

Current efforts are aimed at linking a gas-liquid chromatograph to the electrophysiological set up which will help in the monitoring, identification and delivery of odour stimuli during olfactory studies. This approach is particularly important when dealing with pheromone and other odours influencing insect pest behaviour. In order to expedite the recording of electrophysiological data, and its analysis, computer hardware and software for data acquisition, control and analysis will be acquired soon. Behavioural studies will be enhanced by acquiring insect behaviour facilities such as wind tunnel and insect behaviour-monitoring devices.

ELECTROPHYSIOLOGICAL AND BEHAVIOURAL EFFECTS OF POTENTIAL ANTIFEEDANTS

S.M. Waladde, A. Hassanali and S.A. Ochieng

There is evidence which indicates that some limonoids are potential antifeedants against *Spodoptera exempta*, *Eldana saccharina* and *Maruca testulelis*. This assumption is based on feeding-bioassay studies employing the leaf disc technique. The compounds tested include citrus limonoids, their derivatives and limonoids isolated from the East African plant, *Harrisonia abyssinia*. These complex organic compounds are insoluble in water making it difficult to use electrophysiological techniques to find out how taste cells respond to the compounds. It is essential to find out how the limonoids act at the sensory level, and we must find a suitable medium in which to present such compounds to the taste sensilla.

Taste cells respond to feeding deterrent and stimulant compounds by generating characteristic action potentials which are transmitted directly to the central nervous system via their afferent axons. The central nervous system deciphers the information and dictates the appropriate behavioural response via the efferent pathways. In order to stimulate and record taste-cell responses, the stimulating substance must dissociate into negative and positive ions which facilitate transmission of the electrical signals to the recording and amplifying instruments. If it is a non-dissociating compound like sucrose it must be soluble in an electrotyle solution such as NaCl. These requirements make it relatively difficult to conduct electrophysiological studies using complex organic compounds.

This difficulty was overcome by using a 1:1 mixture of tetrahydrofuran and 0.1 M NaCl solution as the vehicle for the limonoids. Stock solutions were made by dissolving the limonoids in specified volumes of undiluted tetrahydrofuran and later adding equal volumes of the salt solution, thus making the 1:1 mixtures containing the required concentrations of the limonoids. Serial dilutions of the stock solutions were made using a freshly prepared tetrahydrofuran NaCl mixture. This mixture provides a suitable medium because tetrahydrofuran maintains the

limonoids in solution while NaCl provides the necessary electrical conductivity.

All the electrophysiological tests were done on the lateral and medial maxillary styloconica sensilla of *E. saccharina*. These sensilla are easy to approach, and they are strategically located to taste whatever the larva is biting into. Preliminary tests showed that tetrahydrofuran has no noticeable adverse effect on the taste cells. This was confirmed when we compared responses of the taste sensilla to sucrose dissolved in 8×10^{-2}M NaCl and that dissolved in the tetrahydrofuran NaCl mixture (Figure 1). Since sucrose is a good stimulant of the 2 sensilla, we wanted to find out whether incorporating the limonoids in the sucrose solutions would modify the response patterns of those sensilla to sucrose. The effects of the limonoids were assessed in terms of the initial impulse frequency responses, subsequent adaptation rates and spike amplitude patterns. In the presence of pedonin, obacunone and deoxylimonin, responses of the lateral sensillum to sucrose were diminished. However, in the medial sensillum, pedonin enhanced the responses to sucrose whereas deoxylimonin and obacunone had the opposite effect (Figure 2). On the basis of spike amplitudes it appears that sucrose stimulated 4 types of cells in the medial and lateral sensilla. In both cases, the high amplitude spikes (1.5-3 mv) had higher frequencies than the low amplitude spikes (0.5-1 mv). In the presence of antifeedants the frequencies of the high amplitude spikes were reduced and in some cases completely inhibited. At the same time, the frequencies of the low amplitude spikes were increased (Figure 3).

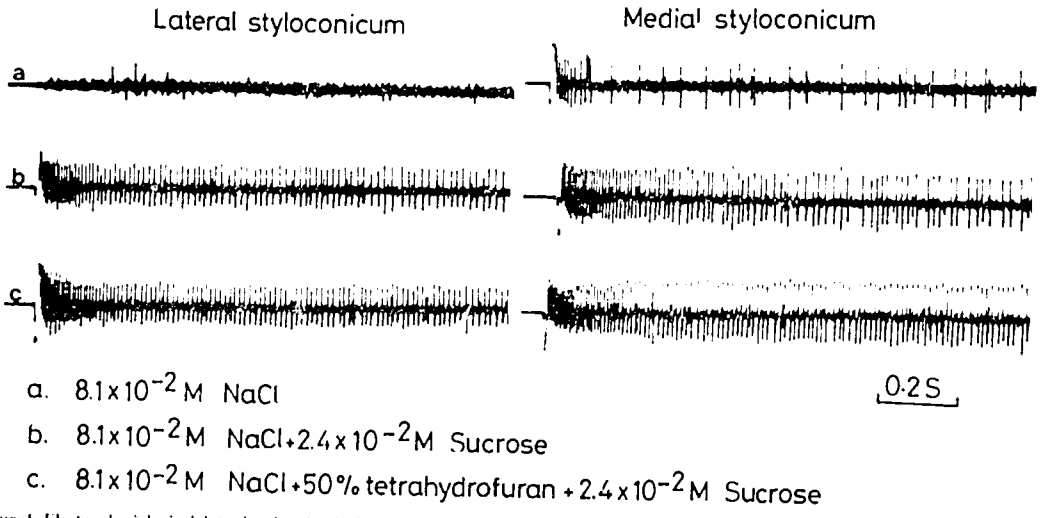


Figure 1. Electrophysiological data showing that in *Eldana saccharina* the medial and lateral maxillary styloconica sensilla are sensitive to sucrose; adding tetrahydrofuran to the stimulating solution did not alter responses to sucrose.

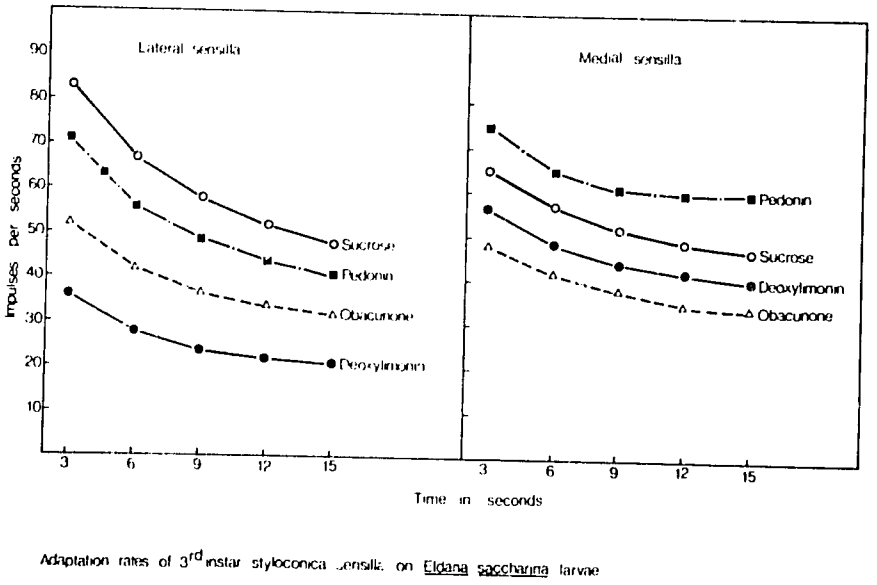


Figure 2. Impulse frequencies and their adaption when the lateral and medial styloconica sensilla of *Eldana saccharina* are stimulated with sucrose alone and sucrose mixed with limonoids (Pedonin, obacunone and deoxylimonin respectively).

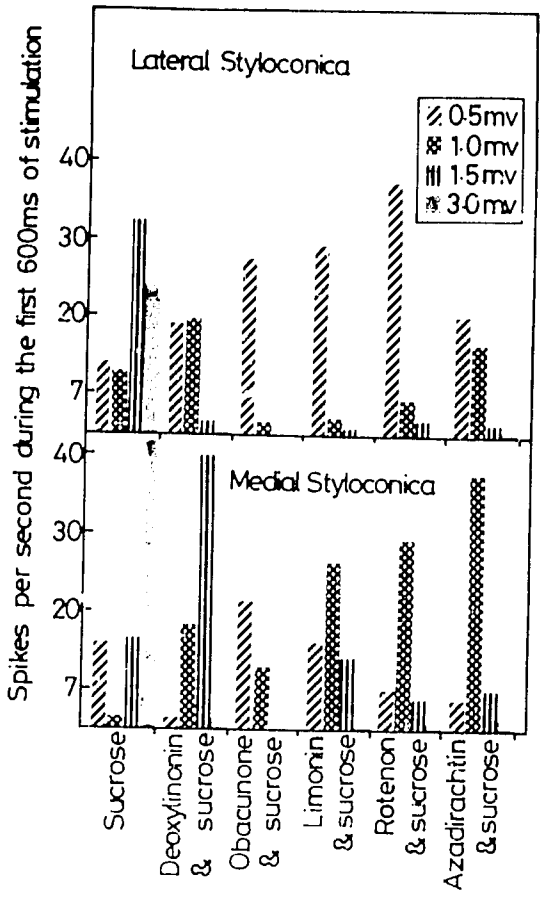


Figure 3. Frequency of spike-amplitude patterns of *Eldana saccharina* styloconica sensilla: response to sucrose and to sucrose plus antifeedants.

It has been suggested by other workers that at the neurone level an antifeedant chemical may act in 1 or 2 ways. it may inhibit the input from receptors responding to phagostimulants, or it may stimulate a cell or group of cells whose input signals are interpreted as inhibitory within the central nervous system. In the case of the limonoids the 2 modes may be operating simultaneously. It is interesting to note that obacunone, regarded as a good antifeedant, strongly inhibits the high amplitude spikes in both sensilla (Figure 3). It can also be observed that the overall effect of deoxylimonin is to depress the frequency of the high amplitude spikes, but preliminary feeding-bioassay data showed that increasing concetrations of this compound did not prevent *Eldana* from feeding (Table 1). This observation concurs with results of leaf disc feeding bioassay tests which showed that deoxylimonin is a poor antifeedant. It is therefore unwise to generalize or assume that a compound inhibiting high amplitude spikes is an antifeedant. More data on the limonoids are needed to enable us to identify those compounds employing their effect at the sensory level and those which exert their effect via other physiological levels leading to cessation of feeding.

Table 1. Effect of deoxylimonin on the feeding activity and weight gain by *Eldana saccharina*.

Deoxylimonin concentration in substrate	Wt. substrate chewed/eaten (feeding activity)	Wt. gained (mg)	Percent (%) Wt. gained
(.001 mg (28)	55.73	5.6	4.0
0.002 mg (9)	50.00	1.7	8.9
0.004 mg (9)	49.05	4.3	21.0
0.008 mg (9)	58.03	6.7	20.0
0.016 mg (19)	48.01	3.3	9.9
0.032 mg (9)	35.03	7.0	5.6
	F = 1.9 ns	F = 7.5 ns	F = 2.7 ns

In order to do this we need the combined input of electrophysiological and behavioural bioassay. In these studies tetrahydrofuran proved to be an efficient solvent of the limonoids and had no immediate adverse effect on the taste receptor. This solvent may be useful in electrophysiological studies involving other organic compounds of host and non-host plant origin.

STEM BORER RESPONSES TO HOST PLANT EXTRACTS

S.M. Waladde, H. Kahoro and S.A. Ochieng

Introduction

Chilo partellus larvae are reported to show non-preference towards resistant maize and sorghum varieties, but the chemical factors responsible for susceptibility or resistance in the 2 host plants are unknown. The objective of the ongoing work is to obtain components of the host extracts from susceptible and resistant varieties and concurrently develop a larval in vitro feeding bioassay to investigate stem borer responses to the host-plant varieties. This work will hopefully provide information about the feeding stimulant or deterrent characteristics in the extract components. The electrophysiological tests are intended to show whether the lateral and medial styloconica sensilla on the maxillary galeae can be used to distinguish between extract components from susceptible and resistant varieties.

Experimental procedures

Preparation of plant materials

Sorghum and maize plants, 5 to 6 weeks old, were obtained from the ICIPE Mbita Point Field Station. Varieties of sorghum were: 2146 resistant, Serena – susceptible, 18363 susceptible, while those of maize were: CMT 324 resistant (ICZ2-CM) and Inbred A – susceptible. As soon as the plants were collected from the field their leaves and sheaths were removed. The clean stems of each variety were immediately chopped into small pieces, approximately 2 cm long, put into polyethylene bags and kept in a cool box containing dry ice. The frozen plant material were transported to Nairobi and dried using a lyophilizer. A portion of each maize variety was ground into a fine powder and later used in the extraction procedure. All the plant material was stored in sealed containers kept in a deep freezer.

Preparation of extract components

Five grammes of maize-stem powder were extracted in 4 steps using 100 ml of the following solvents: diethyl ether, 8:2 chloroform-methanol mixture, ethanol and distilled water. After each extraction the supernatant was separated from the residue by centrifugation. The supernatant was then filtered through glass microfiber filters to remove unwanted debris or suspension. The 4 extraction steps yielded 4 components. The 3 components in the organic solvents were separately concentrated to 20 ml each with the aid of rotorevaporator. The fourth component in water was frozen and lyophilized to produce a solid which was redissolved in 20 ml of antibiotic-antifungal solution (0.1% V/V formaldehyde and 0.05% W/V sorbic acid). The procedures outlined above were successfully used to obtain extract components from susceptible and resistant maize varieties. The visible and ultraviolet absorption spectra of the 4 components were read on a Beckman Du50 spectrophotometer.

Feeding bioassay

In the bioassay tests lyophilized pieces of Inbred-A maize stems were used as feeding substrates. Each maize piece used was weighed, its weight recorded and then placed in a labelled vial (2 cm diameter, 3.8 cm long). Maize pieces in the controls were merely hydrated with the antibiotic-antifungal solution. Those in the treatments were impregnated with 1 of the 4 extract components namely: diethyl ether, chloroform-methanol, ethanol or water. Impregnation was done by dipping each piece of lyophilized stem in the extract until it was thoroughly soaked. At this stage, those feeding substrates treated with the aqueous extracts component were ready for infestation with larvae. However, those substrates impregnated with any of the 3 extract components in organic solvents were set aside to dry for at least 4 hours. This permitted complete evaporation of the solvents and the substrates were then hydrated with antibiotic-antifungal solution and then infested with the larvae.

Third to fourth instars of *Chilo* larvae, reared on a mixture of beans and sorghum leaves powder, were obtained from the ICIPE insectary and starved for 12 hours before commencement of the bioassay test. Each larva was weighed, put in a vial containing a feeding substrate and left to feed for 5 days. All the vials, stoppered with cotton wool, were kept in a light-proof incubator at 30°C and 70% relative humidity. During the course of the experiment all the substrates were hydrated once more on the third day.

On the fifth day each larva was pulled out of the substrate and reweighed. The weight of the larva before feeding was subtracted from what it attained after feeding. With these values, it was possible to determine weight loss or gain thus providing data to relate larval weight changes to the various treatments.

Pieces of each substrate left intact after larval feeding were separated from the frass, transferred to a clean vial and left to dry in the oven at 70°C. The dried substrate pieces were then weighed; that weight was subtracted from the initial weight of the intact substrate. Percentage difference calculated from the above values represented the larval feeding activity.

Impregnating the substrate with extracts increased the initial weight of the substrate, especially for those treated with the aqueous extracts. On the other hand, drying the substrate in the oven reduced the initial weight of the substrate by 5-10%. In order to get the correct substrate weight differences attributable to larval feeding activity, it was necessary to compensate for weight changes arising from impregnation and drying.

From the data collected in the aforesaid process, it was possible to analyze the effects of the treatments on the larvae. The results were examined in terms of larval weight gain or loss and substrate weight chewed or eaten. The same technique was used to find out whether *Chilo* larvae feed better on Serena and 18363, the susceptible varieties of sorghum, as opposed to 2146, a suspected resistant sorghum variety.

Electrophysiology

Electrophysiological tests were done on the medial and lateral styloconica sensilla of *Eldana* and *Chilo* larvae. Materials tested were sucrose and aqueous component of extracts from the susceptible Inbred A and the resistant CMT 324 maize varieties. The aqueous extracts were lyophilized to obtain the solid used in making the test solutions. The concentration range of either sucrose or extract solutions was 0.02%-5.2% W/V.

Observations

Absorption spectra

Differences between the susceptible and resistant maize varieties were found in the absorption spectra of chloroform-methanol and ethanol-extract components. The diethyl ether and equeous components did not show differences between the 2 varieties (Figure 4). Further investigations are in progress to establish the factors contributing to the differences in the absorption spectra. This work will concentrate on those maize or sorghum varieties currently regarded as very susceptible or resistant.

Feeding bioassay

Results of the feeding bioassay were reproducible and the means of the 7 treatments shown in Table 2 were significantly different. Feeding activity of the larvae was expressed as substrate weight chewed/eaten. The mean value for the feeding activity on the substrate treated with susceptible aqueous extract was significantly higher than corresponding values of the other treatments. Similarly, percent weight gained in this treatment turned out to he significantly high (Table 2).

It is interesting to note that there was a significant difference between the effects of the susceptible and resistant aqueous extracts. In addition to bearing better feeding stimulants, it is likely that the aqueous extract from the susceptible cultivar improves the nutritional value of the substrate.

All the chloroform-methanol extract component treatments invariably caused poor feeding activity and weight gain. The values observed were much lower than those for the control. It appears that this extract component contains compounds which prevent larval feeding. On the

Table 2. *Chilo partellus* larvae feeding activity on Inbred A maize stem pieces impregnated with extract components obtained from Inbred A (susceptible) and ICZ2-CM (resistant) maize cultivars.

Extract component impregnated in substrate (Inbred A stem)	Mean values + S.E		
	Wt. substrate chewed/eaten (Feeding activity)	Wt. gained (mg)	% Wt. gained
Controls (126)	33.2±2.6	5.3±0.8	32.3± 6.0
Susceptible (99) "A"	40.8±3.0	8.3±1.0	50.9± 6.0
Resistant (103) "A"	33.4±3.0	5.3±1.0	30.6± 8.0
Susceptible (58) "E"	31.6±4.0	6.5±1.2	34.1± 8.0
Resistant (57) "E"	37.5±4.0	7.4±1.2	35 ± 8.0
Susceptible (38) "C"	18.9±4.8	2.2±1.6	15.6±10.0
Resistant (32) "C"	17.3±5.2	2.8±1.8	17.1±10.0
	F = 17.1**	F = 11.3**	F = 8.9**

"A", "E" and "C" refer to Aqueous, Ethanol and Chloroform-methanol extracts respectively. Number of replicates in brackets

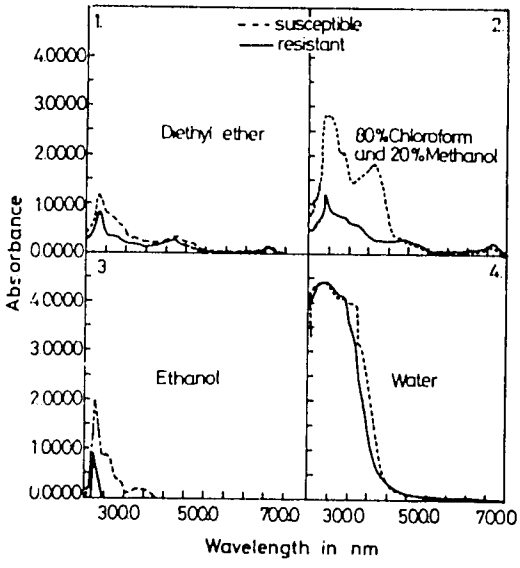


Figure 4. Absorption Spectra of 4 extract components from 2 maize varieties; Inbred A — susceptible — and ICZ2-CM—resistant

other hand, the ethanol extract component does not seem to have a feeding stimulant or deterrent. Observations at Mbita Point Field Station showed that *Chilo* larvae feeding in the whorl of maize plants developed better on the susceptible Inbred A than on the resistant ICZ2-CM. Factors partly responsible for this difference may be in the aqueous component. It is possible that the aqueous extract from Inbred A is more nutritious than that from ICZ2-CM thus accounting for the higher percent weight gain effected by the Inbred A extract. The bioassay test on sorghum clearly showed that Serena is very susceptible and possibly more nutritious than variety 18363, and it was noticed that variety 2146 is not as resistant as previously claimed (Figure 5). This concurs with recent observations at Mbita Point Field Station where it has been shown that 2146 is indeed not resistant and therefore adds to our confidence in the bioassay test.

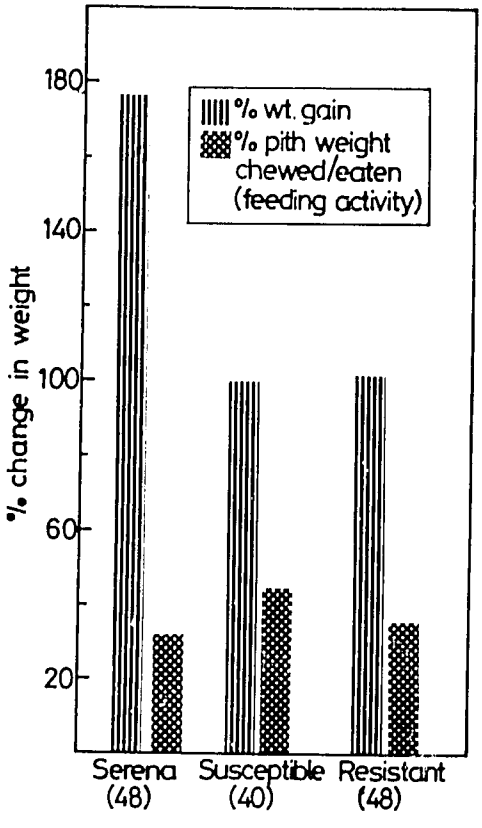


Figure 5. Responses of *Chilo partellus* larvae feeding on stems of sorghum varieties (Serena — very susceptible, 18363 — susceptible and 2146 — claimed to be resistant). Percent weight chewed/eaten is regarded as a measure of feeding activity.

Electrophysiology

In *Chilo* the resistant aqueous extract components evoked higher responses from the lateral styloconicum sensillum than in the medial styloconicum sensillum. The reverse was true for the susceptible aqueous extract component (Figure 6). This kind of response pattern differs

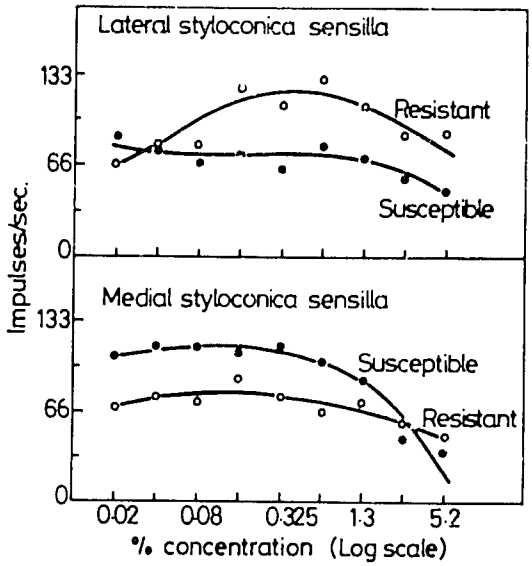


Figure 6. Response curves of *Chilo partellus* maxillary styloconica sensilla to aqueous extract components from Inbred A – susceptible – and ICZ2-CM – resistance – maize varieties

from that evoked by sucrose where the lateral styloconicum sensillum responds with increasing spike frequency to increasing concentrations of sucrose, while the medial sensillum does the reverse (see Twelfth Annual Report, 1984). In *Eldana* both sensilla responded positively to sucrose but the lateral sensillum is more sensitive than the medial sensillum. However, the 2 sensilla showed no differences in their responses to the susceptible and resistant maize aqueous extracts (Figure 7). One-way analysis of variance on the data for *Eldana* confirmed that the overall effect of sucrose was better than that of the extracts (Table 4), and it was shown that increasing concentrations of the aqueous extracts did not increase the response frequency of the 2 sensilla (Table 5). Differences in the response curves of *Eldana* and *Chilo* are not entirely unexpected. It is most likely a reflection of the feeding preferences of the 2 species. In the field, it is mainly *Chilo* larvae which attack the young maize plants used in the present experiment. *Eldana* larvae are usually found in much older maize plants.

Table 3. Overall response of *Eldana saccharina* sensilla to the three treatments

Treatment	Mean impluses per second generated by the two sensilla	
	Lateral	Medial
Sucrose	152±16.6	106±13
Susceptible extract	92± 4.6	110± 6
Resistant extract	102± 5.2	111± 5.2

$F_{2,216} = 30.7$

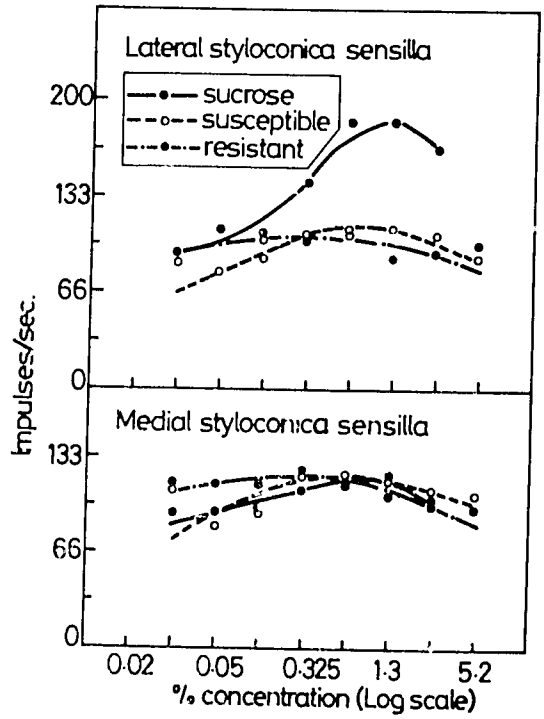


Figure 7. Response curves of *Eldana saccharina* maxillary styloconica sensilla to sucrose and aqueous extract components from Inbred A — susceptible — and ICZ2—CM — resistant maize varieties

Table 4. Overall response of *Eldana saccharina* styloconica sensilla to concentrations of the three treatments

Treatments	Mean impluses/second evoked by different concentrations (% WT/VL)					
	0.08	0.16	0.325	0.65	1.3	2.6
Sucrose	109±12.6	103±14	125±21	154±36	153±34	131±36
Susceptible extract	83± 9	93±12	109± 4.3	112± 9.6	107± 8	104± 7
Resistant extract	101± 8	113± 8	115±10	112± 8	98± 8	96± 8

$F_{10,216} = 2.87^{*}$ (for treatment x concentration interaction
Sucrose showed more than the other two treatments

The available data show that the *Eldana* maxillary sensilla detect no differences in the aqueous extracts tested. On the other hand, the antagonistic responses of similar sensilla in *Chilo* suggested that *Chilo* may use such subtle differences to discriminate between susceptible and resistant host-plant varieties.

Methods and results reported here are part of an extended study; the feeding bioassay will continue to be improved and will be used in studying the effects of host and non-host plant extract components. Electrophysiological tests will continue to be part of these studies. Special emphasis will be placed on collecting data from the palpal and labral taste sensilla of the borer larvae.

CHEMOCOMMUNICATION IN TSETSE FLIES

R.K. Saini

Studies are in progress, in cooperation with the Chemistry and Bioassay Research Unit and the Tsetse Research Programme, to identify the chemical factors involved in host and mate selection in tsetse flies. Screening of a large number of synthetic chemicals and natural products is being undertaken to identify attracting and repelling chemicals for possible use in tsetse control.

Studies on pheromone communication in tsetse showed that a fly can raise its pedicellus and flagellum on stimulation with the odour of the cuticular female sex pheromone. Behavioural studies were designed to observe whether similar antennal responses would occur on stimulation with various chemicals.

To facilitate these observations, tsetse flies were tethered and stimulated with odour emanating from 5 ml glass syringes containing a piece of filter paper (13 x 13 mm) onto which 10 μl of the chemical to be tested had been pipetted. Three chemicals were tested on each batch (comprising 10-13 flies) at any one time. In order to avoid any sequence effect, a paired 3 x 3 latin square design was used. The sequence of stimulation was alternated between the odour of a clean filter paper (control) and odour of the test chemical.

Initially, 10 different chemicals: acetone, methylethylketone, acetophenone, methylvinylketone, formaldehyde, urea, 1-octen-3ol, lactic acid, pentanal, and hexanal, were tested. It was observed that as soon as an attractive odour was applied in front of the antennae, the fly may raise its antennae from its normal resting position. Figures 8 and 9 show the percent antennal responses of 1 week old males and females respectively to 10 μl each of various chemicals tested. Responses are expressed as a percentage of responses to the odour of clean filter paper. It should be noted that since 10 μl of each test chemical was used, differences in volatility between the test compounds were not corrected for; therefore any comparisons among the antennal responses elicited by the various chemicals are relative.

In the males (Figure 8), acetone, methylethylketone, methylvinylketone, formaldehyde, 1-octen-3ol and pentanal showed activity which was 2-3 times more than the control. These chemicals were subsequently checked for attractancy

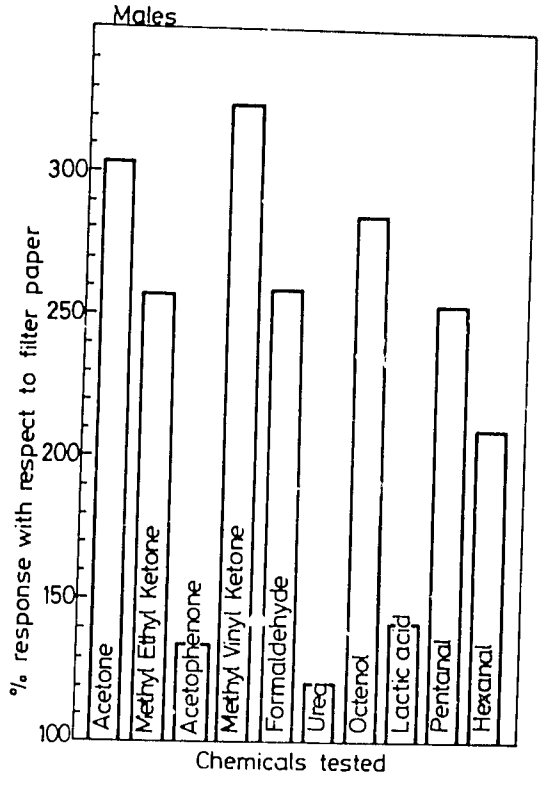


Figure 8. Behavioural (antennal) responses of 7-10 day old male *G. m. morsitans* to 10μl of various chemicals tested. Responses are expressed as a percentage of responses to the odour of clean filter paper (control).

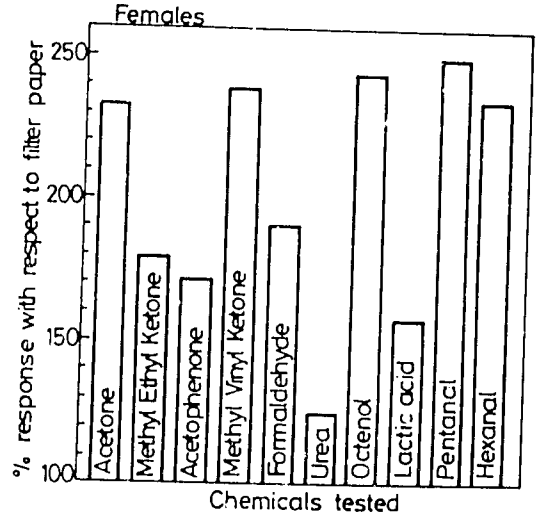


Figure 9. Behavidoural (antennal) responses of 7-10 day old female *G. m. morsitans* to 10μl of various chemicals tested. Responses are expressed as a percentage of responses to the odour of clean filter paper (control).

under field conditions in Nkuruman where it was confirmed that acetone, methylethylketone and octenol were attractants and increased trap catches 2-3 times. Methylvinylketone did not significantly increase trap

catches. In this case, the chemical underwent a marked colour change when exposed to field conditions suggesting that it was unstable under those conditions and possibly explaining its lack of activity in the field. Further laboratory experiments indicated that immediately after purification methylvinylketone was more active and elicited more antennal responses than when the chemical was aged and colour change had commenced. Filed-trapping experiments in Zimbabwe indicated that methylvinylketone, formaldehyde, and pentanal are attractants which double trap catches. Urea, lactic acid and acetophenone elicited antennal responses which were not significantly different from the control. It is interesting to note that lactic acid and acetophenone have been confirmed to be repellents by Vale in Zimbabwe.

Female antennal responses basically showed the same trend as the male though overall responses of females were significantly fewer than those of males (Figure 9). Since results of the antennal responses show a certain degree of correspondence to results obtained by field-trapping experiments, antennal responses may be used to identify various attractants instead of using flight chambers and olfactometers which are time consuming and usually unsatisfactory.

Electroantennograms (EAG's) also provide a quick idea about the sensitivity of olfactory receptors to odours. EAG's from tsetse antenna were recorded by inserting one electrode into the pedicel of an amputated antenna while another electrode was brought into firm contact with the tip of the funiculus without piercing it. No EAG's could be recorded when the funiculus tip was pierced by the electrode. When odours were blown over the antenna (in this case an odour was injected into a constantly flowing air-stream) an EAG response was elicited. Figure 10 shows EAG recordings from 1 week old male stimulated with the air stream from a clean filter paper and filter papers loaded with 10μM of 11 different chemicals. The EAG's amplitudes of about 4 mV were evoked by chemicals such as 1-octen-3ol, methylpropylketone acetone and methylvinylketone. Other chemicals like acetophenone and lactic acid evoked EAG amplitudes of less than 1 mV. EAG values varied slightly between antennae and were highly reproduciable within 1 antenna. It should be noted that an EAG gives no indication about the behavioural effect associated with the stimulating odour and reception and essential odour components may evoke weak responses because only a few specific receptors for those components may be present on the antenna.

Electrophysiological and behavioural techniques are being used to identify the attractive substances in buffalo urine which has been shown to be a potent attractant for tsetse flies in the field. Structure-activity studies on 1-octen-3ol analogues are also in progress. The objective of these studies is to find ways of optimising the activity of this compound.

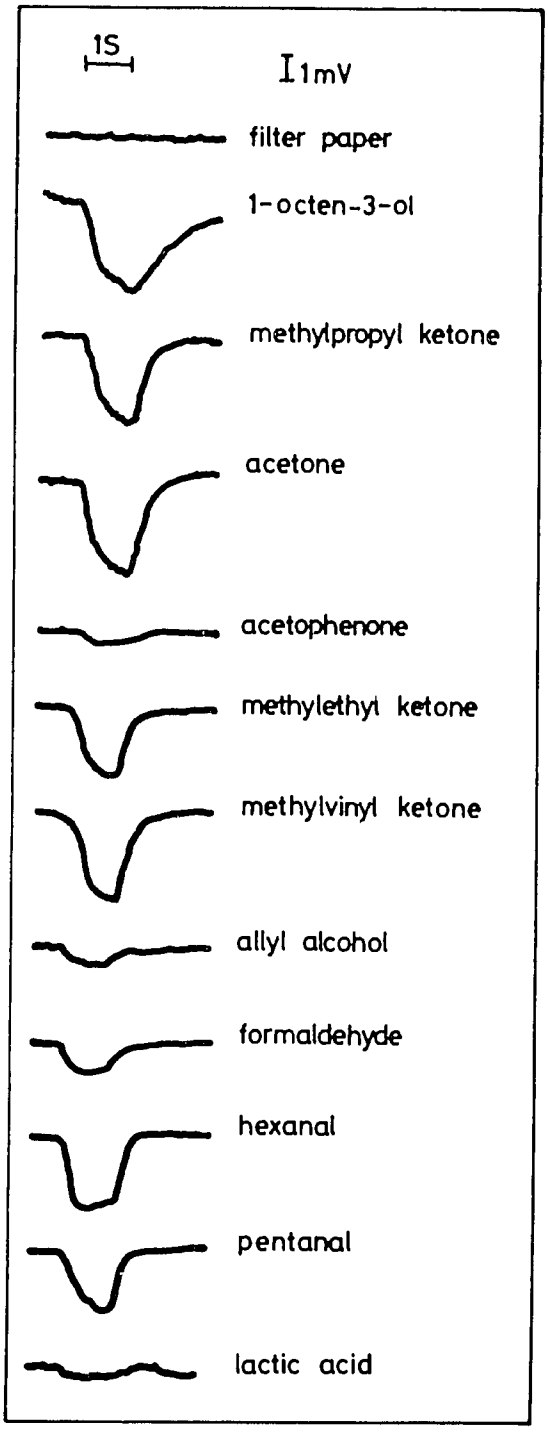


Figure 10. EAG recordings from a 1 week old male *G. m. morsitans* on stimulation with the odour of clean filter paper and 10 μM of 11 different chemicals. Horizontal line above the records: duration of stimulation; vertical calibration refers to all recordings

Outreach and Training

Outreach and Training Unit

Although ICIPE has been conducting short group training courses since 1977 the extension of these efforts to higher level long-term training was only started in 1983 with the postgraduate programme. Collaborative Research and Development was initiated to facilitate the testing of pest and vector management strategies at the national level through national programmes as well as at regional and international levels. The development of an African Regional Pest Management Research and Development Network as one aspect of such collaborative work is seen as a major step forward in the sharing of the continent's scarce scientific material and manpower resources for the development of pest and vector management strategies to reduce hunger, malnutrition and disease. The outreach programmes are essentially interactive programmes that take results, ideas and techniques from their origin to new areas. The origin of these new techniques may be the ICIPE or other collaborating institutions. There are three outreach programmes: PESTNET, CORD and FAMESA.

OUTREACH AND TRAINING PROGRAMMES

Overview

One of the guiding principles underlying ICIPE's outreach and training activities is to establish research cooperation with key international research centres and laboratories throughout the world as well as with national programmes, particularly those in Africa, to facilitate research on the testing and demonstration of pest control strategies. ICIPE continues with its objective intention of proiding training in research methods and techniques for predoctoral and postdoctoral fellows as well as young practitioners in insect science and technology.

Our two distinct but complementary programmes are summarised below:

Outreach principally covers collaborative research and development activities and comprises:

- Cooperation with national research and extension services
- Collaboration with international research centres, and international and regional agencies
- Relationships with laboratories throughout the world engaged in research and development in areas related to insect science.

Training at the ICIPE is conducted in three major areas:

- Professional scientific training is undertaken at both the postgraduate and postdoctoral levels. The former is done in close collaboration with several African universities except, in special circumstances, when students are permitted to conduct research exclusively at the ICIPE.
- Short-term training courses in pest and vector management are offered to practising insect scientists and technologists from the developing world.
- A programme for the upgrading of special skills and techniques is extended exclusively to the staff of the ICIPE.

COLLABORATIVE RESEARCH AND DEVELOPMENT

The fundamental objective of the ICIPE research is to provide solutions to food production problems of resource-poor farmers and improve rural human health in the tropics. Within this general focus, the Centre has given increased attention and special emphasis to collaborative R & D activities.

Scientists at the ICIPE, in their respective research programmes, work closely with related world wide research laboratories and institutions. Collaboration can be on a one-to-one basis between individual scientists, or through a

more structured agreement between the ICIPE and other research centres or institutions.

ICIPE's Collaborative R & D programme seeks to test, disseminate and promote application of the information and technology developed by the Centre. We can identify the programme's specific objectives as:

- To facilitate further testing in the field of ecological-integrated pest and vector control strategies at both the national level and at selected international centres.
- To develop a regional or international network to expedite the testing and application of information and technology packages developed by ICIPE.
- To develop national capacities to enhance ICIPE's objectives, utilisation of pest management technology and the creation of contact points with national research and development systems.

Cooperation with National Research and Extension Services.

The major thrust of our collaboration with national programmes is through the African Regional Pest Management Research and Development Network (PESTNET). Arrangements exist for cooperation with national research and extension agencies through PESTNET to conduct multilocation trials and field tests of results from various ICIPE research programmes. Collaboration with national programmes is most often at invitation to ICIPE by national government agencies, including universities, regional and local research institutions.

African Regional Pest Management Research and Development Network (PESTNET)

The designated objectives of the PESTNET are:

- To test in association with national programmes the performance and adaptability of new knowledge and technology developed at ICIPE, under varying ecological conditions, different farming systems and varied socio-economic circumstances.
- To facilitate the dissemination of research information to national programmes.
- To generate (in collaboration with specific national institutions) location-specific information and technological packages.
- To facilitate the sharing of information among PESTNET member countries.
- To create a mechanism for effective understanding, through feed-back responses from national programmes, of pest management problems as well as analysing the impact of PESTNET activities.

To realise these objectives, ICIPE works with and bases its collaborative activities within national institutions. PESTNET activities are organsied to comprise three components, namely information generation, information verification (testing and field scale trials), and training. The well-focussed networks are: the livestock ticks PESTNET with their major thrust towards field scale trials of the knowledge ICIPE has gained in the immunisation of cattle against the tick *Rhipichephalus appendiculatus*; and the crop PESTNET where emphasis is on multilocational tests of information/knowledge gained on varietal resistance to stem borers of sorghum and maize, mixed cropping system as components of pest management in cowpea-based and maize-based cropping systems, and the application of biological control in subsistence-farming conditions.

A satisfactory base already exists, particularly in the training compoent of the PESTNET, for establishment of the tsetse, the medical vectors and the Pest Management Information networks. An exciting element of the future of PESTNET is the technical cooperation among the network countries, particularly the interchange of scientists within the PESTNET zone.

Collaboration with CGIAR Centres

Priority is given to close-working relationships with the majority of the Consultative Group for International Agricultural Research (CGIAR) centres. ICIPE has inter-institutional cooperative research and training agreements with the International Rice Research Institute (IRRI) in the Philippines and the International Institute of Tropical Agriculture (IITA) in Nigeria. Effective linkages exist between the ICIPE and other CGIAR international centres including International Laboratory for Research in Animal Diseases (ILRAD) (Kenya), International Livestock Centre for Africa (ILCA) (Ethiopia), International Wheat and Maize Improvement Centre (CIMMYT) (Mexico), International Centre for Research in Semi-Arid Tropics (ICRISAT) (India), and also International Council for Research in Agroforestry (ICRAF) (Kenya).

Cooperation with Donor and other International Agencies

Research capabilities of national systems, in vital areas of insect science particularly pest and vector management, are strengthened through collaboration among ICIPE, donors, and other international agencies.

For example, ICIPE presently collaborates with:

European Economic Community (Research and Training)
GTZ (Research and Training)
International Atomic Energy Agency (Research and Training)
International Development Research Centre (Training)
International Fund for Agricultural Development (Research)
Overseas Development Administration (Research and Training)
United Nations Development Programme (Research and Training)
United Nations Environment Programme (Training)
UN Food and Agriculture Organisation (Research and Training)
UN Scientific and Cultural Organisation (Information Systems)
United States Agency for International Development (Research and Training)
UN World Health Organisation (Research and Training)

Linkages with Centres of Excellence and Research Laboratories

ICIPE, in furtherance of the principal objectives of its mandate to undertake fundamental research, establish research cooperation with key institutions throughout the

world, provide advanced training in research methods and techniques, as well as providing a forum for exchange of knowledge to promote the growth of the scientific community in the tropics, identified a clear need for close linkages with centres of excellence and research laboratories throughout the world to complement its own research capacity, particularly in the area of basic research. Collaboration with these institutions emphasises the exchange of scientists and technicians, the provision of services and the use of each others' laboratories to investigate critical scientific problems. The aim is to enhance scientific excellence, increase inter-institutional flow of new ideas and talent and to broaden the experience of scientists and technicians involved in exchange programmes of collaborating institutions.

COLLABORATIVE PROJECTS

ICIPE already has a number of both formal and informal collaborative linkages with national programmes in the areas of research and training on maize and sorghum resistance to stem borers (Kenya, Somalia), sensory physiology and fine structure research in relation to crop borers (Ivory Coast), research and training in relation to tsetse and ticks as vectors of livestock diseases (Kenya, Zambia, Tanzania, Uganda) and research on leishmaniases (Kenya, Sudan). Collaborative projects undertaken with international centres and research institutions include research on the brown plant hopper (International Rice Research Institute, Philippines), germplasm testing and development of the cowpea-based mixed cropping component of pest management (International Institute of Tropical Agriculture, Nigeria), testing of maize germplasm against major crop borers (International Wheat and Maize Improvement Centre, Mexico), testing of sorghum germplasm against stem borers (International Centre for Research in Semi Arid Tropics, India), training in research methodologies applied to tsetse and livestock ticks ecology (International Livestock Centre for Africa, Ethiopia), research on immunological aspects of livestock ticks (University of Neuchatel, Switzerland), and plant protection research and training (OAU InterAfrican Phytosanitary Council).

In affiliation with OAU Scientific, Technical and Research Commission and IITA, ICIPE is actively involved in the Africa-Wide Project for Biological Control of Cassava Pests (ABCS). ICIPE's main contribution is in the area of applied basic research and training; its input is complementary to the on-going IITA research efforts in the ABCS project.

Some of ICIPE's knowledge on environmentally safe and economically feasible strategies for crop pest management in subsistence farming conditions is being tested in Kenya, in collaboration with the UN Economic Commission for Africa and FAO. This is a joint project on the reduction of food losses through insect pest management and the use of small scale cost-effective farm equipment.

Continuing Relationship

From the beginning ICIPE has recognised that development is a process. With this in mind, we endeavour to maintain a policy of ongoing contact with national and international collaborators to ensure permanent partnerships in development.

TRAINING PROGRAMMES

The primary objective of ICIPE's various training programmes is to build up and strengthen scientific and technological capacities of tropical developing countries. Specifically, through its training programmes, ICIPE seeks to enhance national human resource capabilities in the areas of insects and insect-related sciences, facilitate interaction and the exchange of experiences and information among national programme scientists, and to develop a dependable collaborative competence in national research and extension systems.

Training programmes at the ICIPE are of mutual benefit. While increasing the number of competent research workers for the tropical developing countries, they also contribute effectively to the research resource capacity of the ICIPE. Another advantage is to provide the Centre's scientific staff with the opportunity to keep in close contact with the increasing number of national scientists and technicians and to understand better pest and vector problems crucial to agriculture and rural health in the tropics.

Categories of Training Offered by the ICIPE

PROFESSIONAL SCIENTIFIC TRAINING PROGRAMMES

African Regional Postgraduate Programme in Insect Science (ARPPIS). ARPPIS is a collaborative programme between ICIPE and a consortium of African universities. M.Sc. graduates in insect or insect-related sciences, from any of the African countries, register for a Ph.D. degree of a University in the consortium, but undertake course and research work at ICIPE under the supervision of ICIPE scientists. The three-year ARPPIS academic programme includes a six-month period of compulsory coursework, and a research study on a topic which is carefully selected to fit the background and interest of the student, but also acceptable to the ICIPE and the collaborating university. The participating universities and the ICIPE cooperate in finding scholarships for those who are accepted for admission to ARPPIS.

Postdoctoral Research Fellowship Programme. The postdoctoral research fellowships are meant to offer young Ph.D. graduates the opportunity to develop research-related experience in insect or insect-related sciences. The fellowships are tenable at the ICIPE or at participating institutions and in areas where ICIPE has established collaborative projects. The fellowships are open, on merit, to qualifying graduates particularly from tropical developing countries.

Postgraduate Attachments. In special circumstances ICIPE permits students to register with a university for a Master of Science or Ph.D. degree and to conduct their research at ICIPE, under the collaborative supervision of a

senior ICIPE scientist and the student's academic supervisor. Before negotiations for postgraduate attachments are entered into, the candidate must be registered at a university, have a university advisor, be assured a source of funding and have chosen a research project which both falls within the already existing programme of the ICIPE and is acceptable to the university.

SHORT-TERM TRAINING COURSES

Group Training Courses. Three categories of Group Training Courses in the area of pest and vector management are organised at the ICIPE for young scientists from the tropical developing world; they are:

- International Group Training Course in Pest and Vector Management Systems
- ICIPE-EEC Training Courses on the Management of Vectors for the Control of Trypanosomiasis and East Coast Fever in Livestock Production
- The International Training Course on Insect Growth, Development and Behaviour
- Training for Self-Reliance in Ecological Pest Management in Africa

The objectives of these courses are to acquaint young scientists from the tropics, employed in agriculture as well as livestock and public health, with recent advances in the field of pest and vector management and environmental ecology; and to demonstrate the efficacy of new technologies in insect population management with emphasis on ecologically sound approaches to pest and vector control.

Participants for the Group Training Courses are drawn from every part of the tropics. Consequently, this series of courses serves to provide a forum for the exchange of ideas and experiences among individuals from different parts of the world and to establish relationships between scientists and experts in the relevant fields. Group Training Courses last two to three weeks.

In-Service Training and Fellowship Schemes. In-service training courses are aimed at meeting specialised training needs of technical and scientific workers from national and selected international institutions. They provide mid-career scientists with the opportunity to gain experience through working with more experienced scientists on on-going research programmes and acquainting national scientists and technicians with appropriate techniques and methodologies in pest management research as well as with recent developments, approaches and techniques for use in their areas of expertise and employment. In-service training programmes are designed and developed jointly with cooperating institutions.

Training in Financial and Administrative Management of Research Projects (in collaboration with the Programme FAMESA). This training is aimed at developing the management skills of scientists engaged in national scientific research institutions and is conducted in collaboration with the ICIPE-based Project on Financial and Administrative Management of Research Projects in Eastern and Southern Africa (FAMESA). The training was initiated after recognition of the critical need for qualitative scientific leadership in national institutions; capable of planning, designing, executing and evaluating the performance of scientific and industrial research projects.

Participants at the Yaounde course in a group picture taken around a cocoa plant with many pods at Barombi-Kang Cocoa Research Station during a field visit.

Biostatistics and Computer Services

ACCOMPLISHMENTS

H.F. Magahi

The Biostatistics and Computer Service Unit became fully operational during 1983 with 2 full-time and 1 part-time employees and remained so staffed until late in 1984, when 3 additional staff members were hired. However, the operation of the Unit was decentralised in 1985 with the installation of 7 microcomputers (2 at Mbita, 4 at Chiromo and 1 at Duduville) and 4 2200 LVP work stations at Chiromo. The following were accomplished during the past year:

(i) Provided biostatistics and computer facilities to Mbita Point Field Station (MPFS). Installed 2 microcomputers at MPFS in January and conducted training on computer applications in February. The Data Systems Manager (DSM) taught FORTRAN 77 for 5 days in February to MPFS research staff. Word Processing and BASIC were also taught to research staff at MPFS by Mrs. P. Alila and Mr. J. Maina. Mr. F. Obeya, a full-time operator/programmer, was assigned to work with MPFS staff. He maintains programmes used by the scientists, assists in data analysis and supervises training on software packages including Introduction to Word Processing. The DSM regularly visits MPFS for consultations in programming, designing of experiments, data processing and analysis.

(ii) The DSM has developed and written 28 FORTRAN programmes for data processing and analysis and is testing 7 others in the mainframe computer, VS 80. Sixteen programmes from R. Davies Computer Programming and 6 from Biometry were rewritten and tested and are presently being adapted for the microcomputers. As can be seen from the section on computer softwares/packages, the Unit has acquired and is testing many software packages for statistical analysis. With the supplement of the above FORTRAN programmes the Unit is able meet the computing needs of our research scientists.

(iii) Provided a computerised data storage system for the research scientists so that raw data can be stored in appropriate formats prior to analysis. The Tsetse and the Crop Pests Research Programmes have accumulated massive amounts of data in the mainframe computer and the Medical Vectors Programme has just completed inputing several years' research data. Two-way tables have been constructed and analysis of variance were completed using a Least Squares procedure. Graphics using the PC are also being used to analyse this data. Programmes for data inputing, merging, frequency tabulations and least squares were developed and written for this data by the DSM. These programmes were made so user friendly that only the file names are needed to run them, since the parameters needed are in the default.

(iv) Simulation models are being developed for the tsetse and certain crop pests populations. A random number generator was developed for the PC to simulate modeling in crop pests. Shortly a simulation model will be started in livestock ticks population. The simulation models being developed have still to use both the field research and weather data. There is still much to be done in this area; considerable coordination is needed among the research scientists, the programmer and the DSM.

(v) Courses in computer programming and biostatistics were offered to ARPPIS students and other training groups at the ICIPE. The DSM conducted the following:

- (a) a one-week series of lectures in FORTRAN 77 to MPFS research staff, February 1985
- (b) a two-hour lecture-demonstration on Microcomputers to the ICIPE staff at Chiromo, April 1985
- (c) a two-hour lecture-demonstration on Computer and Data Analysis to the 8th International Group Training Course at DIGC, July 1985
- (d) a 5 day (2-hour lecture and 4-hour laboratory per day) course in computer programming in

FORTRAN 4 for ARPPIS and other training groups, August 1985

(e) a three-hour lecture-demonstration on microcomputers to the African Association of Science Editors Conference, at Silver Springs Hotel, Nairobi August 1985.

Dr. R. Dransfield taught a biostatistics course for the ARPPIS students for 10 days in August 1985. Dr. Dransfield continues to give assistance to the research staff of the ICIPE on experimental design, data processing, analysis, interpretation and presentation of results. His invaluable assistance is herewith acknowledged with gratitude by the Unit.

(vi) Training in computer operations and applications is given to the staff of the ICIPE, selected institutions in Kenya (refer to collaborative arrangements) and other African countries (ARPPIS and African Association of Science Editors, at present). The Senior Computer Programmer conducted the following: (a) training of Administrative staff on the features of the Wang PC, hands-on experience and database, January 1985; (b) training of the Communications Division staff on graphics and database, July 1985; (c) training of 19 Research Division staff on graphics and MSTAT, September to October 1985; (d) training of 5 staff members from the Director's Office on the operations of the PC, and an introduction to different software packages of the PC for 3 days in October 1985.

(vii) Computer support is being extended to international conferences. The Protozoology Congress was assisted by the Unit last June and the International Development Research Centre (IDRC) similarly was given computer support for the African Association of Science Editors Conference in August 1985. A mailing list was completed for the International Conference on Tropical Entomology to be held in Nairobi during 1986. Mrs. Ssebunnya participated in systems analysis, programming and data preparation for the VII International Congress for Protozoology and the Tropical Entomology Conference. In both conferences the data are inputed in the VS 80 and then transferred to the PC (microcomputer) at the conference site. Mrs. Ssebunnya has also developed a computerised list of books, periodicals, and journals for the Special Committee for Africa.

(viii) A personnel information system is presently in operation in the Administration Division using data base in microcomputer. A payroll system is running in our mainframe computer, the VS-80 system, producing about fifteen reports for the Financial Division. Mrs. Ssebunnya and Mr. Maina, working together, reduced the time for data input and processing of the payroll from 3 to 1 day. The provident and gratuity fund system was completed in December 1985 by Mr. Maina using 10 BASIC programmes in the Wang PC. This system will be operational by January 1986, after inputing of data, checking of the printouts and training of the Accounts staff. This system will run concurrently with the manual system for several months to check for possible errors in the computerised system. Mrs. Alila input the data using word processing for the ICIPE 1986 budget as prepared for the Planning and Development Unit. She conducted hands-on training in Word Processing for 28 staff members of the ICIPE and completed the first draft of the Word Processor Manual and the rules and regulations for using the PCs and OIS. Mrs. Alila also coordinates, together with the DSM, the interfacing of the WPI/CRTronic (phototypesetter) to the Wang PC's Word Processor.

(ix) The Unit is now preparing a manual of rules, regulations and guidelines to be used in operating the VS 80, OIS, PCs and the 2200 LVP computers. The first draft was completed in December 1985.

COLLABORATIVE ARRANGEMENTS

(i) Three students from the Kenya Polytechnic were trained for 5 months (April to August 1985) on computer operations and applications (refer to achievements).

(ii) Collaboration with the Ministry of Agriculture and Livestock Development for the 1-month training of 2 biostatisticians, August 1985 (refer to achievements).

(iii) Collaborative arrangements were made with ILRAD and ICRAF to study hardware and software requirements in the computerisation of bibliographies. As a result of information gained from this association, a software package was purchased for the Library and Documentation Department which is presently being tested on a Wang PC.

(iv) Computer support is being extended to International Conferences (refer to achievements section).

(v) The Planning and Development Unit, in collaboration with the Outreach and Training Unit and the Biostatistics and Computer Service Unit, submitted a proposals to the British Council to fund in-house training in Statistics and Data Analysis for research staff at both Chiromo and MPFS.

COMPUTER SOFTWARE

(a) Mainframe (VS 80):

- Installed the FORTRAN 77 in September 1985, the newest and most powerful FORTRAN available. It will replace the FORTRAN 4 presently being used. The FORTRAN 77 can create random access files and incorporates COBOL formats. However, this computer language requires a significant amount of computer memory.
- Used FORTRAN and BASIC for scientific applications. This solved the problem of lack of software in the mainframe. A manual for the 28 FORTRAN programmes which were developed and written is being compiled.
- System utilities such as DATAENTRY, CONTROL, EZFORMAT, INQUIRY and REPORT, and COBOL

are being used for data processing. This is useful in the Payroll System, for the inputing of data for the mailing lists.

(b) Microcomputers

- Used BASIC, MULTIPLAN and LOTUS 1-2-3 for Financial Management Applications (refer to achievements).
- Graphics package for all applications.
- Database for Personnel Information System (refer to achievements).
- MSTAT, a statistical package, installed at MPFS in January and at Chiromo in June. This is being used by many research scientists for data input, editing and analysis. A new enhanced version of MSTAT arrived in December and is being evaluated by the DSM before it replaces the old version.
- Used FORTRAN and BASIC for analysing data of scientists which MSTAT is unable to process. Many of the FORTRAN programmes for the mainframe are be written for the microcomputers.
- P EA, a database with statistical programmes, was installed in October at Chiromo and is being utilised by the Ticks Research Programme. This software package is being studied for use with other research data.
- ST TISTICIAN, another statistical package is being tested. Some of the subprogrammes from this package are being rewritten for the mainframe computer, the VS 80, to add to the list of scientific application programmes. It will take about 3 months to complete the evaluation of this software package.
- IN-MAGIC, a database, was acquired in November for bibliographic material applications. This is being tested and training for the use of this package will begin in January 1986.

EQUIPMENT

- Purchased 4 removable 15 MB disks to alleviate data storage problems in the mainframe computer caused by the entry of enormous data from 3 research projects.
- Installed 5577V dot matrix printer in the VS 80 system to increase the printing speed in word processing and will upgrade our graphics capability with the purchase of a graphics package for the mainframe computer.
- Installed 7 Wang PC microcomputers (2 at MPFS, 4 at Chiromo and 1 at DIGC). This brought the computers closer to the user and links the PC to the VS. This increased the data and word processing capabilities of the CIPE. The Personnel Information and Financial Management systems are being developed and run in the PCs.
- Installing 2 2200 LVP microcomputers (1 at Chiromo and 1 at MPFS) in January 1986 after testing the hardware at Chiromo for several weeks. Each microcomputer will have 2 printers and 4 work stations. This will solve the shortage in word and data processing work stations experienced at Chiromo and MPFS. The LVP for Chiromo is being tested and the softwares are being loaded into the system. Some software packages are being converted from 308 KB to 1.2 MB diskettes by Computer Applications Limited in their 2200 LVP computer.
- Installed the Uninterruptible Power Supply (UPS) in July to protect the data as well as the VS 80 system hardware from power outages.

Communication and Information Division

The main highlight of the year was the celebration of the ICIPE's 15th anniversary, which coincided with the Annual Research Conference and Governing Board meetings, held at Duduville International Guest Centre from 14th — 22nd April 1985. The official opening was performed by Dr. John W. Koehring, Director, USAID — Regional Economic Development Services Office (REDSO) for Eastern and Southern Africa, and was witnessed by an unprecedented number of invited guests and observers from all over the world. The week's programme included special guest lectures in addition to the usual Annual Research Conference presentations. The guest lectures will be published in book form, and will be part of a regular series within the publishing programme of the proposed ICIPE Science Press.

Another significant highlight for the Communication and Information Division was the Planning Workshop for the African Regional Pest Management Research and Development Network (PESTNET), which is discussed in greater detail elsewhere in this Report. PESTNET is a major point of departure, as the ICIPE seeks to streamline and strengthen its interactive linkages with the national programmes in Africa. The Network will bring new challenges in terms of the Centre's communication needs, and its success may well depend on how effectively it is able to disseminate and utilise the information generated by its members, with the ICIPE playing a pivotal role.

The video documentary mentioned in last year's Annual Report was launched during the year and has been very well received. Apart from being used by the Conference and Liaison Department to give visitors a general overview of the Centre's activities it has also been screened at donor meetings in Washington, D.C., Rome, Stockholm and Copenhagen. The documentary will be updated from time to time, and it is hoped that funds will be available to enable the Centre to make more extensive use of this very effective communication channel.

In general the year was one of consolidation, after the agressive initiatives of 1984 which were aimed at improving the dissemination capability of the Centre. Further new initiatives are planned, with the proposed ICIPE Science Press as the main focal point. These new initiatives, together with the advances already made over the last two years, should enable ICIPE's information-related services to match the excellent standards already achieved in research and training.

CONFERENCE AND LIAISON
R. Washika

15th Annual Research Conference

The 1985 Annual Research Conference marked the climax of activities planned in celebration of ICIPE's 15th Anniversary. The Conference programme was more elaborate this year featuring, in addition to the annual review of the ICIPE's entire programme of research and training, a series of guest lectures that ran every evening of the Conference week. The official opening, by Mr. J.W. Koehring, Director of USAID's Regional Economic Development Services Office for Eastern and Southern Africa (REDSO/ESA), was followed by a special guest lecture on "The Origins and Model Role of the ICIPE in the Third World" fittingly presented by Dr. Victor Rabinowitch, a staunch supporter of the ICIPE at its founding and presently the Executive Director, Office of the International Affairs, U.S. National Academy of Science.

Other lectures delivered during the 15th Annual Research Conference included:

Developmental Biology and Molecular Genetics: Perspectives and Contributions to Insect Science, *Professor Klaus Kalthoff, University of Texas at Austin.*

Host Immune Response to Feeding by Ticks: A Natural Biological Control Mechanism, *Dr. Stephen J. Brown, University of Illinois.*

Molecular Events in Insect Patterning and Morphogenesis, *Dr. Danny L. Brower, University of Arizona.*

Insect Growth Control, *Professor Peter J. Bryant, University of California, Irvine*

The Conference week also marked the inaugural presentation of ICIPE's Medal for Innovative Research, a medal to be awarded each year for innovative or creative work in fundamental or applied research. The first winners were Dr. M.J. Mutinga, Programme Leader, Medical Vectors Research Programme, for his work on "The Epidemiology of *Leishmania major* in Marigat, Baringo District, Kenya" and Dr. R.K. Saini, for his work on "A Novel Method for Screening Chemicals for Attractancy in Tsetse". Several of the ICIPE staff received Long Service Awards having given 15 years of continuous and dedicated service to the ICIPE.

Taking advantage of the presence during the period of the 15th Anniversary Conference of a cross-section of the scientific community and science administrators, the following meetings were simultaneously held at Duduville:

- Scientific meeting of the East African Society of Parasitologists.
- Planning meeting for the 1986 International Congress on Tropical Entomology.
- First meeting of the Sub-regional Executive Committee of the African Biosciences Network.

Visitors

During 1985 a number of distinguished guests visited the Centre including:

- Mr. Edward V.K. Jaycox, Vice-President for Eastern and Southern Africa Region of the World Bank, accompanied by Mr. James Adams, Director of the World Bank Resident Mission in Kenya. They held wide-ranging discussions with the ICIPE Management and invited Kenya Government officials to lunch at Duduville, the proposed site for ICIPE's headquarters which is being partly funded by the World Bank.
- The Ambassador of the Federal Republic of Germany, His Excellency Mr. Johannes von Cocano, accompanied by Embassy Officials, spent a day at Mbita Point Field Station visiting with staff of the Crop Pests Research Programme.
- Dr. Sam Nilsson, Executive Director of the International Federal of Institutes for Advanced Study (IFIAS) gave a well-attended seminar entitled *"IFIAS and the Major Global Issues"*.
- Over 15 Senior Officials from the Ministry of Agriculture and Livestock Development, Kenya, led by the Director of Agriculture, Dr. S.N. Muturi, and the Director of Research, Mr. W.W. Wapakala, spent a day at the ICIPE Headquarters and a day at Mbita Point to acquaint themselves with research activities at the ICIPE and discuss strategies for collaboration in research and testing of research results.
- Mr. A.L. Mbiele, Scientific Secretary, OAU Inter-African Phytosanitary Council (IAPC), Yaounde, Cameroon, discussed the ICIPE's training collaboration with IAPC.
- Prof. B.M. Honingberg, Director of the Centre for Parasitology at the Marrill Science Centre, University of Massachusetts, Amherst Centre, USA, worked at ICIPE for 6 months in the Tsetse Research Programme.
- Dr. Guy Chauvet and Dr. Michel Remillet, a visiting mission from ORSTOM, discussed longterm funding of the ICIPE by ORSTOM.
- Mr. W.D. Guthrie, United States Department of Agriculture, Iowa, and Professor Larry L. Murdoch, Purdue University, West Lafayette, reviewed the ICIPE's Crop Pests Research Programme for IFAD.

International Study Workshops

Planning Workshop on the African Regional Pest Management R. & D Network (PESTNET) for Integrated Control of Crop and Livestock Pests.

This Workshop was held at the Duduville International Guest Centre (DIGC) 7 to 9 October, 1985, attracting over 30 participants from various national and international institutions concerned with crop and livestock production. Among the presentations were status reports on crop and livestock research at the ICIPE as well as research from all the countries represented at the Workshop.

The following recommendations emerged from the workshop that:

- PESTNET be formed to cover the areas of crop pests and ticks.
- A Secretariat be organized, based at the ICIPE, to facilitate and coordinate PESTNET activities.

- The goal must be to reach the full exchange of scientific information and experiences among participating members of PESTNET.
- PESTNET to identify methodologies, information and technologies to meet the needs of national programmes.
- Both the crop pests and tick sub-networks need to define the extent of the individual networks by specifying the pests and specific problems to be studied and identify the countries and organizations who would benefit from and contribute to the network.

To facilitate implementation of the above recommendations, two committees were formed, one on crop pests and the other on ticks. These committees are already active and have completed many of the details specified in the above recommend .tions.

Planning Workshop for the Establishment and Promotion of Neurosciences Activities in Africa.
On behalf of the International Brain Research Organization (IBRO), the ICIPE, in conjunction with KEMRI, acted as the local organizers, coordinators and hosts of the Planning Workshop for the Establishment and Promotion of Neurosciences Activities in Africa held at Dudubille International Guest Centre on 18 and 19 December 1985. The Workshop was sponsored by IBRO and UNESCO and attracted more than 15 participants from various parts of Africa

Among the important recommendations made was the formation of an African Neurosciences Task Force under the chairmanship of Prof. Thomas R. Odhiambo. Director of ICIPE. This task force was charged with a number of duties, among them the compilation of a directory of neuroscientists in Africa and the formulation of a draft constitution for the proposed Society of Neurosciences in Africa.

EDITORIAL AND PUBLICATIONS

Joy Mukanyange, Winnifred Oyuko, Newton Mwanga

The unit continues to render services to the Centre in the areas of graphics, photography, editorial and publications production.

The above services showed marked improvement during the year under review. New techniques for preparing slides (with colour), illustrations and posters were adopted, greatly enhancing the visual presentation of the Centre's work.

The Twelfth Annual Report, 1984, was produced with a 4-colour cover for the first time in many years. The Dudu, our quarterly newsletter is now more focused, concentrating on a specific aspect of ICIPE's research for each issue. During the year, the newsletter highlighted research on tsetse fly and chemistry and biochemistry.

Other publications were produced including, the ARPPIS Third Annual Report, a brochure for the Group Training Course as well as the proceedings of the planning workshop on the African Regional Pest Management R & D Network (PESTNET) for Integrated Control of Crop and Livestock Pests.

The unit also is responsible for editing scientific papers for publication in international journals as well as translation, particularly, translation of abstracts for papers being published in the journal, *Insect Science and Its Application*. A list of ICIPE staff publications appears at the back of the report.

Scientific Editorial Unit

Professor Thomas R. Odhiambo, Editor-in-Chief, Serah Mwanyeky, Esther A. Opere

The Unit continues to receive many manuscripts for the journal, *Insect Science and Its Application*, from all over the world. The adopted refereeing system has been very successful and the period between receipt of manuscript and its publication has been reduced considerably.

The journal in its sixth volume is bimonthly and publishes mini-reviews, original research papers, book reviews, information on new patents related to insect control, obituaries of prominent insect scientists, and from this year, the Software Survey Section has been launched. The journal also carried the announcement of the Internation Conference on Tropical Entromology held in Nairobi from 31 August to 5 September 1986, whose secretariat is the ICIPE.

The proceedings of the International Study Workshop on Host Plant Resistance and Its Significance in Pest Management, convened and held by the ICIPE in Nairobi, 10 - 15 June 1984, have been published in special issue of the journal (Volume 6 Number 3) entitled *Host Plant Resistance and its Significnace in Pest Management.* Guest editors were K.N. Saxena and J.K.O. Ampofo.

LIBRARY AND DOCUMENTATION

The aim of the service is to enhance and facilitate research on crop pests and medical vectors of tropical diseases by providing an effective library and documentation service to the researchers, trainees and other ICIPE staff, and to cooperate with other organizations for maximum use of research results. This goal continued to guide the library's action programme during 1985 as outlined hereunder.

Acquisition

The library added 331 books and monographs but maintained the number of its current periodicals at 130 titles out of which 90 are subscriptions. Some subscriptions were substituted with exchange, as donations and exchange with our publications, *Insect Science and Its Application, Dudu* and *ICIPE Annual Report,* grew. In addition, 75 reprints of articles by ICIPE authors were received.

Archives

An archival collection of ICIPE publications and other important mementos and documents was set up with plans to have it fully operational by the end of 1986.

Publications

A quarterly *ICIPE Library and Documentation Bulletin* was launched at the beginning of 1985 to cater for new

accessions and other current awareness bulletins. All the four issues of the first volume came out on schedule.

Services

The achievement of a full staff establishment at the end of 1984 led to a general revitalization of the services. Besides the current awareness bulletin mentioned above, Selective Dissemination of Information (SDI) was started. Journal and other in-coming publications are scanned for information that may be of interest to the researchers on a selective basis. Profiles of researchers were generated and maintained to facilitate the matching of particular information to particular users. Through purchases from the British Library Document Supply Centre and continued cooperation with other libraries and institutions, 944 photocopy and reprint requests were fulfilled. Eight bibliographic computer searches were carried out for scientists through assistance of the Commonwealth Agricultural Bureau (CAB). In addition, the library maintained an ICIPE profile with Dialogue for SDI searches as CAB Abstracts Database. Loaning of books and reader enquiry services were also maintained.

Computer Application

Towards the end of the year the library acquired a Wang PC and started laying ground to apply it in some of its projects, particularly documentation, cataloguing, SDI and the exchange mailing list. Several meetings were held with other library documentation authorities in and around Nairobi to share and exchange ideas on computer application and pave the way for future collaboration.

Management

The management and administration of core research programmes and units of the Centre as a whole remained at its 1984 full strength and level with the exception of the sudden loss of the Deputy Director; the late Dr. Mathew Paton Cunningham, in August 1986 whose position was filled in an acting capacity by Dr. Mutuku John Mutinga for the remaining part of 1985.

Steps initiated in 1984 to streamline administrative procedures and strengthen management systems through wide-ranging measures such as training of selected administrative and research staff, recruitment on a competitive basis, salary revisions, allocation of vehicles to programmes, etc, began to show positive results in increased efficiency and productivity throughout the Centre.

International Professional Staff

The International Professional Staff level was reviewed by the Governing Board and revised at 42-man-years.

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

Amenities and Social Welfare Units

The ICIPE's amenities and social services in support of its core activities remained the same and continued to operate effectively and efficiently during the year; except that Duduville International Guest Centre (DIGC) nevertheless managed to function with sub-optimal equipment.

The International Guest Centre System construction work at Mbita Point Field Station was nearly 90% complete to be utilised early in 1986 during the Annual Research Conference.

Average occupancy at the Duduville International Guest Centre rose to 65% during the year, and its expansion programme was rescheduled to the Duduville Phase III Capital Development Programme.

Mbita Point International School's additional physical facilities were completed to cater adequately for the expansion of classrooms.

The Mbita Point Field Station Clinic was renamed St. Jude's Clinic and the extension work to the maternity and laboratory service wings was finally completed. The clinic was able to treat an average of 445 cases per month during the year.

CAPITAL DEVELOPMENT

Mbita Point Field Station

The major capital works carried forward into 1985 were limited to the Guest Centre complex and completion of fittings and furnishings of the School and Clinic extensions, most of which were completed during the year.

Duduville Phase II Capital Development

Efforts to raise funds for this project continued through this year. Following the modest allocation of funds annually by the Board from core funds and the World Bank's support of US$2.5 million and the host country's contribution of US$0.286 million, Masterplanning for Phase II was started by mid-1985 in readiness for the start of construction work in the following year.

RELATIONS WITH THE HOST COUNTRY

The Centre's relations with the Government of the Republic of Kenya continued on very good and cordial terms. The formal discussions on the new Headquarters Agreement progressed very well and have been carried forward to 1986.

1985 Seminars

Speaker	Titles
Dr. Sam Nilsson, Executive Director International Federation of Institutes for Advanced Study (IFIAS), Sweden	IFIAS and the Major Global Issues
Dr. Brian Johnston, Pharmacia Biotechnology Uppsala, Sweden	Modern Approaches to Protein Purification
Dr. P. G. Kaaya, the ICIPE, Nairobi	Recent Advances in Knowledge of Insect Immunology
Dr. A.A. Latif, ARPPIS, the ICIPE, Nairobi	Effects and Basis of Naturally Induced Resistance to Tick Infestations in Rabbits: A Case for *Amblyomma variegatum* and *Rhipicephalus appendiculatus*
Dr. L. Irungu, the ICIPE, Nairobi	Some of the Factors which Influence the Establishment and Development of Filariae in Mosquitoes
Dr. J.H.P. Nyeko, ARPPIS, the ICIPE, Nairobi	Prophylaxis and Induction of Drug Resistance in *Trypanosoma congolense* during cyclic transmissions through *Glossina morsitans morsitans*
Professor C. Pavan, Genetics Department, University of Campinas, Sao Paulo, Brazil	Control of Human Bottly and Screwworms in Brazil
Professor [illegible]aw, Department of Biochemi[illegible]niversity of Arizona	Insect Storage Proteins
Professor John B. Campbell, Department of Entomology, University of Nebraska-Lincoln, USA	Integrated Pest Management Project for Control of Stable Flies at Confined Livestock Facilities
Dr. Leonard H. Otieno, the ICIPE, Nairobi	Some Aspects of Protozoan Research in the USSR
Dr. Bhupinder P.S. Khambay, Insecticides Department, Rothamsted Experimental Station Harpended, Herts AL5 2JQ, UK	Trends in the Design of Newer Synthetic Pyrethroids
Professor Charles K. Levy, Biology Department Boston University, Boston, Massachusetts, USA	Ecological Field Studies Using High Resolution Gamma-spectroscopy in Termites and Other Animals
Dr. Alf Bakke, Norwegian Scientist	Pheromone Ecology of Bark Beetles

1985 Publications

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Awiti, I.R.S., Odhiambo, T.R. and Ogoma, N.T., An electron microscopic study of the aortic wall and corpus cardiacum in relation to their neurohaemal function in the tsetse, *Glossina morsitans morsitans* Westwood. Insect Sci. Applic. **6**(4), 489-501.

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Challier, A. and Turner, D.A., Methods to calculate survival rate in tsetse fly (Glossina) populations. Ann. Soc. belge Med. Trop. **65**, 191-197.

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Chiera, J.W., The distribution and climbing behaviour of *Rhipicephalus appendiculatus* Neumann on grass stems. Insect Sci. Applic. **6**(2), 213-215.

Chiera, J.W., Newson, R.M. and Cunningham, M.P., Cumulative effects of host resistance on *Rhipicephalus appendiculatus* Neumann (Acarina: Ixodidae) in the laboratory. Parasitol. **90**(2), 401-408.

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De Castro, J.J., Effects of tick infestation on Boran (*Bos indicus*) cattle immunized against theileriosis in an endemic area of Kenya. Res. Vet. Sci. **39**, 279-288.

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Den Otter, C.J. and Saini, R.K., Pheromone perception in the tsetse fly *Glossina morsitans morsitans*. Entomol. exp. appl. **39**(2), 155-161.

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