Notes of a Consultation on the Establishment of a Solitary Locust Colony

Nairobi 26th–28th September 1989

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INTRODUCTION

Background

ICIPE is in the process of entering locust research and has identified two major areas of study, semio-chemicals and biological control. Before such research can commence a supply of both gregarious and solitary locusts are required.

A gregarious colony has been established using insects from the DLCO colony in Addis Ababa and this colony is now in the process of expanding.

ICIPE has only limited expertise in the culture of locusts and it is well known that establishing a successful solitary colony is difficult. A consultative group was therefore assembled to advise on the establishment of such a solitary colony. The invited members of the group were Professor P. Pener and Dr. Pritam Singh. The terms of reference of the consultation are given in Appendix 1.

The ICIPE members of the consultation were:-

Dr. P. B. Capstick - Chairman
Dr. M. F. B. Chaudhury
Prof. A. Hassanali
Dr. B. Williams
Dr. W. A. Otieno
Dr. J. P. Ochieng'-Odero
Dr. R. S. Ochieng'
Dr. G. P. Kaaya
Mr. R. C. Joshi
Ms. E. Oladimeji

Phased Introduction

The terms of reference refer to the establishment of solitary colonies of Locusta* and Schistocerca as soon as possible and the need for recommendations on the building and operation of a purpose-built insectary. There is an immediate need to establish these colonies and have solitary insects available as soon as possible. After discussion it was agreed that there was no time available for experimentation in cultural conditions to ensure the latest techniques were used in the new insectary, and the following strategy was agreed; there would be a Phase I in which an

* Locusta always means Locusta migratoriz migratorioides, the African migratory locust which has no egg neither reproductive dispuse. Other subspecies of Locusta migratoriz may have either kind of diapause, or both

initial colony would be established based on known successful rearing methods. Alongside the initial colony ICIPE would initiate a small experimental unit to develop improved housing and cultural conditions and make recommendations for a purpose built insectary. This new insectary would be Phase II.

It was estimated that the Phase I colony could be producing insects for experiments within three months of cages being available if there were no major problems.

OFFTAKE REQUIREMENTS

These are naturally not hard demands at the moment but the following figures provided the basis for sizeing the colony:-

CBRU requirements for semio-chemical studies 10-20/week of both species.
SPRU requirements for behaviour studies and bioassays 100/week of both species.

A total output of 250 insects of both species per week was taken as the basis for further calculations of equipment, space etc.

LOCATION OF PHASE I COLONY

The group inspected the new insectaries at Duduville and the laboratories available at Chiromo. The experts were not in favour of Chiromo as the buildings would require extensive renovation to make them suitable and management and supervisory activities would be divided between the two sites. There is a total of 14 rooms available at Duduville and it was agreed that the Phase I colony and experimental unit would be located there.

Duduville was also inspected for sites for a wheat growing green house and a plot of Kikuyu grass (see on page 8 for details). Suitable sites were located and these will be requested from the ICIPE management.

INSECT SOURCE FOR ESTABLISHING THE COLONIES

It was agreed that there should be two solitary colonies. There was debate on the source of the insects from which to start the colonies. It was the general experience that insects started from gregarious colonies provided a useful means of getting started. Indeed such insects seemed to go quickly into the solitary phase. However, the only insects currently available were those from the DLCO gregarious colony and these were of

unknown history. In the first instance the solitary Schistocerca colony would be started from these insects.

At a time when Schistocerca and Locusta are available in the field these colonies should be estabished and the change in insect characteristics recorded for several generations. The latter has never been examined and the Schistocerca colony could then be compared with that established from the DLCO Addis stock. This would be a suitable subject for a Ph.D. thesis (see page 14).

In both Schistocerca and Locusta the colonies from the field should be established by collecting egg pods, not insects. 5 - 10 pods should be collected of each stock to ensure an adequate gene pool. Eggs from each pod should be pooled, incubated in isolation and sterilised after 8-9 days incubation in 2% peracetic acid for 20 minutes. They should then be washed well in distilled water and transferred out to sterilifed wet and incubated at 30 EC.

Vertical transmission of disease in locusts has not been recorded, thus this method of establishing a colony ensures minimum disease introduction into the insectary.

New introductions should be quarantined for the first four generations to ensure freedom from disease and suitable hatching capabilities.

Only locusts from Africa should be used to establish the colonies. Mali is a suitable place from which to collect Locusta, while Schistocerca can be obtained from the Red Sea winter breeding area of the Sudan.

SOLITARY LOCUST BIOLOGY

Duration of life cycle

Development is highly temperature dependant, for example:-

Egg hatching times and temperatures for Locusta and Schistocerca

	Locusta	Schitocerca
30-31 EC	12 days	13 days
27 EC	16 days	17 days
20 EC	50 days	51 days

Temperature control is a useful way of slowing down the colony if required*. However, the sand must not become dry during incubation. But it is also one of the major factors contributing to insect size variation and must be standardised for the experimental use of insects.

The following table gives the accepted times for the major stages of the cycle for both species in the solitary phase and if bred under an equal 12 hour photo-period (L:D=12:12) at temperatures of 37 EC during light periods and 27 EC during dark periods. These temperatures should be the highest in any part of the cage.

Stage	Locusta	Schistocerca
Egg hatch at 30 EC*	11-13 days	12-14 days
Larval development to adult moult	24 days	25-29 days*
Time to sexual maturity	7-9 days **	14-35 days ***

* Schistocerca usually has one extra moult in the solitary phase. 90% of females have extra moults, males 60%. Each insect that undergoes an extra moult has an extra eye stripe.

** Locusta has a sexual maturity time of 10-19 days in the gregarious phase. High colony density slows maturation time.

*** There is a wide variation between individuals. Males tend to be rather more regular at 14 days. High colony density decreases maturation time.

Humidity requirements have been poorly explored. It should be maintained between 40 - 50% RH. Further work needs to be done in this area (see experimental programme). High anibrent humidities (80% RH or more), should be avoided.

* However, the sand must not become dry during incubation.

Expected Yields

In general fecundity in locust colonies is high, and is never a limiting factor on production unless disease is present in the colony. The yields given below for each stage are deliberately reduced from that which will be attained. Colony numbers should be based on the following minimum colony build-up statistics.

Each female will produce 4 egg pods
Each egg pod will produce 50 eggs
Each egg pod will produce 20 males and 20 female hatchlings
Each 1000 solitarious phase hatchlings ** should produce 900 adults
5 mating pairs will produce 800 hatchlings
5 Schistocerca breeding pairs maintained at -27 EC will produce 720 sexually mature adults every 51-78 days
5 Locusta breeding pairs maintained at -27 EC will produce 720 sexually mature adults every 42-45 days.

* Incubator temperature, not related to cage temperature(s).
** The ratio 200 adults from 1000 hatchlings tilts the crowded colony.

FEEDS AND FEEDING

In general food quantity is not a problem. A high quality of food is required. Care must be taken to ensure that food is completely insecticide free. It is wise to wash all food as a routine. For experimental purposes insects should be on the same diet. Faeces contaminated food should be regularly removed from the cage.

Fertiliser has no apparent effect on locusts and can be used on Kikuyu grass and cabbage plots.

Basically there are two types of food - dry foods, and green or growing food.

a) Dry Food - This can be of crushed grain or flaked oats. It is important to wash and dry before flaking. For optimum results available grains should be compared and evaluated.

Dry foods are fed on an ad libitum basis. They should be always available and should be removed and cleaned out every two days. Suitable containers for dry food are glass or plastic Petri dishes. These should be as small as possible to reduce faeces contamination.

b) Green Foods - Many growing plants have been investigated.

i. Cabbage is essential for Schistocerca which is a polyphagous plant feeder. Remove outside leaves. Essential to establish aclean source of cabbage or grow ones own. (Note that Rao et al. reported that cabbage was harmful). Cabbage can be made available every two days. Sukumawiki (kale) has been used as it is easier to grow. However there are reports that it can be inhibitory.

ii. Kikuyu Grass - Should be grown on site if proper quality control is to be established. 120 m E is adequate to feed about 20 cages of gregarious locusts. There should be no insecticides. Wash if in doubt. As routine dry feed should always be accompanied by Kikuyu grass for both species and with additional cabbage or Schistocerca. Grass or growing grain feed should be available daily.

Grass can be cut and kept in the fridge for one week. However daily cutting is better.

iii. Germinated wheat is an excellent daily food. It can be grown in pots in an open building until approximately 25 cm high. The growing stalk is then cut off and placed in a container with water in the cage. It should be replaced daily. In young (hatchling) cultures the container should be blocked off with grass to prevent drowning of hatchlings and excessive humidity. Over all stages of a colony an average of approximately 1 gram of wheat daily per insect is required. The germinated wheat and the kikuyu grass are interchangeable.

iv. Maize leaves have been used, but reduced reproduction is reported after 4 generations.

v. In many colonies antibiotics and other additives are used to suppress disease and increase performance. Nothing is known about the effect these substances have on other insect parameter or on gut flora. It was agreed that in the first instance the ICIPE colonies would not use other additives. They would be used if subsequently needed for disease control.

HOUSING

Many different cages have been used for solitary locust rearing, only one or two designs have been consistently successful. Each locust is reared in a seperate cage in isolation. It should not be able to see any other insect or its own image. This will cause it to lose some solitary characteristics. Accoustic factors do not appear to affect solitariness.

No details are available as to the precautions that need to be taken to avoid contamination with pheromones etc. Good air exchange is recommended, but actual flow rates per cubic foot of colony are not available. The colony should be run at a negative pressure, (actual psi not known). In general air exhaust systems from gregarious and solitary colonies should be widely seperated to prevent cross contamination.

Cage design

Plastic cages have been used and are satisfactory. The design was based on the Pener cage. However no models are available and there are no commercially available locust cages of any material.

It was agreed that the ICIPE Phase I colony cages would be based on those proven to be successful in Professor Pener's colony for the last 20 plus years. Detailed photographs of the cages were made available by Professor Pener and these have subsequently been translated into working drawings which are attached at Appendix 2. The original cages are of steel, but the ICIPE versions will be of aluminium.

These cages come in banks of eight individual cages. Each block of cages is held at a convenient height for servicing*. They are not stacked one on top of each other, because of potential semio-chemical contamination. Each bank of 8 cages has two bulbs suspended over the top of four cages. This provides light during the 12 hour photo period and also heat. The height of the bulbs from the top of the cages is also variable and provides a convenient way of regulating cage temperature above 27 EC.

It was agreed that the ICIPE cages would be held on trolleys to minimize space requirements. They would have attached cloths (curtains) to prevent visual contact.

It was suggested that ICIPE should obtain quotations, with samples, from three local manufacturing engineers.

Room Requirements

Each bank of cages measures 40 cm wide x 20 cm deep x 25 cm high. Each available insectary room measures 1.5 m wide x 4.5 m long. If two cages are kept on one trolley, 12 trolleys can be kept in each insectary if they are subjected to "library" type stacking.

Trolleys will be linked by an electrical supply system to provide power to the lights. The electrical light system in each room will have a time switch accurate to 1 minute capable of switching at 15 minute intervals to provide a variable photo period.

Such a stocking density seems feasible to the experts and would give 192 solitary insects per room. Atthe predicted reproduction and development rates this means that four rooms of Schisticerca (colony size 768 insects) would provide 120 insects per week. Three rooms of Locusta

* Eye level of a person comfortably sitting on an ordinary chair.

(colony size 576 insects) would provide about 120 adult insects per week for experimental purposes. The larger Schisticerca colony is required because of the additional moult and longer period of sexual maturation; therefore increased development time in this species.

This output is only slightly below the estimated requirement. It is the best that can be achieved with the available space, and will probably be adequate if some hoppers are used experimentally.

Thus 7 rooms are required for the two colonies. In addition one room is required for washing and cleaning cages. One room is required for food preparation. One room is required for materials and equipment storage. Three rooms are required for the experimental programme. These 13 rooms are available in the insectary.

Room Modifications

Colony insectaries will have background electric fan heating variably adjustable to maintain the basic temperature at 27 EC. Heating to 37 EC will be by individual cage lighting. Each room will have an individual temperature recorder. (Note; it is essential that all rooms in a colony are run at the same temperature).

The Experimental insectaries will be similarly modified but humidity control equipment should also be installed, as there is little knowledge of the critical humidity requirements of a solitary colony.

Ducting to extract air will be specially constructed. The extract motor will probably be situated in the roof space with air discharge at ground level to minimize semiochemical spread. Individual room extraction rates (and thus individual room negative pressures) will be adjustable using door grilles.

The washing-up room will require to be stripped of its present benching and sinks. Sinks of a size to allow washing and sterilising of 16 cages simultaneously should be installed.

The corridor will have a lab coat changing area constructed.

Solitary and Gregarious Colony Seperation

The gregarious colony is on the same floor as the proposed solitary colonies. It is proposed to separate them by a sealed door constructed in the corridor near to room 31-15. It is recommended that this degree of seperation in

conjunction with seperate air ducting should be adequate. For details of staffing, see Colony Management.

COLONY MANAGEMENT

The following are comments relating to colony management given by the consultants.

Disease minimisation

Samples of insects should be passed on a regular basis to Pathology for routine screening for disease. Every year for solitary insects and every 3 months for gregarious.

Each insectary should be swept every day and be maintained spotlessly clean.

There should be adequate spatial and personnel separation between locust user groups (eg. Biocontrol) and the rearing colonies.

All dead insects should be removed immediately from the cages and sent to insect pathology.

All equipment should be sterilised either by UV, boiling or 2% peracetic acid.

Sand for egglaying should be well washed, dried and sterilised at 120 EC for three hours. Such sand may be either sharp or soft as long as it is clean.

Eggs can be sterilised after 8-9 days incubation in 2% peracetic acid for 20 minutes. They should then be transferred to nutrient agar or to clean wet sand and incubated at 30 EC.

Cages should be regularly cleaned each day using a small vacuum cleaner to remove faeces*.

Cages should be sterilised after each generation by steam or pressurised hot water cleaning, then soaking overnight in 5.25% sodium hypochlorite or 0.5% chlorocresol. Cages should be well rinsed after that treatment.

* The bottom of the cages, <u>beneath</u> the false floor should be vacuum cleaned. Vacuum cleaner cannot be used, when locusts are present in the cages; it will sweep out the locusts!

Egg Laying

The sand must be kept moist in the cage or the females will not lay and/or the hatch will be poor. Too much moisture is undesirable as it induces fungal growth.

Wet sand slowly until a hand pressed ball of sand dropped from 5" breaks into a few pieces.

Sand should be evenly filled into the laying container with no spaces. Hatchlings are not geotropic and will follow the line of least resistance after hatching. If holes exist in the sand they will collect there rather than at the surface.

Remove 0.5" of sand from the top of the laying container on the day of laying to leave room for the hatchlings when they emerge.

Close the hole in the floor when the laying chamber is removed. Egg-laying should be checked once a day! Do not leave vessels with sand in cages for more than one day.

Incubation

Egg will go into quiescence and ____ die. For moistening a hole should be made (for example, by a pencil) in the sand, far away from the egg pod and the water should be added slowly into the hole. They can be moistened if too dry.

Pods can be stored at 20 EC for up to 50 days.

Examine laying chamber daily on days 10 and 11 and closely from day 12 of the incubation period. Cover chamber with a transparent petri dish to allow visual screening for hatchlings during incubation.

Hatchlings

Separate as soon as possible. Reduce movement to aid separation either by chilling or CO^2 or N^2 anaesthetisation. N^2 is best.

Rearing

Hatchlings can be in 2nd stage within 3-4 days. During first two stages provide green food every day. Then change to 3 x weekly before and after adult moult.

Mating

7-10 days after adult moult put male into the female cage and place oviposition vessel in cage. Remove male after one day to prevent reversion of both to gregarious phase. Repeat the procedure once per week.

Check daily for egg pods. Change oviposition vessel once a day. Remove top 0.5" and the pod plug can then be seen. If an egg pod is laid remove the container, cover it and transfer it to the incubatoor (30 E).

Use different males on the same female. Put males in each week for copulation and remove next day.

Female can be reused up to 4-8 egg pods. Do not keep adults breeding stock after 3-4 months.

QUALITY CONTROL

There are over 50 characteristics identified with solitariness. The consultants were unable to recommend which ones should be included in the ICIPE colony management.

However, uniformity of size, colour, number of moults and good health are first requisites on which any system should be based. ICIPE should then develop its own Q.C. criteria.

EXPERIMENTAL SECTION

An experimental colony is planned to commence operating three months after the comencement of the solitary colonies. The terms of reference of the experimental section should be to develop cultural and building/environmental recommendations for the purpose built solitary insectary in Phase II.

The following factors are some of the investigations that need to be carried out:-

The effect of humidity on colony performance and solitary characteristics.

Improvements in cage design.

Improvements in the routine handling of the colony.

Establishment of solitary quality control characteristics.

Comparison of solitary characteristics of colonies that have been recently established from the field and those that have been in culture many years.

Recording the changes that occur when a solitary colony is established from field insects.

Note: The last three items could form the basis of a Ph.D. thesis for a suitable ARPPIS student.

PERSONNEL

The number of staff required for full operation of a 250 insect/week solitary colony is given in Appendix 2.

Recruitment

i) When recruiting for the colony all applicants should be asked to pick up several free locusts. This is a useful way of weeding out those who are frightened of large insects.

ii) Enquiries should be made for a past history of asthma problems. Such people should not be employed as they are susceptible to allergies.

Clothing

i) Working at 37 EC is exhausting. Provision should be made to supply light T-shirts and trousers/shorts.

ii) A water cooler should be available to provide cold liquid.

Allergic Reactions

i) There are two types of allergic reactions, cutaneous and respiratory*. Both are serious and require the affected person to be withdrawn from the colony, although cutaneously affected staff can remain for a while if gloves are used continuously.

ii) Recovery is rapid and the allergy reverts after a few days separtion from the colony. However, the affliction is permanent and will reappear rapidly if the person re-enters the colony.

iii) Respiratory* allergy is probably acquired by inhalation of minute pieces of cuticle. Such pieces contaminate the faeces, so the latter are also allergogenic.

iv) Allergy develops after 6 months to several years.

v) Insectary staff allergies have been increasing in incidence over the last 20 years - reason unknown. However, only one case has been recorded from the DLCO in Addis and Nairobi.

vi) It is recommended that ICIPE has a rule that all insectary staff wear both gloves and face masks (of the surgical type). It should be recognised that many staff will be unable to abide by this rule due to the heat in the insectary.

RESOURCE REQUIREMENTS

i) A Personnel budget is attached at Appendix 2

ii) Equipment Expenditure. A proposed budget is attached at Appendix 3.

* Respiratory is a better term because it includes the trachea and the mucous membranes of the nose, phnynx etc.

Appendix 1

TERMS OF REFERENCE

1. Selection of appropriate sites, within Duduville Campus for:
   a) Insectary
   b) Screenhouse for growing plants for locust feeding
2. Design of a Functional Insectary, in terms of:
   a) Architectural and Structural Design
   b) Design of Isolation, Airflow and Human Traffic System
3. Chart Rearing Methods, in terms of:
   a) Origin of locust material
   b) Genetic variability and diversity
   c) Quarantine process and procedure
   d) Colonisation method
   e) Techniques of rearing, adequacy of diets and nutrition
   f) Instrumentation and Automation
4. Design of a Quality Control Programme, in terms of:
   i. Assessment of the quality of the locust.
      a) Biological parameters to be assessed
      b) Design of a feedback process
      c) Pathology
   ii. Assessment of the quality of the rearing process
      a) Design of process control procedures
      b) Trouble shooting systems

5. Definition of an Insect Rearing Management Programme (IRM) for Phase Solitaria.

a) Resource Allocation

* Financial
* Physical
* Personnel

b) Insect Order and Supply Systems

c) Colony Build Up and Regulation System

Appendix 2

Personnel Requirements for Solitary Locust Colony - Phase I

Not all the staff detailed below will be required at start up. The requirements given are for the first full year of operation after both Locusta and Schistocerca solitary colonies have been established and the Experimental Unit is operational.

The following are the main activities that require to be carried out.

COLONY

Servicing of the 7 colony rooms

2 x T4	Technicians
2 x T5	Technicians

Food Preparation

1 x T5	Technician

Washing and Sterilising

1 x T5	Technician

Food Growth

1 x T6	Field Assistant (will also assist in Gregarious food production)

EXPERIMENTAL UNIT

1	Scientific Officer	M.Sc.
1	Technician	T4
1	Field Assistant	T6
1	ARPPIS Student	

Appendix 3

Equipment Budget for Solitary Locust Breeding

Number	Description	Amount
1	Corn Crusher for making flaked maize or wheat	
2	Oven - Sterilising Operating temperature 120 - 140oC	
1	Hot Water Pressure Cleaner	
1	Humidifier. With controls to set room humidities.	
1	Sickle or grass shears for cutting Kikuyu Grass	
1	S/s Instrument Steriliser	
2	Hatching incubators with fans and false wall circulation. Operating temp. plus/minus loC.	
220	Cages	
220	Screens between trolleys/cages ("curtains")	
220	Trolleys	
9	Vacuum cleaners - small	
1	Refrigerator	
9 prs	Calipers	
1 doz	Maximum/minimum thermometers	
9	Thermohygrograph (with weekly chart) for each room	
10	Variable Extract Fans	
10	Fan heaters with infinitely variable rheostat settings. Should have rotating head.	
10	Temperature thermostats. Sensitive to 0.5oC.	
1	Electrically operated cage cleaning brush	
11	Time switches. 24 hours, 15 minute periods. To switch 48 x 100 watt bulbs.	
1	Waring blender - commercial type	

1 Balance Plus/minus 0.1 gm for weighing food additives.

Vessels for egg laying and lids

Jars for grass

Small Petri dishes for dry food