AGENCY FOR INTERNATIONAL DEVELOPMENT
PROJECT AUTHORIZATION AND REQUEST
FOR ALLOTMENT OF FUNDS

9311038-4
PD-AAG-227-E1

1. TRANSACTION CODE: A (A ADD, C CHANGE, D DELETE)

9311038 00 PAF
4901

2. DOCUMENT CODE: 3

TYPE A, Worldwide, RDA-G5 - DS/AGR/Livestock

DOCUMENT REVISION NUMBER: Original

PROJECT NUMBER (7 digits): 931-1038

BUREAU OFFICE: A. SYMBOL: DSB; B. LEVEL: 36

PROJECT TITLE (Maximum 40 characters): Physiology and Ecology of Ticks

ACTION TAKEN: A APPROVED, D DISAPPROVED, TE DEAUTHORIZED

3. EST. PERIOD OF IMPLEMENTATION: YRS. 0 3 MTHS. 0

6. APPROVED BUDGET AID APPROPRIATED (000 OMIT)

APPROP. ACTIVITY	B. PRINCIPLE PURPOSE CODE	PRIMARY TECH. CODE		E. 1ST FY ___		H. 2ND FY ___		K. 3RD FY ___	
		C. GRANT	D. LOAN	F. GRANT	G. LOAN	I. GRANT	J. LOAN	L. GRANT	M. LOAN
FN	111	967		244		120			
			TOTALS	244		120			

N. FY ___ O. FY ___ P. LIFE OF PROJECT

O. GRANT	P. LOAN	Q. GRANT	R. LOAN	S. GRANT	T. LOAN
				364	
				364	

U. PROJECT FUNDING AUTHORIZED THRU FY 80

UNDER APPROPRIATE COSTS: LIFE OF PROJECT 364; INCREMENTAL LIFE OF PROJECT 2

V. FUNDS RESERVED FOR ALLOTMENT

W. ALLOTMENT REQUESTED (ENTER ZERO)

X. ALLOTMENT REQUESTED

Y. GRANT Z. LOAN

AA. DIRECTOR NAME (TYPED NAME)

SIGNATURE

DATE

DETAILS

BB. CODE ORIGIN OF GOODS AND SERVICES: 000 [ ] 941 [ ] LOCAL [ ] OTHER ___

CC. AMENDMENT, NATURE OF CHANGE PROPOSED

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FILE

DD. ACTION OFFICER (Typed name)

EE. ACTION DATE

FF. ACTION REFERENCE DATE

PROJECT AUTHORIZATION AND REQUEST FOR ALLOTMENT OF FUNDS

PART II

ENTITY : DS Bureau

PROJECT : Physiology and Ecology of Ticks

PROJECT NO.: 931-1038

I hereby authorize a Grant of not to exceed two hundred and forty-four thousand United States dollars ($244,000) to help finance the first two years of the three-year Physiology and Ecology of Ticks research project as described in Attachments A and B from the International Center for Insect Physiology and Ecology (ICIPE). These revised documents of June 22, 1978 cover the physiology and ecology components respectively.

The broad objectives of this project are to reduce the spread and damage of ticks and tick-borne diseases and to increase livestock productivity in the tropics. The general purpose of the ecological portion is to develop or identify efficient ecological methods of control of ticks. A basic component of this research will be controlled ecological studies on Rhipicephalus appendiculatus which is possible now that tick-infested paddocks under auspices of the Joint Tick Program of ICIPE and EAVRO (the Kenya Agricultural Research Institute) have been established. New or improved methods will be integrated into a systematic approach for tick control based on ecological data.

The purpose of the physiology portion is to learn more about the tick endocrine mechanisms in order to (1) identify natural tick hormones and pheromones and (2) possibly develop endocrine regulators which would be effective in control of certain tick species.

I approve the total level of AID appropriated funding planned for this project of not to exceed three hundred and sixty-four thousand United States dollars ($364,000), including the funding authorized above, during the period FY 78 through FY 80. I approve further increments during that period of grant funding of up to one hundred and twenty thousand United States dollars ($120,000) subject (a) to the availability of funds in accordance with AID allotment procedures; (b) to favorable review by the Research Advisory Committee (RAC) of additional information to be supplied by ICIPE at the RAC

October 1978 meeting; and (c) to favorable results of the evaluation by RAC personnel following an indepth review at ICIPE approximately one year from the commencement of project research.

This is a scientific research activity which will have no adverse environmental impact. (See Attachment C. Environmental Threshold Dicision).

Sander Levin
Assistant Administrator
for Development Support

9-22-78
Date

Clearances:

for DS/AGR/1, NKonnerup		date 21SEP78
DS/AGR, FJWilliams		date 21SEP78
DS/AGR, MMozynski		date 21 Sept 78
DS/AGR, DPeterson		date 21 Sept 78
for DS/PO/RES, MRechcigl		date 21 Sept 78
DS/PO, RSimpson		date
DAA/DS/FN, TBabb		date 22.9.78

DS/AGR/L,CSMcClusky:vdw:revised 9/19/78:ext. 51275

SEP 21 1978

ACTION MEMORANDUM FOR THE ASSISTANT ADMINISTRATOR, DEVELOPMENT SUPPORT BUREAU

Dean F. Peterson

FROM : DS/AGR, Dean Peterson

SUBJECT : Physiology and Ecology of Ticks, Project 931-1038

Problem: Your approval is required for AID funds in the amount of $364,000 for the three-year Physiology and Ecology of Ticks research project as described in attachments A and B from the International Center for Insect Physiology and Ecology (ICIPE). The proposed AID life of project contributions are as follows: Physiology component $53,000; and Ecology component, $311,000. The proposed AID grant for the first two years is $244,000, of which $36,000 is for the physiology component and $208,000 for the ecology component.

Discussion: The initial research proposal by ICIPE was reviewed and approved by the R&DC during 1976. The Research Advisory Committee (RAC), March 1978, recommended that the proposal be recast, separating the three (then) components, physiology, ecology, and immunology. This has been done. Subsequent review by a RAC Review Subcommittee concluded that the physiology and ecology components should be approved at half the level proposed for AID funds. This Subcommittee has been seeking to have several project related points clarified and has asked that ICIPE submit its response to specific questions of the Subcommittee at the RAC October 1978 meeting. ICIPE has agreeed to do so and is preparing the required material. The RAC has also required that an indepth team review be held at ICIPE approximately one year from the beginning of project activites. This evaluation is being scheduled for October 1979.

The primary research activity on which the consolidated project activity is predicated is in the field of ecology. It is this area which the major part of the ICIPE core staff will be devoting the most energy and time. The ecological studies include identification and population dynamics of ticks in field test plots at the Kenya Institute for Trypano-

somiasis Research (KITR), formerly EAVRO, Muguga. These studies require extensive investigation of ticks on the pasture plots, and on the animals, including observations of cyclic population densities, feeding behaviors at regular intervals by adults and nymphs and an analysis of the animal reactions to various levels of tick infestations. These baseline studies, which form the core requirements for the ultimate project objectives, require a major part of the staff time, consume a large share of funding resources since the field plots, fences, corrals and test animals must be maintained. The segment also requires allocations for transport and communication between the field and the laboratories. As these ecological studies progress, the laboratory trials in tick physiology can commence and the results expected can be related to the animals on the field plots. In fact, the application of results of the physiological research in pheromones, insect growth regulators and other hormones depends on the completion of the research in the ecological sector. ICIPE and other research institutions are at the present time conducting research in all of these areas but this total research in model systems needs to be expanded and accelerated for direct application to field programs.

Recommendation: That you approve $364,000 AID grant funding to finance the three-year Physiology and Ecology of Ticks research project by signing the attached PAF and Environmental Threshold Decision.

Clearances:
DS/AGR/L, NKonnerup
DS/AGR, FWilliams
DS/AGR, MMozynski MEM
DS/AGR, DPeterson
DS/PO/RES, MRechcigl
DS/PO, RSimpson
DAA/DS/FN, ENBabb

DS/AGR/L,CSMcCrosky:vdw:revised 9/21/78

RESEARCH PROJECT STATEMENT

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RESEARCH PROJECT STATEMENT

A. Project Summary

1. Statistical :

Project Title	:	Research on the Physiology and Ecology of Ticks
New or Extension	:	New
Contractor and Address	:	The International Center for Insect Physiology and Ecology (ICIPE) P.O. Box 30772, Nairobi, Kenya
Principal Investigator	:	Professor Thomas R. Odhiambo Director - ICIPE
Duration	:	3 years
Previous Funding	:	None
Proposed Funding	:	First year - 250,000 Second year - 250,000 Third year - 250,000
Project Manager	:	Dr. Nels Konnerup, TA/AGR

2. Abstract

Ticks and tick borne diseases are a major limiting factors in livestock productivity in the tropics. Current control systems, either dipping or straying, plus disease immunization or chemotherapy are costly and time consuming exercises with little acceptability by the average and lower income livestock raisers. Furthermore the tendency for ticks to develop resistance to acaracides requires the development of new materials periodically. Another adverse factor is relative acaracide toxicity to the host animal and residue problems of environmental and health concern. Moreover, many livestock raisers find mobilization of cattle for treatment or disease preventive procedures virtually impossible under extensive grazing conditions. Therefore the losses due to mortality and morbidity are substantial.

Ticks surpass all other arthropods in number and variety of diseases transmitted to man and animals. Only a few of these disease are amendable to prevention or treatment. Thus the only recourse is to control the ticks.

This research would also investigate a new approach to control of ticks by producing in the cattle specific antibodies against the developmental hormones of ticks. To achieve this end, the hormones that control growth and development of two species of ticks will be elucidated and used to develop antibodies in local breeds of cattle which would interfere with normal tick development upon subsequent tick feeding. Tick aggregation pheromones will be utilized to cluster the tick population to achieve more efficient control.

B. Narrative Statement

1. General Background and Rationale

All species of vertebrate land animals are subject to attack by ticks, but mammals are particularly attractive to these parasites that transmit important diseases. The food of ticks consists of blood and lymph, and as rule adult ticks as well as immature forms, both male and female, are bloodsuckers. Some of the factors which account for the capacity of ticks in the spread of disease to man and animals include their persistence as bloodsuckers, their highly protective sclerotized body, their freedom from natural enemies, and their wide host range.

Ticks are a major factor in inhabiting effective livestock production thereby limiting availability of high quality protein for human use. Ticks also transmit serious human and animals diseases and are an economic drain on livestock producers income. Present methods for vector control using acaracides (in stocks dips and sprays) are expensive, inefficient, potentially hazardous, and the development of tick resistance is an increasing problem. An economic, safe biological control system would eliminate these problems and conform to AID objectives.

Ecological studies of Rhipicephalus appendiculatus are underway by ICIPE scientists using paddock facilities provided by EAVRO. The purpose of this work is to determine the role of R. appendiculatus in the transmission of East Coast Fever (ECF), a Theileria parva infection highly fatal to cattle. These studies provide base-line parameters for tick populations studies in the field and permit appropriate quantitative control measures for the disease using an attenuated culture of the organisms along with chemotherapy.

ICIPE is also studying Ornithororos moubata, the tick vector of relasping fever in the man and African swine fever as a model. Adult ticks produce an assembly pheromone which actively aggregates both males and females. The chemical identify of this pheromone is under investigation. This pheromone is not species-specific, inter-species attraction appears to be strong and it may be a general assumbly attractant for the entire argasid family.

That development and reproduction of insects is regulated by hormonal interactions is well known. Ticks, like other arthropods, also develop through a series of moults but the hormone regulating process in ticks has not yet been well studied. It will be necessary to isolate and identify these moulting hormones if they are to be used in control systems.

While the ecology of O. moubata has been studied in fair detail, its physiology with particular reference to hormonal and pheromonal physiology is just beginning.

Although the research concepts related to using pheromones (attractants/repellants) and hormones (insect growth regulators/juvenile hormones/moulting hormones) as tools in the control of pests have been pursued for a relatively long period in insects, very little has been done in these fields in ticks.

However, few scientists at a few institutions including ICIPE have begun work in these areas. For instance three main lines of research are underway: 1) physiology and behavior of hard ticks including identification of assembly pheromones and tick reactions to climatic factors. 2) studies of the growth and persistance of natural tick populations on experimental plots. 3) physiology of argasid (soft) ticks particularly to the role of endocrine secretions in growth and development.

A recent major accomplishment is the isolation of ecdysone (moulting hormone) and the synthesis of a juvenile hormone analogue which may have some influence in embryonic development of ticks.

ICIPE has already accomplished considerable fundamental research related to possible biological control of ticks as an alternative to chemical acaracidal control. ICIPE and the International Atomic Energy Agency (IAEA) have supported research on the Sterile Male Technique, and the USDA had supported similar work at IIBR, and both efforts have resulted in the conclusion that this method of biological control is not likely to be effective in tick control, within the current research development processes, except as a possible adjunct to other systems. Hence one of the main goals of ICIPE is fundamental research

in the structural analysis and synthesis of hormones, anti-hormones and pheromones of tropical ticks. The UNDP has assisted in this line of research on the physiology and ecology of hard ticks at ICIPE and EAVRO and this research needs to be continued and expanded. Physiological studies underway have identified female sex pheromones attractive to males, determined the time of maximum production and succeeded in identifying and synthesizing them. These compounds seem to attract a number of tick species and may therefore be significant components in integrated tick control programs.

2. Research Purpose and Expected Products

The purpose of this project is to develop a means of controlling pest ticks through an integrated biological control method. To achieve this purpose, a step wise set of experiments has been designed. The initial phase of the project will emphasis the isolation and identification of hormones regulating tick growth and development. Concurrently tick aggregation pheromones will be isolated and identified which will greatly aid in manipulating field tick population dispersal tendencies. After the hormones and pheromones have been identified, experiments will attempt to produce immune responses in cattle such that subsequent tick feeding may lead to developmental arrest.

3. Relation to Existing Knowledge

Most experts place the start of "modern" biological control of pests in the latter part of the nineteenth century and these systems have proceeded through a series of techniques that include use of beneficial insects that prey on pests, infectious agents pathological to selected pests, attractants to lure pests to destruction, repellants which protect the host, sterilants which inhibit reproduction and most recently the use of hormones that interfere with the developmental processes of the host. Many insect pheromones and hormones have been chemically identified, synthesized and some have even been commercially produced for use in pest control programs. In the case of ticks such techniques are in the developmental stages of identification and synthesization at a few institutions in the U.S., Australia, Europe, Israel and at ICIPE in Africa. It is only at the two latter locations where much of the work on the principal target species can be carried out.

It has long been known that specific antibodies are capable, under appropriate circumstances, of neutralizing many biologically active macromolecules including toxins,enzymes, peptide hormones and others. Specific antibodies were reported to reverse established cellular effects of insulin and thyrotropin <u>in vitro</u>. Recently, antibodies were also reported to be physiological antagonists to low molecular weight molecules such as steroid hormones, pyridoxal phosphate, histamine, serotonine, chloramphenicol, and cardiac glycosides. By virtue of the specific antibodies capacity to bind these substances, they inhibit certain physiological effects of these compounds <u>in vivo</u> and <u>in vitro</u>.

The growth and maturation of insects (and probably of ticks as well) are governed by 3 main hormones: brain hormone, juvenile hormone (JH), and ecdysone (MH) which are responsible for the complex series of moults that these arthropods undergo during their life cycle. The moults are regulated by the brain hormone which activates production of the moulting hormone, ecdysone. Changes in concentration between juvenile hormone and ecdysone determine the nature of the newly moulted arthropod. Early in the life cycle, relatively large amounts of juvenile hormones are present to maintain the juvenile state, and ecdysone level is low. As the immature grows, the juvenile hormone concentration decreases while the ecdysone level increases, until moulting into the adult is accomplished, at which time the juvenile hormone has dropped to undetected levels and ecdysone reaches its maximum.

The chemical nature of the brain hormone is not yet known. It is assumed to be a protein (M.W. 9000 -30000) synthesized by neurosecretory cells in the brain. Pure preparations of this hormone are not yet available.

Three naturally occurring JH have been characterized. These are sesquiterpenoid, so-called C - JH 1, C - JH 2 and C - JH 3.

Antibodies to C - JH have been recently produced for radioimmune assay of this hormone. By conjugating the hormone to a large carrier protein (such as human serum albumin (HSA) - via the N-hydroxysuccinimide) the hormone is rendered heptenic and can elicit an immunogenic response. Rabbits injected with the conjugate produce specific antibodies at a titer of 1.8 mg/ml blood. Only JH and very close epoxy derivatives react with the antibodies.

Ecdysone, the moulting hormone (MH) of arthropods, is a polyhydroxy steroid.

For the purpose of radioimmune assay, antibodies against this hormone have recently been produced. Borst and O'Connor (1972) have produced the conjugate: ecdysone-bovine serum albumin (BSA) by converting first the ecdysone to the oxime acetic axid ether. The oxime derivative is coupled to BSA by way of the isobutylchloroformate.

Radioimmune assy of ecdysone is already in use in several laboratories, yet the physiological effects of the antibodies on insect development has not been studied. We believe that if antibodies against any of the 3 hormones <u>in vivo</u>, then cattle can be immunized against the tick hormones and this method can then be used as a control against ticks.

4. Relation to Other Research

This research proposal is designed to build on available technology and completed research on tick physiology, ecology and control, and to complement current research efforts.

The research program of the CSIRO Division of Entomology has focused on chemical, immunogenetic and cultural methods of control. They have noted that tick resistance is highly heritable and efforts to breed for resistance are rather encouraging. Research continues to elucidate the mechanisms of resistance.

Research is in progress in CIAT to characterize the physical and chemical factors responsible for reduced tick populations in molasses grass (melinis minutuflora) and to exploit these factors in the relution of pasture species and the development of tick repellants. Similar research is underway at ICIPE and research results will be compared.

The proposed research project complements these and other research efforts in investigating the potential of immunization of ruminants against the developmental hormones of ticks, which could disrupt the reproductive cycle in ticks.

5. Research Project Design and Method

The research activities for this project are aimed at analysis of hormones and pheromone of tropical ticks. These materials would then be utilized in laboratories and field experiments to aggregate tick populations and to evolve the immune system of the vertebrate host to neutralize the ticks hormone system.

There are several reasons to believe that such a control system might be effective against ticks.

1) Many species of ticks are almost exclusively monophagous, and within a given area feed only on domestic animals which could all be immunized. Thus every single tick in this area will presumably be affected.

2) The blood meal ingested by a tick is about 100-fold its own weight and therefore there is a chance that the amount of antibodies ingested with the blood meal might be sufficient to neutralize the hormones.

3) A considerable amount of the globulin ingested by ticks passes through the gut wall serologically unchanged. This may be related to the intercellular digestion typical to ticks. Thus it may be assumed that enough of the ingested antibodies will pass the gut wall into the tick hemolymph where they should interact with the hormones.

4) As these hormones are common to the whole group, a method successful for one species will most probably be effective against other cattle tick species.

A standard procedure has been developed for the analysis of these hormones. Since R. Appendiculatus and O. moubata are of great economic importance, these two species will be used as models with the expectation that research finding will have significant implications to other tick species. Ecological studies will be carried out to determine the host density including wild ungulates requirements for tick maintenance under differing climatic and temperature conditions.

A synchronized mass production of Rhipicephalus ticks will be maintained at EAVRO for pheromone studies as well as extraction and identification of development hormones. Mass production of O. moubata will be carried out at ICIPE while other species, O. tholozani and Argas persicis will be mass produced at IIBR to identify assembly pheromones of this group and to supply material to ICIPE for further studies.

The overall program of work involved includes: (1) establishement of tick colonies at ICIPE and/or IIBR for the purpose of producing biological material for tick control, (2) identification and assay of hormones, antihormones and hperomones; (3) application of these materials in meaningful field trails under simulated natural conditions, (4) assessment of the effectiveness of these materials in reducing or eliminating tick infestations.

C. Contribution to Institution Building

ICIPE is a unique international African Institution working on tropical biological problems with a wide range of support from donors throughout the world. The director has put the Institute contributions very succinctly and well in the preface to the ICIPE 1975 Annual Report. "The task for the future, as we enter our fifth year of research, is a challenging one: to continue to serve the developing world in their critical pest problem areas by carrying on high-quality fundamental research on those vital questions that need to be answered in order that these pests might be controlled on a long-term basis with the least ecological and environmental perturbations. Our task is therefore goal-oriented, tuned to practical application."

ICIPE is in the process of developing further existing facilities and has recently acquired capital grant support from two European donor Agencies for substantial expansion. ICIPE with the support of the Government of Kenya has also acquired two major field stations for outreach programs, established a firm working relationship for field studies on ticks at EAVRO and developed an intimate relationship with other research centers in Africa, Europe, Asia and the Americas.

The tick research program at ICIPE, will provide short-term professional training for Nationals who wish to become proficient in tick research methodology. In addition, the training of young professionals to the M.S. and Ph. D. levels will be undertaken by accepting candidates for 3 to 6 months yearly at ICIPE, with the remainder of the training at the home university. For training technicians, ICIPE will accept

trainees for 4 to 6 months in specialized areas of tick research. About 5 such participants may be trained each 6 months. Such training will be supplemented by appropriate work shops and seminars, so that the ICIPE program will serve as an international center for tick research.

D. Linkages with National, Regional and International Institutions

Research Linkages:

This project will provide participation and active cooperation with important research institutions. ICIPE is associated with the University of Nairobi, Kenya. Studies on the feeding behavior and nutrient requirements of soft ticks are designated for the "Israel Institute for Biological Research (IIBR). The production ticks is an important component of the research plan, with basic research at IIBR, and field testing at the "East African Veterinary Research Organization" (EAVRO) at Muguagu, Near Nairobi. Close collaboration will be maintained with ILRAD, particularly as related to East Coast Fever which is transmitted by ticks.

E. Utilization Plans

This is a new project and definite utilization plans cannot be identified at this stage except that it is expected that a practical biological tick control system may materialize as a replacement for current systems (stock dips and sprays) which have many shortcomings such as limited effectiveness, hazardous consequences and economically prohibitive costs especially to small farmers.

In anticipation of successful research results such as those achieved in biological control systems for certain insect pests a training program for entomologists and biologists has been built into the research work plans. Additionally ICIPE has scheduled seminars on the tick behavioral physiology in 1977 and is planning others related to the research effort.

Biological control of pests must be considered as one of the important tools in pest management and be incorporated in the series of Pest Management Seminars conducted by the University of California in various parts of the world.

F. Research Competence

ICIPE is a relatively new research institution conceived by its present director and a group of internationally eminent scientist to carry out research on tropical pests within their natural ecological niche or environment. The institution is located within the campus of the University of Nairobi with a number of field stations at appropriate locations to conduct research on specific pests problems.

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The Institute has a competent resident staff which is growing and is backstopped by highly qualified and well recognized scientific authorities in a wide variety of scientific disciplines. The institute also engages a number of young, enthusiastic and capable post graduate and post doctoral fellows.

G. Overall Cost Estimates

The included itemized budget sets forth dollar costs for individual line items for the first 12 months of the contract. The total amount is anticipated to remain the same for the second and third years, but line items may change. (See Attachment)

H. Management Considerations

1. Organizationally, ICIPE is a unique institution operating with a full time director and a permanent staff of 24 scientists, 10 experimental officers, 20 technicians and 13 administrative personnel. It has a Board of Governors composed of 14 internationally eminent scientists. The research activities are under the Director of 16 Directors of Research encompassing four scientific disciplines. The Institute also utilizes the guidance of Consulting Directors of Research and Visiting Scientist.

ICIPE is represented internationally by two Committees - an International Committee and an African Committee.

The Governing Board has applied for acceptance and support by the Consultative Group on International Agricultural Centers (CGIAR) and presumably this application will be acted upon in July 1977.

ICIPE has established close linkages with other International Centers notably ILRAD, ILCA, IITA and CIAT, as well as with EAVRO, FAO, UNEP, WHO and IAEA.

ICIPE relationships with European, American and Asian scientific and academic institutions are outstanding.

2. ICIPE research is now scientifically planned and reviewed by the Directors of Research and approved by the Board of Governors annually. The International and African Committees meet annually to review the scientific program and report to the Board of Governors.

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3. ICIPE's current overall institutional support:

Estimated Funding

For ICIPE

Date 1977	Duration	Funding (Thousands of dollars)
UNDP	5 yrs.	996,
UNEP	3 yrs.	347.5
WHO	1 yr.	6
ODM	1 yr.	40
Rockefeller	1 yr.	60
DITH, Netherlands	3 yrs.	210
France, GDOST	1 yr.	18
Dahida, Denmark	3 yrs.	330
Kenya (In kind 85 acres primeland)	1 yr.	10
SIDA, Sweden	1 yr.	140
Swedish Agr. Resource Council	1 yr.	140
Swiss Tech Coop.	1 yr.	108,
IDRC-Canada	3 yrs.	240,
Australian AID	1 yr.	30,
Max Plank Soc.	1 yr.	30,
Capital Grants NORAD (Norway)		$2.5m
Netherlands		1.5m

1. Environmental Consideration

This project is one of several AID supported research activities designed to reduce or eliminate the need for the use of hazardous pesticides or materials that may contribute to undesirable chemical residues. The research is basically oriented to target-specific control with minimum consequence to other species. Since the research design is biological control oriented adverse environmental impact will be negligable.

This project has been subject to a formal Environmental Statement (Appendix -) by a Committee of Experts which basically concludes that this research will have virtually no adverse environmental effect on the test areas and, that if successful, could result in the reduction in use of environmentally hazardous materials in areas where the system may be applied.

J. Internal and External Review

This is a new research project, hence no review has been made. However, the research concept and planning has been reviewed by qualified entomology researchers from the U.S. Department of Agriculture, a number of academic institutions in Europe and the Americas, the Overseas Development Ministry of the U.K., the International Atomic Energy Agency, the United Nations Environmental Program, the International Laboratory for Research on Animal Diseases and other International Centers. The program has also been discussed with the technical staff of the Africa Bureau and the US AID Missions of Kenya and Tanzania. A Study Workshop on Tick Physiological Behavior with proposed international attendance to acquaint participants with the research objectives and goals is scheduled for October 1977 at ICIPE in Nairobi.

K. Project Statement Evaluation

TA/AGR has long regarded ticks as the most universally destructive pests of livestock and the major health constraint on efficient livestock production. We have also been concerned over the use of hazardous pesticides, the development by pests of resistance to pesticides, the problem of residues from the use of chemicals in pest control, as well as for the costs incurred in the use of these materials particularly by the small livestock operators.

TA/AGR has taken cognizance of the benefits derived from the use of biological control systems for insect pest control and the favorable environmental impact resulting from application of such systems.

After careful consultation and review by qualified entomologists TA/AGR has concluded that this research has a high probable potential for reducing losses in livestock operations in areas of the world where high quality protein is critically needed.

AID 1020-11 (9)

PROJECT DESIGN SUMMARY
LOGICAL FRAMEWORK

Life of Project
From FY 78 to FY [illegible]
Total U.S. Funding 75[illegible]
Date Prepared 5/13/77

Project Title & Number

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Program or Sector Goal: The broader objective to which this project contributes. To develop improved methods of control of ticks.	Measures of Goal Achievement. Reduced tick infestation of animals and reduced incidence of tick borne diseases.	Field observation, control [illegible] and incidence of tick borne diseases where tick control [illegible] been applied.	Assumptions for achieving goal targets. 1. New knowledge is generated on tick control. 2. Infrastructure and inputs are available for the application of improved methods of tick control.
Project Purpose: To develop a biological method of control of ticks based on antibodies in cattle which interfere with normal tick development and feeding.	Conditions that will indicate purpose has been achieved: End of project status. Methods will have been developed and/or knowledge generated which will contribute to biological methods of control of ticks.	Same as FOUS; [illegible] report	Assumptions for achieving purpose 1. Outputs are achieved. 2. Outputs will have been integrated into a control system, and/or knowledge generated contributing to biological control methods of ticks. 3. Favorable cost/benefit ratios for control.
Outputs: 1. Establishment of tick colonies. 2. Identification and assay of tick hormones, antihormones and pheromones 3. Field trials with ruminants to develop/test methods of producing antibodies in ruminants which will disrupt the maturation process in ticks. 4. Training of LDC personnel. 5. Publication of results.	Magnitude of Outputs: 1. Tick colonies in ICIPE and IIBR. 2. Complete identification and assay of tick hormones, antihormones and pheromones. 3. Completion of required field trials to achieve output (3). 4. Training: (a) Graduate students:6 (b) Technicians: 6-12. 5. Annual reports, scientific publications.	Same as Magnitude of Outputs; Annual Reports	Assumptions for achieving outputs. 1. ICIPE has the research facilities and technical competency to implement research program. 2. Professional and laboratory technican personnel are available for training.
Inputs: 1. ICIPE analytical laboratories, tick colonies, lab and field personnel. 2. IIBR tick colonies. 3. AID funding and project management.	Implementation Target (Type and Quantity) 1. ICIPE (a) biochemical, physiological, entomological facilities; insectary (b) Personnel: 2 scientists, 2 experimental officer officers, 5 technicians, 12 ancillary staff. 2. IIBR - insectary. 3. AID a) Annual funding - 250,000 b) One project manager.	Same as Implementation Target; Annual Reports	Assumptions for providing inputs: 1. AID project funding. 2. ICIPE administrative support.

EQUIPMENT

1.	Vehicles	14,000	1 New and 1 Replacement
2.	Scientific Equipment	5,000	4000 Nairobi, 1000 Israel
		19,000	
	TOTAL	250,000	Nairobi - 226,000 Israel - 24,000

ATTACHMENT

PERSONNEL COSTS

1.	Scientists/Experimental Officers	78,900	2 Scientists and 2 Experimental Officers.
2.	Technicians	47,900	1 at Israel and 4 at Nairobi.
3.	Lab/Field Assistants	24,300	12 Ancillary Staff, 7 at Muguga, 4 at Chiromo & one Driver.
4.	Daily-Rated	600	Casual employees at Peak periods.
5.	Participant training	17,330	
		168,030	

CONSULTATIONS	3,420	One Consultation per Year-Fares & Accommodations

TRAVEL

1.	Directors of Research	7,090	2 D/R x 2 Visits per year
2.	Sc. Staff	2,950	Sc. Staff to conference & Seminars
		10,040	

MATERIALS SERVICES & EXPENSES

1.	Supplies and Expendables	16,000	Lab. Supplies, Animals, etc. 8000 Kenya; 8000
2.	Field Expenses	4,250	Vehicles Running & Camping etc.
3.	Equipment Maintenance & Insurance	500	
4.	Report Costs	550	Includes photography & reprints for ICIPE Library.
5.	Insectary	2,850	Contribution to Gen. Insectary 2150 + 700.
6.	Fencing	10,000	Ecological Experiments
7.	Field Station - Share of Costs	1,980	Kajiado - 2½% of Sc. Staff Costs.
8.	ICIPE - Share of Costs	15,780	20% of Sc. Staff Costs
		51,910	

PROJECT DESIGN SUMMARY
LOGICAL FRAMEWORK

Life of Project
From FY 78 to FY 80
Total U.S. Funding 750,000
Date Prepared 5/13/77

Project Title & Number

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Program or Sector Goal: The broader objective to which this project contributes: To develop improved methods of control of ticks.	Measures of Goal Achievement: Reduced tick infestation of animals and reduced incidence of tick borne diseases.	Field observations on tick infestation and incidence of tick borne diseases where tick control methods have been applied.	Assumptions for achieving goal targets: 1. New knowledge is generated on tick control. 2. Infrastructure and inputs are available for the application of improved methods of tick control.
Project Purpose: To develop a biological method of control of ticks based on antibodies in cattle which interfere with normal tick development and feeding.	Conditions that will indicate purpose has been achieved: End of project status. Methods will have been developed and/or knowledge generated which will contribute to biological methods of control of ticks.	Same as EOPS; Annual Reports	Assumptions for achieving purpose: 1. Outputs are achieved. 2. Outputs will have been integrated into a control system, and/or knowledge generated contributing to biological control methods of ticks. 3. Favorable cost/benefit ratios for control.
Outputs: 1. Establishment of tick colonies. 2. Identification and assay of tick hormones, antihormones and pheromones. 3. Field trials with ruminants to develop/test methods of producing antibodies in ruminants which will disrupt the maturation process in ticks. 4. Training of LDC personnel. 5. Publication of results.	Magnitude of Outputs: 1. Tick colonies in ICIPE and IIBR. 2. Complete identification and assay of tick hormones, antihormones and pheromones. 3. Completion of required field trials to achieve output (3). 4. Training: (a) Graduate students:6 (b) Technicians: 6-12. 5. Annual reports, scientific publications.	Same as Magnitude of Outputs; Annual Reports	Assumptions for achieving outputs: 1. ICIPE has the research facilities and technical competency to implement research program. 2. Professional and laboratory technican personnel are available for training.
Inputs: 1. ICIPE analytical laboratories, tick colonies, lab and field personnel. 2. IIBR tick colonies. 3. AID funding and project management.	Implementation Target (Type and Quantity) 1. ICIPE (a) biochemical, physiological, entomological facilities; insectary (b) Personnel: 2 scientists, 2 experimental officers officers, 5 technicians, 12 ancillary staff. 2. IIBR - insectary. 3. AID a) Annual funding - 250,000 b) One project manager.	Same as Implementation Target; Annual Reports	Assumptions for providing inputs: 1. AID project funding. 2. ICIPE adminstrative support.

ATTACHMENT A

THE INTERNATIONAL CENTRE OF
INSECT PHYSIOLOGY AND ECOLOGY
P.O.BOX 30772, NAIROBI KENYA

REF: F1/CONTR/USAIDIC/46/70

22nd June 1978

RESEARCH PROJECT ON PHYSIOLOGY OF TICKS

RESEARCH PROJECT STATEMENT

A. Project Summary

1. Statistical

Project Title	RESEARCH ON PHYSIOLOGY OF TICKS
New or Extension	NEW
Contractor and Address	THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY (ICIPE), P.O. BOX 30772, NAIROBI, KENYA
Principal Investigator	PROFESSOR THOMAS R. ODHIAMBO DIRECTOR, ICIPE
Visiting Director of Research	PROFESSOR RACHEL GALUN ISRAEL
PROGRAMME LEADER	DR. M P. CUNNINGHAM PRINCIPAL RESEARCH SCIENTIST TICK PROGRAMME, ICIPE
Duration	THREE YEARS
Previous Funding	NONE

Proposed Funding (in U.S. '000 $)

	AID	UNDP	TOTAL
1st yr.	18.0	67.6	85.6
2nd yr.	18.0	61.5	79.5
3rd yr.	17.0	73.5	90.5
TOTAL	53.0	202.6	255.6

2. Abstract

Initially the physiological programme will concentrate on the further elucidation of tick endocrine mechanisms involved in processes of growth, development and reproduction. Identification of the natural tick hormones and pheromones and the subsequent development of synthetic analogues of comparative biological activity to the natural products, might lead to the formulation of broad spectrum endocrine regulators which would be effective in the control of most external and internal arthropod parasites of domestic animals.

B. Narrative Statement

(Narrative statement (subheading 1-4) are the same for three proposals to assure conciseness and to demonstrate the interrelatedness of research).

1. General Background and Rationale

All species of vertebrate land animals are subjected to attack by ticks, but mammals are particularly attractive to these parasites that transmit important diseases. The food of ticks consists of blood and lymph fluid. Some of the factors which account for the capacity of ticks to spread disease to man and animals include their persistance as blood-suckers, their highly protective sclerotized body, their freedom from natural enemies, and their wide host range.

Ticks are a major factor inhibiting effective livestock production, thereby limiting availability of high quality protein for human use. Ticks also transmit serious human and animal diseases and are an economic drain on the income of livestock producers. Present methods for vector control using acaricides (in dips and sprays) are expensive, inefficient and potentially hazardous; and the development of tick resistance is an increasing problem. An economic, safe, integrated control system would eliminate or reduce these problems and conform to AID objectives.

Ecological studies of Rhipicephalus appendiculatus are underway by the ICIPE scientists at different stations in Kenya, including the East African Veterinary Research Organization (EAVRO), where tick-infested pasture facilities were developed. These studies are providing base-line parameters for tick population control methods which the project is expected to produce.

Some adult tick species produce an assembly pheromone which actively aggregates unfed males and females. The chemical identity of this pheromone is under investigation by the ICIPE. This pheromone is not species-specific; inter-species attraction appears to be strong, and it may be a general assembly attractant for the entire argasid family, and a few of the ixodid species (See references for pheromones in ticks).

It is well-known that development and reproduction in insects is regulated by hormonal interactions. Ticks, like other arthropods, also develop through a series of moults, but the hormone regulating this process in ticks has not yet been identified. There are good indications that MH of ticks is identical or very close to beta-ecdysone (see ref). Yet it will be necessary to isolate and identify the moulting hormones if they are to be used in control systems.

Although the research concepts related to using pheromones (attractants/repellants) and hormones (insect growth regulators, juveline hormones/moulting hormones) as tools in the control of pests have been pursued for a relatively long period in insects, very little has been done in these fields on ticks.

However, a few scientists at a few institutions have begun work in these areas. At the ICIPE, the following main lines of research are underway: (a) the physiology and behaviour of a few tick species including the identification of hormones, assembly pheromones and sex pheromones and tick reactions to climatic factors - (b) studies on the growth and peristence of natural tick populations on experimental plots.

The ICIPE has already accomplished considerable progress in fundamental research related to the possible biological control of ticks as an alternative to chemical acaricidal control. EAVRO and the International Atomic Energy Agency (IAEA) have supported research on the sterile-male technique, and the USDA has supported similar work at IISR; and both efforts have resulted in the conclusion that this method of biological control is not likely to be effective in tick control, except as a possible adjunct to other systems. Consequently, one of the main goals of the ICIPE is fundamental research in the structural analysis and physiology of hormones, anti-hormones, and pheromones of tropical ticks. The UNDP has assisted in this line of research on the physiology and ecology of ticks at the ICIPE and EAVRO and this research needs to be continued and expanded. Physiological studies have been completed which identified the female sex pheromone which attracts the male of R.appendiculatus for mating, determined the time or maximum production and succeeded in identifying them. Of these compounds, 2,6-dichlorophenol attracts the males of a number of tick species and might therefore be of value in integrated tick control programmes.

2. Research Purpose and Expected Products

The purpose of this project is to examine the possible role of hormones and pheromones and cattle immunization as means of controlling pest ticks through integrated biological control methods. To achieve this purpose, a plan of work has been designed which incorporates basic research into critical aspects of the ecology and physiology of the more economically important livestock ticks in East Africa. Experiments will be conducted in an attempt to produce an immune response in cattle which will reduce the number of ticks which feed on them and which could result in arrested development of those ticks which do feed.

The basic population-ecological studies will continue, as these must be the means for interpreting much of the work, and ultimately of assessing its results at the field level. It has been estimated that tick infestations result in losses in livestock production of as much as one-third the actual potential.

3. Relation to Existing Knowledge

Most experts place the start of 'modern' biological control of pests in the latter part of the nineteenth century. These systems have proceeded through a series of techniques that include use of benefical insects that prey on pests, infectious agents pathological to selected pests, attractants to lure pests to destruction, repellants which protect the host, sterilants which inhibit reproduction and, most recently, the use of hormones that interfere with the development processess of the host.

Many insect pheromones and hormones have been chemically identified, synthesized and some have been commercially produced for use in pest control programmes. In the case of ticks, such techniques are in the developmental stages of identification and synthesis at a few institutions in the USA, Australia, Europe, Israel, and at the ICIPE in Africa. It is only at the two latter locations where much of the work on the principal target species can be carried out.

It has long been known that specific antibodies are capable, under appropriate circumstances, of neutralizing many biologically active macromolecules, including toxins, enzymes, and peptide hormones.

Specific antibodies were reported to reverse established cellular effects of insulin and thyrotropin in vitro. Recently, antibodies were also reported to be physiological antagonists to low-molecular weight molecules, such as steroid hormones, pyridoxal phosphate, histamine, serotonin, chloramphenicol, and cardiac glycosides. By virture of the capacity of the specific antibodies to bind these substances, they inhibit certain physiological effects of these compounds in vivo and in vitro.

The growth and maturation of insects (and probably of ticks as well) are governed by three main hormones: brain hormone, juvenile hormone (JH), and ecdysone (MH), which are responsible for the complex series of moults and growth that these arthropods undergo during their life cycle. The moults are regulated by the brain hormone, which activates production of the moulting hormone (or ecdysone). Changes in con - centration between JH and MH determine the nature of the newly moulted arthropod. Early in the life cycle, relatively large amounts of JH are present to maintain the juvenile state, and MH level is low. As the immature grows, the JH concentration decreases, while the MH level increases, until moulting in the adult is accomplished, at which time the JH has dropped to undetected levels and MH reaches its maximum.

The chemical nature of the brain hormone is not yet known. It is assumed to be protein (M.W.9,000-30,000), synthesized by neurosecretory cells in the brain. Pure preparations of this hormone are not yet available.

Three naturally occurring insect JHs have been characterized. These are sesquiterpenoids, the so-called JH-1 (18C), JH-2 (17C) and JH-3 (16C).

Antibodies to JH-3 have been recently produced for radio-immune assay of this hormone. By conjugating the hormone to a large carrier protein (such as human serum albumin (HSA), via the N-hydroxysuccinimide, the hormone is rendered haptenic and can elicit an immunogenic response. Rabbits injected with the conjugate produce specific antibodies at a titer of 1.8 mg/ml of blood. Only JH and very close epoxy derivatives react with the antibodies (Laufer et al 1974).

The MH of arthropods is a polyhydroxy steriod. For the purpose of radioimmune assay, antibodies against this hormone have recently been produced. Borst and O'Connor (1972) have produced the conjugate, ecdysone-bovine serum albumin (BSA), by conjugating the ecdysone to BSA by way of the [illegible] chlorofomate.

Although raido-immune assay of ecdysone is already commonly in use in several laboratories, the physiological effects of the antibodies on insect development have not yet been studied. There is every reason to expect that these hormone systems pertain to ticks as well as insects (see relevant references), and we believe that if antibodies against any of the three hormones can be produced in vivo, then livestock can be immunized against the tick hormones; and this method can be used as a control against ticks, by interference in their development.

4. Relation to Other Research

This research proposal is designed to build on available technology and completed research on tick physiology, ecology and control, and to complement current research efforts.

The research programme of the CSIRO Division of Entomology in Australia has focussed on chemical, immunogenetic, and management methods of control. They have noted that tick resistance is highly heritable, and efforts to breed for resistance are rather encouraging. Research continues on elucidating the mechanisms of resistance.

The proposed research project complements these and other research efforts in investigating the potential for immunization of ruminants against the different antigens and developmental hormones of ticks, which could disrupt the reproductive and other cycles in ticks.

5. Research Project Design and Methods:

Physiology Programme

The main objective of the ICIPE tick physiology programme is to prove or modify the hypothesis that tick growth, moulting and reproduction are controlled by hormones similar to those employed by insects.

Additional objectives are to carryout research on tick pheromones and in specific areas of tick physiology which will support work in the ecology and immunology portions of the programme. In supprt of our main objective we will investigate the possible occurence and role of moulting and juvenile hormones in Ornithodoros moubata and Rhipicephalus appendiculatus.

Ecdysones:

We have already demonstrated that beta-ecdysone and the related compound ponasterone A stimulate supranumerary moulting in O. moubata adults (Mungo, Odhiambo and Galun, 1976).

Preliminary bioassays show that nymphal O. moubata haemolymph contains a factor which induces pupariation in ligatured Musca larvae. This Musca bioassay is a classic system for studying changes in ecdysone titre in insects. Our further investigation on the moulting hormones of ticks will include three aspects.

(1) We will investigate changes in the titre of moulting activity (in Musca units) in the haemolymph of O. moubata and later in R. appendiculatus, during the intermoult period of nymphal ticks and following the adult blood meal. These bioassays will be coordinated with histological and physiological studies to determine the temporal relationship between changes in moulting

hormone-titre and the developmental processes of moulting and reproductive maturation. Particular attention will be paid to the synthesis of new cuticle and to the process of vitellogenesis since they are known to depend on sequential changes in ecdysone titre in insects. When possible the Musca bioassay will be supplemented with the highly sensitive, but less specific radio-immunoassay for ecdysone-like compounds.

(2) We will isolate those compounds in the haemolymph of nymphal O. moubata which show activity in the Musca test so that their chemical nature can be identified. Initial progress has been made in this area and we are now testing the third subfractions of tick haemolymph in cooperation with Professor K. Nakanishi's laboratory at Columbia University.

(3) We will continue to investigate the effects of exogenous ecdysone and related compounds on the supramoulting of O. moubata adults, the acceleration of moulting in fed O. moubata nymphs and the mortality dose-response relationship in all stages of R. appendiculatus. Physiological studies in Israel will concentrate on the metabolism of exogenous ecdysone in O. moubata using the extraction techniques of Karlson and Shuaya (1964) and chromatographic techniques, while the persistance of exogenous beta-ecdysone in the midgut of O. moubata will be followed at the ICTA with the Musca bioassay. Histological studies will also concentrate on the effects of exogenous ecdysone on cuticle synthesis, engorgment success and vitellogenesis.

Juvenile Hormone (JH), its Analogues (JHA) and Antagonists:

Preliminary work (Mungo and Galun, unpublished, has shown that the application of JH has a lethal effect on egg development in R. appendiculatus. JHAs also have a lethal effect on tick egg development (Mansingh and Rawlins, 1977; Solomon and Evans 1977) but JHAs also have been used to break adult ovipositional diapause in Argas arboreus (Bassal, 1974). Still there is no convincing evidence that an insect-like JH plays a role in development or reproduction in ticks. In order to investigate its presence or absence we plan an initial two phase approach.

(1) We will test for the presence of juvenilising activity in tick haemolymph and purified extracts from early nymphal stages of O. moubata and from R. appendiculatus larvae, as well as from adult females of both species during vitellogenesis. The Galleria and Tenebrio bioassay will be used for titre quantification.

If positive findings are made at this stage further experiments will be made to test possible tissues as the source of JH activity utilizing a radiolabelled technique to monitor in vitro production of JH.

(2) Precocene I and II have been shown to inhibit the differentiation of corpora allata in certain insects, thereby preventing JH secretion. Preliminary reports by Sister M.G. Leahy (1977 meeting

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of Ent. Soc. Amer.) show that high concentrations of precocene block the moulting of nymphal ticks and prevent oviposition in adults. Our own preliminary work shows that lower doses, similar to those used in work on the insects Oncopeltus and Locusta (whose copra allata are sensitive to precocene), kill 85% of nymphal O. moubata when application is at the time of ecdysis. Other experiments will be performed to determine the effects of topical and contact modes of application of precocene II on the development rate (number of nymphal instars preceeding the adult moult) and/or moulting success of nymphal O. moubata. Parallel experiments will be performed to test for the effects of precocene treatment on reproductive development in both O. moubata and R. appendiculatus. If the precocenes have any effects on tick development or reproduction, follow up experiments will test whether these influences can be modified or reversed with application of insect JHs or JHAs.

Tick Protein Digestion:

In order to test one of the basic hypothesis of the immunology portion of the tick programme, physiological and fine structural studies will be performed to investigate the passage of various proteins serologically unchanged through the midgut epithelium.

(1) Fine Structure studies on the process of bloodmeal digestion will be performed at ICIPE using tracer molecules such as horseradish peroxidase. These studies will determine the temporal sequence of pinocytotic uptake into the midgut epithelium and determine the fate of bloodmeal proteins; whether it be entirely digestion or include passage into the tick haemolymph

(2) In parallel experiments to be performed in Israel the passage of proteins through the midgut epithelium or their digestion will be investigated with radiolabelled proteins. A series of *I-125 labelled proteins ranging from cytochrome C (MW=12,000), through haemoglobin (68,000) and bovine serum albumen (80,000) to gamma-globulins (MW above 100,000) will be incorporated into the tick "bloodmeal". On recovery, both from the bloodmeal and from tissue and haemolymph of experimentally fed ticks, these proteins will be identified and quantified with electrophoretic, immunologic and immunoelectrophoretic techniques, coupled with radiological techniques for recognition of the *I-125 label. Detection of radiolabel in other fractions will be used as an indication of the digestion/metabolism of "blood meal" constituents.

6. Priorities: Physiological Programme

(1) To test commercially available growth regulators for biological activity in O. moubata and R. appendiculatus. These compound would then be tested for their applicability in control methods.

(2) To elucidate the chemical structure of the moulting hormone of ticks.

(3) To determine changes in the titre of moulting hormone in ticks and to relate these titre changes to physiological processes of moulting and reproduction.

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(4) To follow the metabolic fate of exogenous ecdysones.

(5) To quantitate protein passage through the tick midgut epithelium

(6) To elucidate the chemical structure of assembly pheromones.

Priorities 2 and 6 will be done in cooperation with Professor K. Nakanishi of Columbia University.

TRE OF
INSECT PHYSIOLOGY AND ECOLOGY
P.O.BOX 30772, NAIROBI, KENYA

RESEARCH PROJECT STATEMENT

A. Project Summary

1. Statistical

Project Title	RESEARCH ON THE ECOLOGY OF TICKS AS RELATED TO INTEGRATED PEST MANAGEMENT
New or Extension	NEW
Contractor and Address	THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY (ICIPE) P.O.BOX 30772, NAIROBI, KENYA
Principal Investigator	PROFESSOR THOMAS R. ODHIAMBO DIRECTOR, ICIPE
Visiting Director of Research	PROFESSOR RACHEL GALUN ISRAEL
Programme Leader	DR. M.P. CUNNINGHAM PRINCIPAL RESEARCH SCIENTIST TICK PROGRAMME, ICIPE
Duration	THREE YEARS
Previous Funding	NONE

Proposed Funding (in U.S. '000$)

	AID	UNDP	TOTAL
1st yr.	104.0	17.3	121.3
2nd yr.	104.0	32.3	136.3
3rd yr.	103.0	67.5	170.5
TOTAL	311.0	117.1	428.1

Abstract

Ticks and tick-borne diseases are major limiting factors in livestock productivity in the tropics. Current control systems, either application of acaricides to cattle by dipping or spraying to control the ticks or chemotherapy to control the diseases are costly and time-consuming exercises with low acceptability by the low-income livestock raisers. Furthermore, the well-known ability of ticks to develop resistance to acaricides requires the continuous development of new materials, itself a slow and costly process. Other adverse factors are relative acaricide toxicity to the host animal, and residues in meat and milk, which are of environmental and health concern.

Tick surpass all other arthropods in the number and variety of diseases transmitted to domestic animals. Relatively few of these diseases are amenable to prophylaxis or treatment. Thus, the only recourse is to control the ticks.

To achieve this goal, three approaches will be concurrently investigated: physiological, immunological and ecological. The ecological approach has been separated in the budget exercise from the other two approaches in order to conform to PAC recommendations.

Comprehensive ecological studies on field populations of ticks will be continued, to produce the basic information required to apply and evaluate the efficiency of the different control methods resulting from the investigations of other research. It should perhaps be pointed out that controlled ecological studies on R. appendiculatus only became possible with the establishment of tick-infested paddocks at Muguga, under the auspices of the ICIPE/EAWAO (now known as the Kenya Agricultural Research Institute) Joint Tick Programme.

As new methods of tick control are developed by separate but related research they will be integrated in a systematic approach for tick control based on ecological data; the ecological investigations are the basis on which integrated pest management is built.

Narrative Statement

(Narrative statement (subheading 1-4) are the same for the three proposals to assure conciseness and to demonstrate the inter-relatedness of the research).

1. General Background and Rationale

All species of vertebrate land animals are subject to attack by ticks, but mammals are particularly attractive to these parasites that transmit important diseases. The food of ticks consists of blood and lymph fluid. Some of the factors which account for the capacity of ticks to spread disease to man and animals include their persistence as blood-suckers, their highly protective sclerotized body, their freedom from natural enemies, and their wide host range.

Ticks are major factors inhibiting effective livestock production, thereby limiting availability of high quality protein for human use. Ticks also transmit serious human and animal diseases and are an economic drain on the income of livestock producers. Present methods for vector control using acaricides (in livestock dips and sprays) are expensive, inefficient and potentially hazardous; and the development of tick resistance is an increasing problem. An economic, safe, integrated control system would eliminate or reduce these problems and conform to AID objectives.

Ecological studies of Rhipicephalus appendiculatus are underway by the ICIPE scientists at different stations in Kenya, including the East African Veterinary Research Organization (EAVRO), where tick-infested pasture facilities have been developed. These studies are providing base-line parameters for tick population control methods which the project is expected to produce.

Some adult tick species produce an assembly pheromone which actively aggregates unfed males and females. The chemical identity of this pheromone is under investigation by the ICIPE. This pheromone is not species-specific; inter-species attraction appears to be strong, and it may be a general assembly attractant for the entire ambosid family, and a few of the ixodid species. See references for pheromones in ticks).

It is well known that development and reproduction in insects are regulated by hormonal interactions. Ticks, like other arthropods, also develop through a series of moults, but the hormone regulating this process in ticks has not yet been identified. There are good indications that MH of ticks is identical, or very close, to B-ecdysone (see ref.). Yet it will be necessary to isolate and identify the moulting hormones if they are to be used in control systems.

Although the research concepts related to using pheromones (attractants/repellants) and hormones (insect growth regulators, juvenile hormones/moulting hormones) as tools in the control of pests have been pursued for a relatively long period in insects, very little has been done in these fields on ticks.

However, a few scientists at a few institutions have begun work in these areas. At the ICIPE, the following main lines of research are underway: (a) the physiology and behaviour of a few tick species including the identification of hormones, assembly pheromones and sex pheromones and tick reactions to climatic factors; (b) studies on the growth and persistence of natural tick populations on experimental plots.

The ICIPE has already accomplished considerable progress in fundamental research related to the possible biological control of ticks as an alternative to chemical acaricidal control. EAVRO and the International Atomic Energy Agency (IAEA) have supported research on the sterile-male technique, and the USDA has supported similar work at TIER; and both efforts have resulted in the conclusion that this method of biological control is not likely to be effective in tick control, except as a possible adjuct to other systems. Consequently, one of the main goals of the ICIPE is fundamental research in the structural analysis and physiology of hormones anti-hormones, and pheromones of tropical ticks. The UNDP has assisted in this line of research on the physiology and ecology of ticks at the ICIPE and EAVRO and this research needs to be continued and expanded. Physiological studies have been

completed which identified the female sex pheromone which attract the male of R. appendiculatus for mating, determined the time for maximum production, and succeeded in identifying them. Of these compounds, 2,6-dichlorophenol attracts the males of a number of tick species and might therefore be of value in integrated tick control programmes.

2. Research Purpose and Expected Products

The purpose of this project is to examine the possible role of hormones and pheromones and cattle immunization as means of controlling pest ticks through integrated biological control methods. To achieve this purpose, a plan of work has been designed which incorporates basic research into critical aspects of the ecology and physiology of the more economically important livestock ticks in East Africa. Experiments will be conducted in an attempt to produce an immune response in cattle which will reduce the number of ticks which feed on them, and which could result in arrested development of those ticks which do feed.

The basic population-ecological studies will continue, as these must be the means for interpreting much of the work, and ultimately of assessing its results at the field level. It has been estimated that tick infestations result in losses in livestock production of as much as one-third the actual potential.

3. Relation to Existing Knowledge

Most experts place the start of 'modern' biological control of pests in the latter part of the nineteenth century. These systems have proceeded through a series of techniques that include use of beneficial insects that prey on pests, infectious agents pathogenic to selected pests, attractants to lure pests to destruction, repellants which protect the host, sterilants which inhibit reproduction and, most recently, the use of hormones that interfere with the developmental processes of the pest. Many insect pheromones and hormones have been chemically identified, synthesized and some have been commercially produced for use in pest control programmes. In the case of ticks, such techniques are in the development stages of identification and synthesization at a few institutions in the U.S.A., Australia, Europe, Israel, and at the ICIPE in Africa. It is only at the two latter locations where much of the work on the principal target species can be carried out.

It has long been known that specific antibodies are capable, under appropriate circumstances, of neutralizing many biologically active macromolecules, including toxins, enzymes, and peptide hormones.

Specific antibodies were reported to reverse established cellular effects of insulin and thyrotropin in vitro. Recently, antibodies were also reported to be physiological antagonists to low-molecular weight molecules, such as steroid hormones, pyridoxal phosphate, histamine, serotonin, chloramphenicol, and cardiac glycosides. By virtue of the capacity of the specific antibodies to bind these substances, they inhibit certain physiological effects of these compounds in vivo and in vitro

The growth and maturation of insects (and probably of ticks as well) are governed by three main hormones : brain hormone, juvenile hormone (JH), and ecdysone (MH), which are responsible for the complex series of moults and growth that these arthropods undergo during their life cycle. The moults are regulated by the brain hormone, which activates production of the moulting hormone (or ecdysone). Changes in concentration between JH and MH determine the nature of the newly moulted arthropod. Early

in the life cycle, relatively large amounts of JH's are present to maintain the juvenile state, and MH level is low. As the immature grows, the JH concentration decreases, while the MH level increases, until moulting in the adult is accomplished, at which time the JH has dropped to undetected levels and MH reaches its maximum.

The chemical nature of the brain hormone is not yet known. It is assumed to be a protein (M.W. 9,000 -30,000), synthesized by neuro-secretory cells in the brain. Pure preparations of this hormone are not yet available.

Three naturally occurring insect JH's have been characterized. These are sesquiterpenoids, so-called C_{18}=JH-1, C_{17}=JH-2 and C_{16}=JH-3.

Antibodies to JH-3 have been recently produced for radio-immune assay of this hormone. By conjugating the hormone to a large carrier protein such as human serum albumin (HSA), via the N-hydroxysuccinimide, the hormone is rendered haptenic and can elicit an immunogenic response. Rabbits injected with the conjugate produce antibodies at a titer of 1.8 ng/ml of blood. Only JH and very close epoxy derivatives react with the antibodies (Lauer et al. 1974b).

The MH of arthropods is a polyhydroxy steriod. For the purpose of radioimmune assay, antibodies against this hormone have recently been produced. Borst and O'Connor (1972) have produced the conjugate, ecdysone-bovine serum albumin (BSA), by conjugating the ecdysone to BSA by way of the isobutylchloro-formate.

Although radio-immune assay of ecdysone is already commonly in use in several laboratories, the physiological effects of the antibodies on insect development have not yet been studied. There is every reason to expect that these hormone systems pertain to ticks as well as insects (see relevant references), and we believe that if antibodies against any of the three hormones can be produced <u>in vivo</u>, then live-stock can be immunized against the tick hormones; and this method can then be used as a control against ticks.

4. Relation to Other Research

This research proposal is designed to build on available technology and completed research on tick physiology, ecology and control, and to complement current research efforts.

The research programme of the CSIRO Division of Entomology in Australia has focussed on chemical, immunogenic, and management methods of control. They have noted that tick resistance is highly heritable, and efforts to breed for resistance are rather encouraging. Research continues on elucidating the mechanisms of resistance.

The proposed research project complements these and other research efforts in investigating the potential for immunization of ruminants against the different antigens and developmental hormones of ticks, which could disrupt the reproductive and other cycles in ticks.

5. Research Project Design and Methods

Ecological Programme

In 1973 ICIPE established a joint project with the East African Veterinary Research Organisation (EAVRO) at Muguga, Kenya, on the ecology of hard (ixodid) ticks. Our object was to complement the work being done at EAVRO on vaccinating cattle against *Theileria parva* infection (East Coast Fever or ECF). We have concentrated on the ecology, physiology and behaviour of *Rhipicephalus appendiculatus*, the ECF vector, in order to lay the foundation for developing improved methods of control, including those which do not employ substances of high general toxicity and to enlarge our understanding of its role as a vector.

The principal work to date has involved the establishment and intensive study of infected and uninfected field tick populations and the investigation of *T. parva* persistance in tick populations feeding only on immunized hosts.

Another set of studies has centered on the effects of cattle density on tick population dynamics. A long-term objective of these studies is to develop a population model for *R. appendiculatus* in one or more situations, from which life tables can be derived and an attempt made to distinguish the key factors in its population regulation and the mode of operation.

(1) Effects of Host Density on Tick Populations

These experiments were started in June, 1976, and will be continued for several more years until the tick population levels at the different host densities have become stable or until they show regular patterns of oscillation. The techniques employed in these studies include daily measurements of temperatures, relative humidity, rainfall and cattle rectal temperatures. Weekly records are kept on the number of ticks on the ears of cattle and routine hematological data (hemoglobin, PCV, differential white cell count, thin blood film) are obtained. Monthly records are kept on half-body samples of all feeding ticks (for species, sex and age determination), *T. parva* serology, numbers of ticks collected on standardized blanket drags and data on the vegatation along transects (including leaf height, depth of the litter, % leaf cover, flowering and % litter cover).

(2) Long Term Study of ECF-Infected Tick Populations

When populations of *R. appendiculatus* infected with virulent *T. parva* were allowed to feed only on cattle which have been immunized against ECF, the infection disappeared from the experimental tick population in 10 months. A subsequent study is now under way on the survival of the tick populations and on the peristence of *T. parva* in the control population, in the absence of cattle hosts. ECF-susceptible cattle are introduced at 1-2 month intervals and grazed in the plots for 6 days before removal to quarantine for observation. The tick counts obtained from the introduced cattle, together with data from blanket dragging, provide estimates of tick population levels without contributing to population increase. Monthly records are kept of the vegetation characteristics as outlined above and the experiment will be continued until all stages of the tick populations become extinct.

(3) Field Study of R. appendiculatus and Associated Species and 2 Tick-Borne Theileria Species

Monthly visits are paid to a herd of Masai cattle being kept under traditional husbandry in an area that is ecologically marginal for R. appendiculatus. The species is present in very low numbers but there is nevertheless clinical and serological evidence of ECF. Tick samples are collected from up to 8 marked adult and 15-20 young cattle, with blood samples for serological study and thin blood films for microscopic examination. Extensive samples of ticks from the vegetation at seven sites are also taken.

The first phase of the work (covering 15 months up to August, 1976), established the basic population picture for the ticks and the disease situation. The work has been suspended due to rinderpest quarantine restrictions but will be restarted as soon as possible. The principal tick species is R. pulchellus, plus R. pravus and Amblyomma gemma. A second Theileria, T. mutans, is common in the cattle, and may in fact be causing deaths attributed to ECF. The virulence of the T. mutans strains will be ascertained at KARI, and the likely vectoring of the disease by A. gemma will be investigated by laboratory transmission experiments.

(4) Anti-Tick Effects of Molasses Grass (Melinis minutiflora)

Our preliminary studies have already confirmed that larvae and nymphs of R. appendiculatus and larvae of Boophilus decoloratus are repelled by the grass and are also unable to climb it. The sheaths are thickly covered (leaf with fine hairs, bearing sticky droplets at their tips. Extracts of the grass in 5 solvents have been tested for their effect on R. appendiculatus larvae, and those in benzene have been found to repel strongly in choice tests.

The next step will be to obtain bulk extracts for chemical fractionation, and to test those fractions for activity, finally attempting chemical identification of the most active through collaborative work with the ICIPE Chemistry Unit. This work complements the pasture studies on M. minutiflora in progress at CIAT, Colombia.

(5) Planned Future Research

Comparison will be made in the laboratory on the survival of all stages of the four species being studied in 4(1) above, under favourable conditions (20°, 23°, 26°C and 75%, 85% and 95% relative humidity). Survival will be studied of R. appendiculatus on the ground at Muguga in nylon mesh bags with monitoring of temperature, RH and rainfall. In addition, survival and dispersal of unrestrained ticks will also be assessed using radioactive marking techniques.

C. Contribution to Institution Building

The ICIPE is a unique International African Institution working on tropical biological problems with a wide range of support from donors throughout the world. The Director has put the Institute contributions very succinctly and well in the preface to the ICIPE 1975 Annual Report:

"The task for the future, as we enter our 1_th year of research, is a challenging one: to continue to serve the developing world in their critical pest problem areas by carrying on high quality fundamental research on those vital questions that need to be answered in order that these pests might be controlled on long-term basis with the least ecological and environmental perturbations. Our task is therefore goal-oriented, tuned to practical application."

The ICIPE is in the process of developing further the existing facilities, and has recently acquired grant support from two European donor agencies for substantial capital expansion. The ICIPE, with the support of the Government of Kenya, has also acquired land for two major field stations for outreach programmes, established a firm working relationship for ecological experimental studies on ticks at EAVRO and developed an intimate relationship with other research centres in Africa, Europe, Asia and the Americas.

The tick research program at the ICIPE will provide short-term professional training for nationals who wish to become proficient in tick research methodology. In addition, the training of young professionals to the M.S. and Ph.D. levels already is being undertaken by accepting candidates for 3 to 6 months yearly at the ICIPE, with the remainder of the training at the home university institution. For training technicians, the ICIPE will accept trainees for 4 to 6 months in specialized areas of tick research. About 5 such participants may be trained, each for 6 months. Such training will be supplemented by appropriate workshops and seminars, so that the ICIPE programme will serve as an international centre for tick research.

D. Linkages with National, Regional, and International Institutions: Research Linkages

This project will provide participation and active cooperation with important research institutions. The ICIPE is associated with the University of Nairobi, Kenya. Studies on the feeding behaviour and nutrient requirements of soft ticks are designated for the 'Israel Institute for Biological Research' (IIBR). The production of ticks is an important component of the research plan, with basic research at IIBR, and field testing at EAVRO, at Muguga near Nairobi. Close collaboration will be maintained with ILRAD, in Nairobi, particularly as related to East Coast Fever, which is transmitted by ticks.

E. Utilization Plans

This is a new project and definite utilization plans cannot be identified at this stage, except that it is expected that a practical biological tick control system may materialize as a replacement for current systems (livestock dips and sprays) which have many shortcomings, such as limited effectiveness, some potential hazards, and high cost (especially to small farmers).

In anticipation of successful research results, such as those achieved in biological control systems for certain insect pests, a training programme for entomologists and biologists has been built into the research work plans. In addition, the ICIPE held a workshop on the physiological basis of tick behaviour in October 1977, and is planning others related to the research effort.

Biological control of pests must be considered as one of the important tools in pest mananagement, and be incorporated in the series of Pest Management seminars conducted by the University of California in various parts of the world.

F. Research Competence

The ICIPE is a relatively new research institute conceived by its present Director and a group of internationally eminent scientists to carry out research on tropical pests within their natural ecological niche (or environment). The institute is located within the campus of the University of Nairobi, with a number of field stations at appropriate locations, to conduct research on specific pest problems.

The institute has a competent resident staff, which is growing and is back stopped by highly qualified and well recognized scientific authorities in a wide variety of scientific disciplines. The institute also engages a number of young, enthusiastic and capable post graduate and postdoctoral fellows. The work of the team is coordinated on a day-to-day basis by a Programme Leader (Dr. M.P.Cunningham) recruited in August 1977. (For a description of the staff directly involved in this project, please see attachment.)

G. Overall Cost Estimates

The included itemized budget sets forth dollar costs for individual line items for the 3 years of the contract (see Attachment).

H. Management Considerations

(a) Organizationally, ICIPE is a unique institution operating with a full-time Director and a permanent staff of 28 scientists, 14 scientific officers, 44 technicians, 4 training and communication officers, and 23 administrative personnel. It has a Board of Governors composed of 15 internationally eminent scientists, institute managers, and policy-makers. The research activities are under the direction of 12 Visiting Directors of Research encompassing six scientific disciplinary groupings. The Centre also utilizes the guidance of consultants and Visiting Scientists.

The ICIPE is represented internationally by two Committees - an African Committee, and the ICIPE Foundation (transformed recently from the previous International Committee).

The ICIPE has established close linkages with other International Centres, notably ILRAD, ILCA, IITA, ICRISAT and CIAT, as well as with EAVRO, FAO, UNDP, UNEP, WHO and IAEA.

ICIPE relationships with European, American and Asian scientific and academic institutions are outstanding.

(b) ICIPE research is now scientifically planned and reviewed by the Research and Training Council, and approved by the Governing Board annually. The ICIPE Foundation and the African Committee meet annually to review the scientific programme of the ICIPE and report to the Governing Board.

(c) ICIPE current overall institutional support:

Estimated Funding for ICIPE (U.S.$ '000)

Date	Duration	Funding	
UNDP	5 Years	996.0	(1977 only)
UNEP	3 Years	342.5	(1977 only)
WHO	1 Year	6.0	
ODM, U.K.	1 Year	75.0	
Rockefeller	1 Year	60.0	
DITH, Netherlands	3 Years	210.0	(1977 only)
DGRST, France	1 Year	18.0	
DANIDA, Denmark	3 Years	330.0	(1977 only)
Kenya (also in kind 85 acres prineland)	1 Year	10.0	
SARDC, Sweden	1 Year	140.0	
Swiss Technical Cooperation Agency	1 Year	108.0	
IDRC, Canada	3 Years	208.0	(1977 only)
Australian Development Assistance Bureau	1 Year	105.0	

Capital Grants

NORAD (Norway)	1,927.0	(1978-1981)
UNDP (for hostel only)	250.0	(1978)
DITH (Netherlands)	409.0	(1977/78)
ODM,UK (for equipment only)	422.0	(1977-1979)

I. Environmental Considerations

This project is one of several research activities designed to reduce or eliminate the need for the use of hazardous pesticides or materials that may contribute to undesirable chemical residues which should be supported by AID. The research is basically oriented to target-specific control with minimum consequence to other species. Since the research design is biological-control oriented, adverse environmental impact will be negligible.

This project has been subject, via the AID, to a formal Environmental Statement (Appendix) by a Committee of Experts which basically concludes that this research will have virtually no adverse environmental effect on the test areas and, that if successful, could result in the reduction in use of environmentally hazardous materials in areas where the system may be applied.

I. Internal and External Review

This is a new research project, hence no review has been made. However, the research concept and planning has been reviewed by qualified entomological researchers from the U.S. Department of Agriculture, a number of academic institutions in Europe and the Americas, the Overseas Development Ministry of the U.K., The International Laboratory for Research on Animal Diseases (ILRAD), and other International Centres. The programme has also been discussed with technical staff of the African Bureau and the USAID Missions of Kenya and Tanzania. A Study Workshop on Tick Physiology and Behaviour with international attendance to acquaint participants with the research objectives and goals was scheduled for October 1977 at the ICIPE Research Centre in Nairobi.

K. Project Statement Evaluation

DS/AGR has long regarded ticks as the most universally destructive pests of livestock and the major health constraint on efficient livestock production. We have also been concerned over the use of hazardous pesticides, the development by pests of resistance to pesticides, the problem of residues from the use of chemicals in pest control, as well as for the costs incurred in the use of these materials particularly by the small livestock operators.