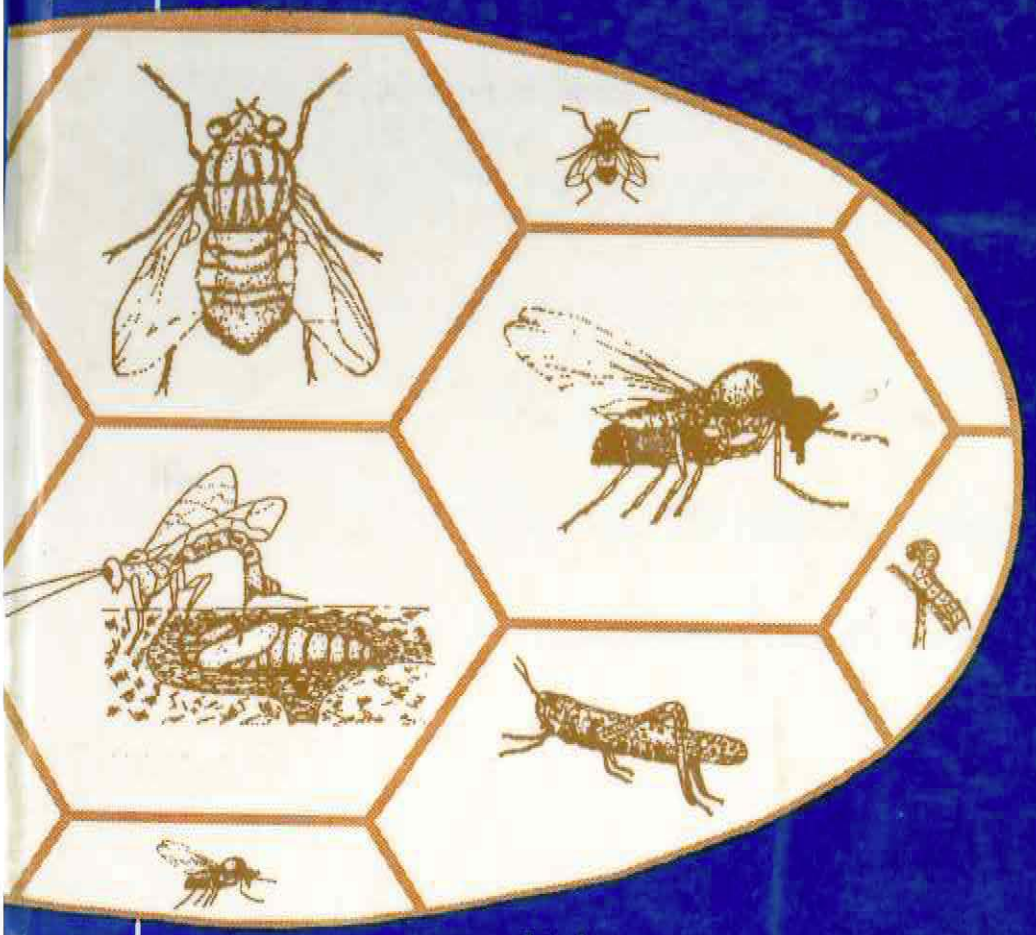
Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies

Edited by
J. P. R. Ochieng'-Odero



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Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies

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Proceedings of the International Group Training Course on Techniques of Insect Rearing
for the Development of Integrated Pest and Vector Management Strategies
16th March–3rd April 1992
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Preface

The ability to colonise and rear various species of target insects is crucially important to all aspects of research in insect science. In many regards it is a pre-requisite to the sustainable and environmentally safe management of many destructive pests and vectors. There are many examples where it is the primary tool in research and management of various insect species. Nevertheless, the overall importance of insect breeding goes beyond that. It is key to the success of various industries, especially those that are related to beekeeping, sericulture, and lac-culture. It is in the wider context that this International Group Training Course on Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies was conceived and held between 16th March and 3rd April 1992, at the International Centre of Insect Physiology and Ecology (ICIPE), in Nairobi, Kenya.

The participants of this three-week course were drawn from various parts of tropical Africa. Countries represented in the course included Ethiopia, Kenya, Tanzania and Uganda (from Eastern Africa); Zambia, Malawi, Mozambique, Swaziland, Zimbabwe and Angola (from Southern Africa); Rwanda, Cameroun (from Central Africa); the Sudan (from Northern Africa); and Nigeria, Senegal and Burkina Faso (from Western Africa). These regions and countries represent several agro-ecological zones, various cultures and socio-economic experiences, all meeting together, for the first time in tropical Africa, to discuss and learn insect rearing techniques for pest and vector management. The course was structured to include formal lectures within the first week, and more emphasis on practical work and individual projects during the following two weeks.

Right from the onset, the ICIPE scientific community felt that we needed to build a firm foundation on the nature of the rationale for insect rearing. It is for this reason that Professor K.N. Saxena, the Deputy Director (Research) of the ICIPE, and Dr. Udo Feldmann, of the International Atomic Energy Agency (IAEA), gave opening papers on the relevance of insect rearing to the management of various pests and vectors. We also decided to show examples of programmes that rely extensively on successful insect rearing practice; and for this reason the participants visited the Medical Vectors and Locust Research Programmes of the ICIPE.

Presentations were also given on the scientific basis for insect rearing. Professor J. W. Smith Jr. of Texas A&M University discussed the ecology of successful parasitism as well as the foraging strategies of parasites. Dr. Gary Hill of the International Institute of Biological Control (IIBC) talked about methods of

colonisation and quarantine of insects; while Dr. Franz Bigler of the Swiss Federal Research Station for Agronomy discussed the usefulness of quality control in insect rearing systems.

The lectures then moved onto the practical considerations of rearing. Dr. Gary Bernon of USDA-APHIS discussed the basic consideration in the design of functional insectary as well as his experiences in the rearing of the dung beetle, the oil palm leaf mining beetle, and the gypsy moth. Mr. Joab Amusan from the International Institute of Tropical Agriculture (IITA) discussed practical aspects in preparation of artificial diets. Various ICIPE scientists discussed the rearing of various plant tissue borers, and of tsetse, mosquitoes and parasitoids. The lectures included presentation on the rearing of other related arthropods, such as livestock ticks and the predaceous mites. Brief introductory remarks were given on the rearing of the silkworm and the honeybee. Finally, a session was included on the breeding of small mammals for the maintenance of blood-feeding arthropods.

Participants were then exposed to the rearing practices developed at the Duduville Headquarters as well as at the Mbita Point Field Station of the ICIPE. Each participant selected an area of interest and worked on it enthusiastically until completion. The projects were varied: for instance, a participant with a background on coffee pests worked on the oviposition behaviour of the legume pod borer; again, Mr. Alioune Beye, the coordinator of a Regional Centre for Survey and Pest Control in Senegal, was able to accomplish three projects in the rearing of the parasitoid *Cotesia*. Mr. Beye found useful information on the effect of temperature and diet on *Cotesia*, its fecundity, and the attraction of Lepidoptera frass to the parasitoid. Yet again, Mr. McDaff Musa Ziba from Zambia was able, in the short time of the project, to colonise the lacustrine species of tsetse, *Glossina fuscipes*.

This course was not designed to turn the participants into expert rearing practitioners instantly; but rather, to sensitise them into the importance of having scientific basis in the development of any specific rearing methodology. We wanted to expose the participants to problem-solving tactics in developing this extremely useful tool in pest and vector management. The course was not structured to give all the answers to the rearing of insects (we could not even if we wanted to, as there are as many techniques as there are species!); but rather to expose the participants to what is being successfully done elsewhere. If each participant went back home, feeling confident that they learnt the right basis for developing rearing techniques and tactics; that they had seen relevant examples and met useful people, then this first Group Training Course could be deemed as a success. In order to allow a wider dissemination of this experience, this Manual

is being produced for use widely by rearing practitioners within the Pan-tropical regions of the world.

Let me thank all the course participants, the various resource persons, and the planning committee for an excellent job done in a timely manner.

Professor Thomas R. Odhiambo,
Director, International Centre of Insect Physiology and Ecology, Nairobi

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E. The Rearing of Beneficial Organisms

Rearing Parasites of Lepidopteran Stalk Borers of Tropical Gramineous Plants

J. W. Smith, Jr. and R. N. Wiedenmann

Department of Entomology, Texas A&M University, College Station, Texas 77843-2475

Abstract

Successful laboratory rearing of the parasites attacking tropical lepidopteran stalk borers requires an understanding of host life history, the process underlying successful parasitisation and an insight into parasite foraging strategies. Traits common to the life history of pyralid and noctuid stalk borers of tropical gramineous crops are reviewed. The sequence of steps required for successful parasitisation are detailed with special emphasis on the steps most important for laboratory rearing. Foraging strategies for different parasite taxa usually subfamily, are related to the parasite's attack method. Elucidating the foraging strategy used by a particular parasite provides the initial clue on rearing methodology to pursue. Seven distinct attack methods are presented for parasites belonging to the egg, larval and pupal foraging guilds. For each attack method, a short summary of parasite biology and anticipated rearing problems are provided. Citations follow to direct the reader into the published rearing literature.

Introduction

Stalk borers attacking tropical gramineous crops are chiefly Lepidoptera of the families Pyralidae and Noctuidae. The more economically important stalk borers belong to the pyralid genera *Chilo* Zincken, *Diatraea* Guilding, *Bissetia* Kapur, *Eoreuma* Ely, *Coniesta* Hampson, *Haimbachia* Dyar, *Tryporyza* Common, *Maliarpha* Ragonot, *Girdharia* Kapur and *Eldana* Walker and the noctuid genera *Busseola*

Thurav and *Sesamia* Guenée. *Diatraea* and *Eoreuma* are Neotropical, the ten remaining genera are almost exclusively Paleotropical. Lepidopteran stalk borers attack rice, maize, sorghums, millets and sugarcane throughout the tropics and subtropics.

Biological control in tropical crops will require successful laboratory rearing of hosts and natural enemies. The level of rearing needed will depend upon the number of natural enemies required by the biological control approach. The classical approach of field-colonisation of an exotic parasite will require fewer individuals than will periodic augmentation. However, rapid range expansion of a successfully field-colonised natural enemy may be as numerically demanding on the rearing regimen as augmentation. Regardless of the quantity of natural enemies needed, rearing procedures must possess the physical and chemical cues needed by the natural enemy for host acceptance and suitability.

The diversity of life history strategies of stalk borer parasites dictates a similarly diverse array of rearing methodologies. Due to space limitations, we cannot detail most components of rearing stalk borer parasites. However, our approach is to present stalk borer life history coupled with parasite foraging strategies as a template for developing rearing methods, which will allow the reader to develop specific rearing methods for a particular parasite of interest.

Life History

Although each stalk borer species has inherent life history traits, a generalised life cycle provides a common template for viewing biological and ecological similarities across genera. The general life cycle also provides a convenient outline for recognising subtle differences between the typical life history and the life history of a specific stalk borer. The general life cycle can be easily modified to accommodate the attributes of a specific stalk borer.

All life stages of the more common stalk boring pyralids and noctuids inhabit the aerial portions of gramineous plants. Adults oviposit on the plant leaves, depositing eggs either singly or in masses ranging from two eggs to several hundred eggs. Pyralid eggs are oval, flattened and scale-like, and are laid in imbricated rows. Noctuid eggs are semi-globular, laid singly or in rows. Eggs are creamy white when first laid and darken before eclosion. The head capsule of the neonate larva is usually visible through the egg chorion just prior to hatching. Usually 3 to 6 days are required for eggs to hatch under tropical temperatures.

Stalk borer larvae occupy two distinct plant microhabitats as a result of age-related feeding behaviour. Early instars superficially mine leaves and leafsheaths, which confines feeding to the stalk periphery. During this period, young larvae are cryptic and cannot be seen without dislodging the leaf sheath from the stalk and exposing the mining activity. In contrast, older larvae excavate extensive feeding tunnels inside the stalk. Tunnel size, length and architecture vary between species; some species tunnel through plant internodes, others exit a tunnelled internode to bore into new internodes, and some species tunnel in only one internode. Tunnel architecture can be longitudinal, transverse or a combination of both. Most borer larvae maintain relatively clean feeding tunnels by regularly depositing the frass outside the tunnel entrance. In contrast, a few species maintain closed tunnels, in which the larvae plug the traversed area with frass and detritus, thus packing the tunnel.

Larval development requires 25 to 45 days. Just prior to pupation mature stalk borer larvae construct a pupal chamber at the terminus of the feeding tunnel. The pupal chamber is excavated slightly larger than the feeding tunnel and access to the feeding tunnel is packed with frass and detritus. To facilitate egress from the stalk upon moth emergence, the mature larva also constructs an exit tunnel from the pupal chamber to the outside of the stalk. The outer layer of the stalk epidermis is left intact and forms a conspicuous "window" as the thin epidermal layer dehydrates. The integrity of the emergence window varies with borer species and host plant. Some windows are intact and sturdy, whereas others are fragile and tattered. After completion of the pupal chamber, tunnelling and locomotor activity ceases, and the mature larva merges into an inactive prepupal stage that may last several days before pupation. The noctuid genus *Sesamia* pupates differently. Instead of constructing a sheltered chamber in the stalk for pupation, mature *Sesamia* larvae usually vacate the feeding tunnel and pupate between the stalk and leaf sheath, secured by loose silken threads. Pupation requires 7 to 10 days for both pyralids and noctuids.

Successful Parasitisation

Successful parasitisation requires a sequence of distinct and consecutive processes. The parasite must locate the habitat that harbours its host and then, within the habitat, must find a host. Once a host is located, it must be acceptable for oviposition and be suitable for completion of parasite development. Finally, the parasite must regulate host physiology to enhance and maintain host suitability.

This sequence of events has to occur, whether under natural conditions or during laboratory rearing. Successful laboratory culturing requires that an acceptable, suitable host be presented in a manner recognisable to a receptive female parasite and that the ovipositing female and/or her progeny regulate the host to prevent rejection of the progeny and enhance host suitability. Recognition of how the sequence of processes occurs in the field is critical; from that recognition, laboratory rearing methods that mimic the sequence of events will enhance successful culture of parasites. Violation of any one of these events will result in failure of the parasite culture.

In the laboratory, the process of habitat finding is fulfilled by placing the parasite and host in close proximity within a rearing cage or container. However, parasitisation will not proceed unless the receptive female parasite receives the proper cues that lead to host finding. Chemical by-products of normal host activity such as oviposition, feeding, defecation, or silk production can act as kairomones that provide chemical stimuli to guide the searching parasite and lead to host location. The physical microhabitat of the host also plays an important role in host recognition, especially for stalk borer parasites, because hosts are often cryptic. Physical cues such as the larval feeding tunnel, moth emergence window, or the larva or pupa enclosed in a stem are often required for host recognition. The exact role a specific physical cue contributes to parasite success may be unknown, but cues are necessary for successful host finding.

Cues associated with host finding in the natural setting also need to be included in the laboratory. Some host finding cues are essential, whereas others may simply enhance productivity of the colony. Recreating the natural host microhabitat and normal activity in the laboratory setting usually will provide the essential cues for host recognition. Often, artificial physical microhabitats that mimic the natural situation can be successfully substituted.

After the parasite has found the host, the process of selecting an individual for oviposition begins. The host selection process is the result of coevolution between the parasite and host, ensuring that the progeny committed to the host have the best chance of surviving. The sequence of habitat selection, host finding, host selection and acceptance progressively narrows the range of potential hosts that a parasite may encounter. That narrowing of potential hosts ensures the host accepted is not one that is physiologically unsuitable for the parasite. Physiological

suitability is an absolute necessity for development of parasite progeny, because of the intimate relationship between parasite progeny and hosts.

In nature, any given parasite will have a narrow host range which may be host-specific and/or stage-specific. Other parasites may have a broader host range, selecting and accepting hosts that meet a more diverse set of criteria. Requisites for host acceptance may include host size, shape, texture, age, odour, behaviour and previous parasitisation status. For laboratory rearing, the first choice for a host is the natural host that has coevolved with the parasite. However, the coevolved host may not be available and alternative hosts must be substituted. Many stalk borer parasites have been successfully reared on factitious hosts, either Lepidoptera that are easily reared and readily available, such as the wax moth (*Galleria mellonella* L.), or the stalk borer species targetted for biological control. Familiarity with the literature provides a basis for determining the potential host range for a parasite.

The final process in successful parasitisation is host regulation. The parasite must regulate certain processes of the host development for the parasite's own advantage, to assure development of parasite progeny is complete. Parasitised hosts differ from their unparasitised counterparts both physiologically and ecologically. Changes in parasitised hosts caused by both the ovipositing female and her developing progeny include altered growth rate, food consumption, development, morphology, behaviour, biochemical and physiological activities.

Host alterations of interest in stalk borer parasitisation are host paralysis and the host immune response. Host paralysis is common among the ectoparasitic Hymenoptera attacking stalk borer larvae in the feeding tunnel, for which parasitism is a two-stage process, starting with paralysis of the host followed by oviposition. Host immune systems and the evasion of those systems by parasites are the subject of recent interest; the wealth of information about immune responses is beyond the scope of this manual. However, for the purposes of rearing parasites, a brief mention is appropriate. The immune system of host insects is a primary means for the host to maintain homeostasis, by attacking invading organisms. In turn, a successful parasite must evade the host immune response. Evasion can consist of active responses by the parasite or responses that are more passive, due to suppression of the immune response.

Foraging Strategies

The processes necessary for successful parasitisation point out the importance of reproducing the ecological conditions for rearing parasites in the laboratory. Those steps, coupled with the stalk borer life history, provide a template for discerning how a parasite may utilise the host. In addition, successful rearing requires understanding the particular foraging strategy employed by the parasite.

A foraging strategy can be defined as the set of behavioural and morphological adaptations that enable a parasite to exploit a particular host effectively. The strategy will include all aspects of the host/parasite association, including the method of attack, disposition of the host (cryptic or exposed), the cues employed to find the host, the host stage attacked and the host stage from which the parasite progeny emerge.

Although a particular parasite species may be very host-specific, there are usually numerous parasite species that will utilise the same host stage. The different parasite species that exploit a specific host stage in a similar manner comprise a guild. Therefore, in the case of stalk borers, each life stage has a guild of natural enemies associated with it. There also can be some overlap between guilds, especially for parasites with complex biologies that utilise more than one life stage, such as egg-larval parasites.

Table 1 shows foraging strategies of stalk borers parasites, arranged approximately phenologically, from egg parasites through pupal parasites. For each foraging strategy, we have identified the method of attack, whether the host is exposed or cryptic, the types of cues thought to be used to find the host, stage attacked, stage from which the parasite emerges, and examples of taxa that employ such a strategy. Note that within a parasite foraging guild, there can be several strategies employed to utilise the host, depending on the age of the host attacked, or the behavioural or morphological adaptation used to attack the host.

The particular adaptation and strategy employed by a parasite will depend primarily on several factors of the host behaviour and life history. The strategy depends on the particular host stage, as well. Stages of stalk borer hosts can be exposed, such as eggs, or cryptic, as are all larval and pupal stages. Even among larval stages of stalk borers, parasites will discriminate between early-instar larvae found in the leaf sheaths, later-instar larvae that tunnel in the plant stalk,

Table 1. Foraging strategies of parasites of tropical stalk borers

Attack method	Host disposition	Proximal cues	Host stage attacked	Host stage emerged from	Examples
Egg Guild					
direct	exposed	host	egg	egg	Trichogrammatidae (*Trichogramma*, *Trichogrammatoidea*), Scelionidae (*Telenomus*)
Larval Guild					
direct	exposed	host	egg	larva in stalk	Braconidae, Cheloninae (*Chelonus*, *Phanerotoma*)
drill & sting	cryptic	frass, tunnel	larva in stalk	larva in stalk	Braconidae, Doryctinae (*Allorhogas*, *Rhaconotus*, *Heterospilus*), Braconinae (*Myosoma*, *Bracon*)
probe & sting	cryptic	damage, frass sheath	larva in leaf	larva in stalk	Braconidae, Macrocentrinae (*Macrocentrus*), Agathidinae (*Alabagrus*)
probe & sting	cryptic	damage, frass, moth emergence window	mature larva in pupal chamber	mature larva	Ichneumonidae, Gelinae (*Mallochia*)

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Table 1. continued

Attack method	Host disposition	Proximal cues	Host stage attacked	Host stage emerged from	Examples
wait & sting	cryptic	frass, tunnel entrance	larva in stalk	larva in stalk	Braconidae, Braconinae (*Digonogastra, Iphiaulax, Euvipio, Stenobracon*)
ingress & sting	cryptic	frass, tunnel entrance	larva in stalk	larva in stalk	Braconidae, Microgastrinae (*Cotesia, Apanteles*), Bethylidae (*Goniozus*)
planidial ingress	cryptic	frass, tunnel entrance	larva in stalk	mature larva & pupa	Tachinidae (*Lixophaga, Paratheresia, Metagonistylum, Descampsina, Sturmiopsis*)
bait & wait	cryptic	frass, tunnel	larva in stalk	mature larva & pupa	Tachinidae (*Palpozenillia*)
Pupal Guild					
drill & sting	cryptic	damage, pupal chamber	pupa	pupa	Ichneumonidae, Pimplinae (*Xanthopimpla*)
ingress & sting	cryptic	damage, frass, moth emergence window	pupa	pupa	Eulophidae (*Tetrastichus, Pediobius, Trichospilus*), Ichneumonidae, Ichneumoninae (*Dentichasmias*), Chalcididae (*Hyperchalcidia*)

or mature larvae that make the pupal chamber and exit window. In addition, the host stage exploited for food by the parasite progeny may differ from the stage attacked.

Insights and Applications

Having a general understanding of the host life history, the steps of successful parasitisation, and now the different methods of parasitisation associated with foraging strategies, we have enough information to pursue parasite rearing. More importantly, Table 1 provides a template for predicting the specific guild or method of parasitisation an unknown parasite species may employ. Identification of a parasite to genus usually provides the best rearing clues, provided the biology of the taxon is known. Often, identification to genus will be difficult for the entomologist working in the field, in the absence of a detailed reference collection or access to a museum. Placement of the natural enemy into the correct family and subfamily by the field entomologist provides a broad base of biological information. For example, identification of an unknown parasite to the ichneumonid subfamily Pimplinae, coupled with the information in Table 1, reveals that the parasite should be a true pupal parasite and, thus, rearing methods can be deduced from the foraging strategy relative to the host life history. As another example, recognition that an unknown parasite is a agathidine braconid reveals the parasite attacks early-instar larvae cryptically enclosed in leaf sheaths, and probably will not accept exposed larvae out of proper ecological context.

Other important rearing clues can be gleaned by observing and collecting biological information, from host collection in the field through parasite emergence in the laboratory, as well as adult parasite behaviour. Age of the host when collected can provide key information on the host stage attacked. Acceptable host stages are easily noted for ectoparasites, since they do not undergo further moults after paralysation. However, discerning the acceptable host stage for endoparasites can be more difficult. At the very least, knowing what age or stage the host was collected can bracket on one end the possible acceptable host stages accepted by the parasite. As an example, we find that a chalcid adult always emerges from the stalk borer pupa. Upon dissection, we find that the chalcid is a primary parasite and not a hyperparasite. However, after checking the collection information, we note that the chalcid has emerged from hosts collected both as pupae and as mature larvae, but never from hosts collected as eggs or early-instar larvae. Thus, we can narrow down the possibilities to a larval-pupal parasite or a true pupal

parasite. Coupling the acceptable host information with the possible attack methods, we can then devise an appropriate method for laboratory rearing of the parasite.

Laboratory Rearing

Successful parasitisation requires the sequence of distinct and consecutive processes that we have just discussed. First the parasite must find the habitat that harbours the host. Next the host must be found, the host must be acceptable for parasitism and suitable for parasite development. Laboratory rearing is most concerned with host finding, host acceptance and host suitability.

Successful rearing of stalk borer parasites requires that an acceptable, suitable host be available in a manner recognisable to the parasite. Presenting the proper host stage in the proper manner requires general knowledge of host life history, parasite biology and parasite foraging strategy. Developing a successful rearing strategy for a specific parasite is enhanced by being familiar with the general biology of stem borers, the general foraging strategies of parasites and parasite taxonomy. Observations concerning host age and condition during field collection and host age at parasite emergence in the laboratory are important for developing successful rearing techniques when specific rearing methods are unknown.

The larval parasite guild exhibits the most diverse foraging strategies and contains the most parasite species. These parasites have developed complex strategies to exploit the large, mature larvae as a food source, and thus are the most challenging to rear. However, diversity of this guild can be simplified for the purpose of rearing, by exploiting similarities in foraging strategies. Initially classifying parasites by a common attack method (e.g., drill-and-sting, probe-and-sting) provides mechanistic groups. Further subdividing the attack methods by life stage attacked simplifies the diverse array of strategies, as the parasites largely fit into natural taxonomic units, either as families or subfamilies. Now a common rearing procedure can be developed for each parasite genus, based on matching the method of attack with taxonomic affiliation.

Parasite rearing procedures are organised by attack method without foraging guild subdivisions. Table 1 provides the most complete reference for foraging strategies. The more salient features of rearing groups of parasites are highlighted, specific details can be obtained from the references. Although parasite

rearing is presented in a cursory manner because of space constraints, we hope this approach will help simplify rearing of this diverse group of parasites and serve as a guide toward developing the specific, detailed laboratory rearing methodology needed for rearing the parasite of interest.

Direct Attack

Several genera in the hymenopteran families Trichogrammatidae, Scelionidae and Eulophidae contain entomophagous species that exploit the eggs of stalk borers as a food source. The trichogrammatids and scelionids are primary, gregarious, endoparasites of stalk borer eggs. Taxonomy of *Trichogramma* and *Telenomus* is very difficult and has resulted in a confusion of specific names in these genera appearing in the literature.

In nature, most stalk borer eggs are deposited exposed on leaf surfaces, thus in the laboratory *Trichogramma* and *Telenomus* readily recognise and oviposit in host eggs deposited on almost any substrate when parasite and host are confined in close proximity. The trichogrammatids and scelionids are easy to culture. Many *Trichogramma* species are highly polyphagous in the laboratory and many lepidopteran eggs are acceptable and suitable factitious hosts for laboratory rearing. Adult *Telenomus* are 2 to 3 times as large as *Trichogramma* and appear to be more host-specific. However, this observed host-specificity may only be a manifestation of the restrictions that host size forces on host acceptance and suitability. Host eggs that produce 4 to 5 *Trichogramma* adults will produce only one *Telenomus*.

Female *Trichogramma* and *Telenomus* usually oviposit in all ages of stalk borer eggs. However, eggs in the early stages of embryonic development appear to have greater acceptance and suitability. Hosts in the latter stages of development, especially when the neonate larva is discernible through the egg chorion, usually produce fewer parasites than do younger eggs. *Trichogramma* readily respond to increased host size by increasing the number of eggs the parasite lays in each host. Superparasitisation is not a problem during culturing. The initial female marks the host after parasitism and marked eggs are subsequently avoided by searching parasite females.

Completion of larval parasite development and onset of pupation causes a darkening of the parasitised egg, as the parasitised eggs turn dark brown to black. This dark coloration is retained after the adult parasites emerge. The first parasite to mature to the adult stage chews a round hole in the chorion of the host egg and emerges. Subsequent adults in the egg use the same hole to exit the egg. Males emerge first, remain on the egg mass and mate with the females when they emerge. Emergence of both sexes of parasites from single eggs or from the egg mass, coupled with immediate mating upon emergence, insures adequate mating frequency for production of females and continuous laboratory culturing. *Trichogramma*, *Trichogrammatoidea* and *Telenomus* are reared similarly. Detailed methodology for rearing *Trichogramma* are found in the references and can be used to rear the other egg guild parasites.

The Cheloninae are solitary, internal, egg-larval parasites that have evolved a complex route to exploit the maturing stalk borer larvae as a food source. These solitary endoparasites oviposit in the stalk borer egg, the first-instar parasite larva discontinues development after hatching and "hitches a ride" in the growing stalk borer larva. When the stalk borer larva has begun to mature and is tunnelling in the stalk, the parasite's growth quiescence is broken and the parasite larva begins to develop and consume the host larva. The mature parasite larva spins a cocoon and pupates in the tunnel near the host cadaver. The emerging adult uses the host feeding tunnels for egress.

We have reared *Chelonus sonorensis* Cameron on *Eoreuma loftini* (Dyar), but have never documented the specific biology. Laboratory rearing of *Chelonus annulipes* Wesmael on the temperate stalk borer *Ostrinia nubilalis* (Hübner) is well-documented and can be followed for rearing the tropical chelonines.

Drill-and-Sting

The drill-and-sting attack method is manifested in the larval guild by the braconid subfamilies Braconinae and Doryctinae, and in the pupal guild by the ichneumonid subfamily Pimplinae. Searching larval-guild females initially locate host larvae tunnelling in stalks. The exact mechanisms of locating their cryptic hosts is unknown, but searching females recognise that hosts are feeding or traversing certain areas of infested stalks. When hosts are located, locomotion ceases and females drill into the stem with their strengthened ovipositor. Once ovipositor contact with the larva is made, the female injects a venom that induces a

permanent paralysis. When drill-and-sting strategists attack stalk borer larvae and miss direct contact with the host as they drill into the feeding tunnel, they wait passively for the stalk borer larvae to traverse the tunnel and contact the ovipositor. Multiple eggs are subsequently deposited either on the host or near the host in the tunnel. The parasite larvae consume the host and pupate communally near the host cadaver in the larval tunnel. Mating apparently occurs in the larval tunnel soon after adult parasite emergence. The behaviour and biology of drill-and-sting pupal parasites is similar except they are solitary endoparasites.

Host acceptance by drill-and-sting strategists requires adequate duplication of the natural physical microhabitat containing hosts. In the field, drill-and-sting strategists recognise hosts enclosed in plant stems, thus for host acceptance in the laboratory, hosts must be enclosed either in plant stems or placed in an artificial physical microhabitat that mimics the natural situation. The important cues appear to be associated with an acceptable host being physically separated from the ovipositing female by a barrier that can be penetrated by the drilling ovipositor. This barrier requirement can be met by placing hosts in the natural tunnelled grass stem, in a paper or plastic drinking straw, or under cotton muslin cloth or paper. Acceptable hosts not enclosed in a grass stem or reasonable facsimile are not recognised by drill-and-sting females. In fact, acceptable hosts not located in an appropriate microhabitat context (e.g., exposed rather than cryptic) do not elicit host recognition or searching responses from female parasites (e.g., no antennation, locomotion or ovipositor drilling). However, when these same hosts are presented in tunnelled grass stems, responsive females immediately begin locating and parasitising the hosts in the stems.

The main ingredient for successful laboratory rearing of drill-and-sting parasites is presenting acceptable hosts, cryptically, to ovipositing females. Detailed rearing procedures for the drill-and-sting strategists *Allorhogas pyralophagus* Marsh, *Rhaconotus roslinensis* Lal, *Myosoma chinensis* Szépligeti and *Xanthopimpla stemmator* Thundberg are given in the references.

Probe-and-Sting

Parasites employing the probe-and-sting method use their ovipositor for probing into crevices and through frass or thin plant epidermal layers to attack host larvae, as opposed to actively drilling through thick layers of stalk tissue with the ovipositor, as practiced by drill-and-sting parasites. Unlike drill-and-sting para-

sites, which will wait passively for stalk borer larvae to contact the inserted ovipositor, probe-and-sting strategists continue to probe actively with the ovipositor for larvae until contact is made.

The probe-and-sting attack strategy is represented by genera in the Braconidae and Ichneumonidae. The two braconid subfamilies, Macrocentrinae and Agathidinae, oviposit in the early larval instars feeding in the leaf sheaths, but utilise the mature stalk borer larva as their food source. The Agathidinae are large wasps with a long ovipositor that permits them to probe into the cracks and crevices around the leaf sheaths for early-instar hosts. The Macrocentrinae are small-bodied with an ovipositor slightly longer than the body. Unlike the agathidines, the macrocentrine parasite's small size does not prohibit access to the cryptic leaf sheath mines of the early-instar stalk borer larvae. The agathidines are solitary endoparasites. The macrocentrines are also internal parasites, but are polyembryonic, as each egg undergoes divisions to produce multiple progeny.

In the natural habitat, agathidines and macrocentrines attack early instar stalk borer larvae feeding in the leaf sheaths of gramineous plants. The elements of this natural setting that are essential for host recognition and successful parasitisation must be reproduced in the laboratory. The microhabitat necessary for host recognition by the parasite can be supplied by excising the green, leafy tops and at least one formed internode from corn, sorghum or sugarcane plants. Long leaves can be trimmed to extend 3 to 5 cm from the stalk and the stalk placed in a vial partially filled with water. A cotton plug is placed between the stalk and vial lip to prevent small larvae from crawling into the vial. First-, second- and third-instar larvae, those instars that naturally feed in the leaf sheaths, are placed on the leafy top and allowed to move to the leaf sheaths and begin feeding. The infested leafy top and water supply are secured in a normal upright position and exposed to ovipositing females. The females are initially attracted to the plant and are further attracted to the hosts by tissue damage from larval feeding and larval frass. When the female contacts larval feeding perforations or frass, she probes into the damaged area with her highly flexible ovipositor. Upon contact with a host, the ovipositor is inserted into the larva and oviposition proceeds within seconds.

Detailed biology and rearing procedures for the agathidine *Alabagrus stimaterus* (Cresson) attacking tropical stalk borers, and the macrocentrine *Macrocentrus gifuensis* Ashmead are documented in the references. Although *M. gifuensis*

attacks the temperate stalk borer *Ostrinia nubilalis*, the rearing procedure is easily adapted for tropical macrocentrines such as *M. prolificus* Wharton.

Mallochia pyralidis Wharton is a member of the large Ichneumonid subfamily Gelinae, which are primary ectoparasites of Lepidoptera larvae feeding in cryptic microhabitats. However, few Gelinae utilise stalk borers as hosts. *Mallochia pyralidis* Wharton is a solitary ectoparasite that uses the probe-and-sting attack method to attack the mature stalk borer larva and prepupa by probing the ovipositor through the moth emergence window. The cue for successful host finding by *M. pyralidis* in the laboratory is the moth emergence window. The searching female parasite locates the moth emergence window, probes through the window, paralyses the mature larva, then oviposits on or near the paralysed larva. Only mature host larvae associated with the moth emergence window are recognised as acceptable hosts by the ovipositing female. Mature larvae placed in grass stems or artificial paper stems will construct the pupal chamber and moth emergence window necessary for host acceptance. The biology and rearing of *M. pyralidis* is detailed in the references.

Wait-and-Sting

Parasite females employing the wait-and-sting host attack method are represented in the Old World genera *Iphiaulax*, *Euvipio* and *Stenobracon* and the New World genus *Digonogastra*. Wait-and-sting females contact and parasitise the host larva in the feeding tunnel with their ovipositor. The attack is very similar to probe-and-sting, except the wait-and-sting method is a more passive approach. This attack method differs from drill-and-sting in that the long, slender ovipositor is flexible and is used to probe for hosts in the feeding tunnel through natural openings, whereas the more robust ovipositor of the drill-and-sting parasite is used to drill actively through plant tissue. Host contact is made either by the searching females waiting at the tunnel entrance for larvae to transport frass as a normal activity of tunnel cleaning, or by probing through breaches in the stalk periphery created by the tunnelling larvae. Tunnelling behaviour such as clean tunnel maintenance or breaching the outer wall are usually specific to stalk borer genera or species. Regardless of where the parasite waits, it detects the presence of the larva at the tunnel entrance or near a breach, inserts the ovipositor and stings the larva when it traverses the opening.

Successful rearing of parasites using this foraging strategy requires acceptable, suitable hosts be presented to searching females in a cryptic manner with unobstructed avenues for ovipositor access. Ovipositor access can be provided by making 0.5 to 1.0 mm diameter holes in the substrate enclosing the cryptic larva. The ovipositing female must be able to reach the host larva by unsheathing the ovipositor and introducing it through the hole constructed in the substrate, thus the length of the ovipositor access tunnel must not place the host larva beyond ovipositor reach. Acceptable, suitable hosts are medium to large larvae that would normally be tunnelling in the stalk. Oviposition is a two-stage process, i.e., paralysation of the host larva followed by deposition of eggs on the host integument. Parasite larvae feed ectoparasitically and pupate adjacent to the host cadaver.

The paleotropical *Stenobracon deesae* (Cameron) and the neotropical *Dignogastra kimballi* (Kirkland) have well documented rearing procedures.

Ingress-and-Sting

Parasites using the ingress-and-sting host attack method are small in size, which allows them easy access to open host tunnels. Both the larval and pupal guilds are represented in this group. The gregarious, endoparasitic, microgastrine braconids belonging to the genera *Cotesia* and *Apanteles* and the gregarious, ectoparasitic, bethylid *Goniozus* attack the latter-instar larvae tunnelling in the stalk. Access to the host is gained by entering the tunnel entrance or a tunnel breach. Stalk borer larvae that maintain clean feeding tunnels are more prone to attack by ingress-and-sting parasites in the larval guild.

With a few exceptions ingress-and-sting larval parasites can be reared easily in the same manner as direct attack parasites. Adult females readily parasitise naked, exposed hosts. Even though the parasites in nature have to enter the host excavations to parasitise the host, there is no need to replicate this microhabitat in the laboratory. Further additions of frass to the ovipositional arena does not appear to enhance larval parasitisation. Numerous rearing methods have been used successfully by a variety of workers with *Cotesia* and *Apanteles* parasites of stalk borers. These genera are easily reared with the gregarious habit providing adequate mating. The chapter in this manual on rearing *Cotesia flavipes* coupled with the provided references adequately detail rearing procedures for *Cotesia* and *Apanteles*. *Goniozus* rearing is found in Conlong *et al.* 1984 and Ndoye 1980.

Ingress-and-sting parasites in the pupal guild gain access to hosts through the moth emergence window. The gregarious, endoparasitic, eulophids *Tetrastichus*, *Pediobius* and *Trichospilus*, are quite small and, upon gaining access, easily traverse the pupal excavations of most stalk borers. The integrity of the moth emergence window appears to be the physical restraint that limits access of these parasitic eulophids to the pupa in the natural microhabitat. *Hyperchalcidia soudanensis* Steffan has functional mandibles and cuts a hole in the moth exit window of its hosts. In contrast, an intact moth emergence window prevents *Pediobius furvus* Gahan from reaching the acceptable and suitable host *Eoreuma loftini*, whereas *P. furvus* successfully enters the tattered moth emergence window of *Diatraea grandiosella* Dyar and parasitises the enclosed pupae. Access to the pupal chamber by the ichneumonine *Dentichasmias busseola* Heinrich, is not restrained by the apparently intact construction of the moth emergence window of *Chilo partellus* Swinhoe.

The eulophids *Pediobius furvus*, *Tetrastichus inferens*, *T. israeli* Mani and Kurian, *T. ayyari* Rowher, and *Trichospilus diatraeae* Cherian and Margabandhu, are all easily reared by exposing host pupae to ovipositing female parasites in small containers. Generally, pupae in any stage of development are acceptable hosts, but pupae in the first half of their development are the most suitable hosts. These eulophids are much like the trichogrammatids and will utilise many factitious hosts in the laboratory. However, field collections do not reflect the polyphagy observed in the laboratory, and apparently is an artifact of laboratory conditions.

Dentichasmias busseola appears to have a much narrower range of host suitability than *Tetrastichus*, *Pediobius* and *Trichospilus*, and also requires reconstruction of the natural ovipositional microhabitat for successful host location. Natural hosts of *D. busseola* in Africa appear limited to *Chilo partellus*, *Chilo* sp. and *Haimbachia ignefusalis*, although *Busseola fusca*, *Sesamia calamistis* and *Eldana saccharina* are acceptable and suitable hosts under certain laboratory conditions. New World factitious hosts that are acceptable in the laboratory include *Diatraea saccharalis*, *D. grandiosella* and *Eoreuma loftini*. *D. busseola* females will parasitise these stalk borer pupae when hosts are provided in sugarcane or corn stalks. The proportion of the hosts parasitised is unknown; however immature development is marginal and the sex ratio is skewed to males. Laboratory colonies of *D. busseola* reared on these hosts progressively degenerated with each generation and could not be maintained for more than three generations.

Planidial Ingress

Numerous tachinid flies attack tropical stalk borers around the world, perhaps most notably the genera *Lixophaga, Metagonistylum, Descampsina, Paratheresia* and *Sturmiopsis*. The tachinid genus *Palpozenillia*, which uses a different attack method, will be discussed in a later section of the manual. Tachinid host-finding is a two-stage process that is unique among parasites of stalk borers. Female flies are initially attracted to host frass at the tunnel entrance, where the planidial first instar maggots are deposited. Planidia are negatively phototropic, which guides them to the proximity of the host larvae feeding in the enclosed tunnel. Upon host contact the maggots penetrate the host cuticle, usually through the intersegmental membrane, with their mouth hooks. Maggots spend some time living freely inside the host body, but then attach themselves to a tracheal branch or main trunk.

Rearing methods for several tachinids are well documented and can be used as general rearing models for the lesser documented species. In general obtaining adequate mating is the major problem in rearing. Once females have mated and become gravid, they are generally dissected and the planidial maggots placed on host larvae.

Bait-and-Wait

For this attack method, the adult fly is separated both spatially and temporally from the host. Unlike the Tachinidae that use the planidial-ingress method for host finding, the bait-and-wait strategists use a completely passive host-finding approach. Instead of the parasite finding the host, in this case, the host finds the parasite. This strategy is exemplified by the New World genus *Palpozenillia*. The female parasite apparently finds the host tunnel by detecting frass, then extends the ovipositor into the host tunnel, where the eggs are deposited. As the host larvae traverse the tunnel and feed, they ingest the parasite eggs, which then hatch. First-instar larvae feed initially within the gut, then enter the host fat body. During the third (ultimate) instar, feeding by the larval parasite increases as it consumes the remainder of the host. Mature parasite larvae emerge from the host and form puparia in the host feeding tunnel.

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Rearing of Predatory Mites Using Artificial Food Source: A Case Study of the Phytoseiid Mite *Neoseiulus teke* Prichard and Baker

Raphael Sumba Ochieng'

Department of Zoology,
Moi University,
P.O. Box 3900, Eldoret, Kenya

Abstract

Several diets for rearing **Neoseiulus teke** ***were developed and tested. Of these, the liquid diet ICD 286 based on commercial bee honey, milk powder, egg yolk, Wesson's salt and water proved to be the best.***

The artificial diet "ICD 387" described below proved to be more superior. This diet contained natural host inclusions. With this diet behavioural anomalies associated with rearing on artificial diets were eliminated.

Introduction

Predaceous mite of family Phytoseiidae are effective natural enemies of various phytophagous mites. Several species of *Typhlodromus neoseiulus* and *Phytoseiulus* are known to be associated with populations of the cassava green spider mites and other tetranychid mites.

Many artificial diets have been developed for insects but few have been tested on predaceous *Acarina*. Development of immature stages has been obtained on

artificial diet but the resulting female adults have failed to produce viable eggs. They have been smaller in size and have had a shorter life span than predators fed on natural mite prey.

Prior to the diet described hereunder, investigations on oviposition rates and development of immature stages, showed that they were low and development of immature stages was poor on artificial diet as compared to mites prey or pollen as food sources. These results suggested that artificial diets could be developed, particularly for polyphagous species. Predator cultures maintained on the artificial diets showed a gradual decline in viability, with adult females of some species failing to develop in subsequent generations.

The artificial diet described in this chapter was developed with the objective of sustaining phytoseiids in transit to release sites, and before they locate the target host mite, the cassava green spider mite *Mononychellus tanajoa* (Bondar) in biocontrol campaigns.

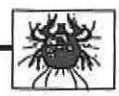
Rearing Methods

Steps	Key points
1. Starting an initial predator population	• Maintaining a large culture of the red spider mite, *Tetranychellus* sp. on beans *Phaseolus vulgaris* L. in the screen house. • Use the spider mite to feed the phytoseiid mite *Neoseiulus teke.* • Maintain a colony of the phytoseiid mite in petri dishes. • Change the leaves by placing the fresh leaves under the old ones in the petri dish so that the mites can move onto the fresh leaves.
2. Preparing the artificial diet	• Prepare the following ingredients. The quantity listed below is sufficient for a 100 ml diet.

Steps	Key points		
	(i)	Milk powder	10 g
	(ii)	Bee honey	10 g
	(iii)	Egg yolk	30 g
	(iv)	Wesson's salt	1 g
	(v)	Distilled water	50 g
	• Apparatus used in preparation of diet consists of:		
	(i) Plastic medicine cups		
	(ii) Measuring cylinder		
	(iii) Kenwood blender (small size)		
	(iv) Blender jar		
	(v) Container for keeping the diet (beaker).		
	• Wash the apparatus for preparation of diet in 40% sodium hypochlorite and dry in the oven at 60°C.		
	• Mix the solid ingredients of the liquid diet (i.e. milk powder, honey, Wesson's salts) in the blender jar.		
	• Add 50 ml of water.		
	• Blend the mixture to a homogeneous substance for 5 minutes using a Kenwood blender.		
	• Dispense the diet into the container.		
	• Liquid diet intended for future use can be kept in the refrigerator at -16°C for up to 30 days.		
3. Preparation for phytoseiid rearing	• Dispense the liquid substance on the bottom of a small plastic medicine cup, turned upside down using a 5 ml disposable syringe.		

Steps	Key points
	• Stretch a parafilm over it to provide a thin feeding membrane for the mites.
	• Place a paper comb on the parafilm to confine the mites on the substrate and to provide refuge for the mite.
	• Use a piece of plasticene to firmly fix the medicine cup onto the bottom of plastic lunch box (16 cm long, 10 cm wide, 6.5 cm deep).
	• Place cotton wool at the bottom of the lunch box to cover about 1.5 cm thickness.
	• Soak the cotton wool with water to provide a barrier against mite escape.
	• Using a fine camel hair brush (number one) introduce male and female phytoseiids on the paper comb on the parafilm. (Best results are obtained when culture is initiated from eggs).
	• Cover the top of the lunch box with black cotton cloth firmly fixed with a plastic lid with six holes, (each hole 1 cm in diameter) to provide ventilation.
	• Keep away on the shelves to incubate and develop.
4. Maintenance of the colony	• Allow 2 to 3 generations to develop on the same diet on the medicine cup, before changing.
	• Check diet daily for fungal development.
	• Add more water on the cotton wool in the lunch

Steps	Key points
	box so that it does not dry up to allow the phytoseiids to escape. • When contaminated with fungus or when mite population is too large, separate onto new diet containers using a camel hair brush. • No antibiotic necessary.
5. Monitoring the colony performance	• Determine the colony performance diet suitability by conducting comparative tests on the natural diet (i.e. spider mites). • Record the following biological criteria on both artificial and natural diets: (a) development period (days) (i) Egg (ii) Larva (iii) Protonymph (iv) Deutonymph — adult (v) Total life cycle (egg–adult) (b) Fecundity (egg/female) (c) Eclosion rate: (%) (d) Sex ratio (male: female) (e) Longevity rate (adult female, days) (f) Survival rate (g) (%) eggs per adult
6. Remedial measures to generation decline	• After some generations when the predator fails to recognise the host, incorporate 4 g of mite compound.
7. Preparation of mite compound	• Wash the host mite from the leaves. • Weigh the washed mites and blend with the diet above.



Steps	Key points		
	Thus composition of the new diet is as follows		
	(i)	Egg yolk	30 g
	(ii)	Milk powder (Lactogen)	50 g
	(iii)	Honey	10 g
	(iv)	Wesson's salts	1 g
	(v)	Distilled water	100 g
	(vi)	Mite compound	4 g

Conclusion

Composition of the novel liquid diet described above include the mite component. The quality of the diet as a medium for the development and production of the phytoseiid predator *N. teke* has been demonstrated by biological data (Ochieng' *et al.* 1987). Eighty-seven generations have been obtained from this diet. The rate of development of *N. teke* was shown to be relatively slower on the diet than on the natural prey. The fecundity, eclosion rate, sex ratio, longevity and stamina of this mite was comparable to that on the prey.

The paper comb placed on the parafilm platform provided a suitable dark and confinement for feeding on the arena, while plasticine held the cup in position sites. The best results were obtained when the culture was initiated at the egg stage. An important feature of the diet formulation is that it can last for up to 35 days, thereby supporting several generations of *N. teke* at room temperature even with a little fungal growth. However, no antibiotic was incorporated in the diet.

The development of this rearing technique is considered an important technological breakthrough which opened up for the first time the scope for mass rearing of phytoseiid mites as a group.

Like many other insects that change their behaviour when reared continuously on artificial diet, it was observed that the phytoseiid had difficulty in recognising the natural host after a few generations. This anomaly was, however, overcome by including ground parts of the natural host. The diet decribed above was called

"ICD 387". With ICD 387 there is no change in the behaviour of the phytoseiid predator. The predator still recognises the natural host even after several generations of continuous rearing on the diet.

The inclusion of ground parts of the natural host to overcome the behavioural change of phytoseiid *N. teke* in recognising the natural host is another important development in this work with the phytoseiid mite. Now, field application using mites reared artificially can be undertaken. Previously this was not possible as the laboratory reared predator would not recognise the prey in the field.

This work therefore opens up a whole new approach to field work using the phytoseiid mite.

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The Rearing of *Trichogramma*

F. Bigler

Swiss Federal Research Station for Agronomy,
8046 Zurich, Switzerland

Abstract

***Many species of the genus* Trichogramma *are mass reared and released for biological insect control on more than 15 million hectares worldwide per year. Most species used are produced on eggs of factitious hosts because rearing of their natural host(s) is often difficult and/or expensive. The production system depends on factors like the release period, the number and stage to be released, the release system and the availability and costs of labour and technical equipment. The production system described here refers to the unit used in Switzerland for mass rearing* T. evanescens *which is applied against the European corn borer,* Ostrinia nubilalis.**

Introduction

The genus *Trichogramma* comprises a relatively large number of species known today. Voegele and Pintureau (1982) assume, however, that a much higher number are still unknown or undetermined. All *Trichogramma* species are egg parasitoids. On a worldwide basis *Trichogramma* attack more than 400 insect species in 203 genera, 44 families and 7 orders (Bao and Chen 1989). By far the most important number of hosts belong to the Lepidoptera. *Trichogramma* are distributed over the world to all kinds of habitats, from aquatic to desert and from natural to agricultural ecosystems.

Worldwide interest in the mass production and utilisation of *Trichogramma* for control of numerous lepidopteran pests has continued for a long time. At present, various species are used annually in inundative releases on over 15 million hectares of agricultural and forest land (Olkowski and Zhang 1990). Large areas of crops like maize, sugarcane, rice, cotton, soybeans, sugarbeets, vegetables and

pine trees are treated in the former USSR, China, Mexico, Columbia, Brazil and the Philippines. Relatively limited areas are treated in European countries and North America. However, important research programmes for utilisation of these parasitoids are in progress.

Equipments and Methods for Mass Rearing

General Considerations

A variety of systems are used for mass rearing *Trichogramma*. The establishment of one or the other production system depends much on the *Trichogramma* species used, the availability of natural and factitious hosts, the length of the release period, the crop, and the resources available, i.e. qualified technicians, labour costs, technical equipment etc.

Most species released are produced on eggs of factitious hosts because rearing of natural hosts is in most cases difficult and/or expensive. Commonly used hosts are the Angoumois grain moth, *Sitotroga cerealella* Olivier, the mediterranean flour moth, *Ephestia kuehniella* Zeller and the rice moth, *Corcyra cephalonica* Staint. In China, the voluminous eggs of the two silk moth species *Bombyx mori* L. and *Antheraea pernyi* Guerin are used in many *Trichogramma* production units. Cropping patterns and length of the release period determine to a large extent whether a continuous or periodic production with long and/or short time storage is needed. In climates with short cropping seasons an economic production must rely on long term storage of either the parasitised and/or unparasitised host eggs. In tropical areas with continuous cropping patterns a constant production without storage may be adequate.

The release system itself determines the production to some extent. Whether the *Trichogramma* are released without carriers (widespread, loose release) or on carriers (cards or small containers) may influence the choice of the parasitisation and storage techniques.

The availability and costs of labour and sophisticated technical equipment are important features. Inexpensive labour may compensate partly for the lack of high technical standards of the equipment. Sophisticated, mechanised processes are being developed nowadays by private industry. Costs will be reduced substantially and make *Trichogramma* production more economic.

Rearing of *Trichogramma evanescens*

The following specifications of a mass rearing system relate to the procedures developed in Switzerland for the production of *Trichogramma evanescens* (= *T. maidis*) used against the European corn borer (ECB) in maize.

Figure 1 is a schematic presentation of the different production steps. Two organisations are involved. The Swiss Federal Research Station for Agronomy (FAP) has—besides the scientific support—the objective to maintain and develop

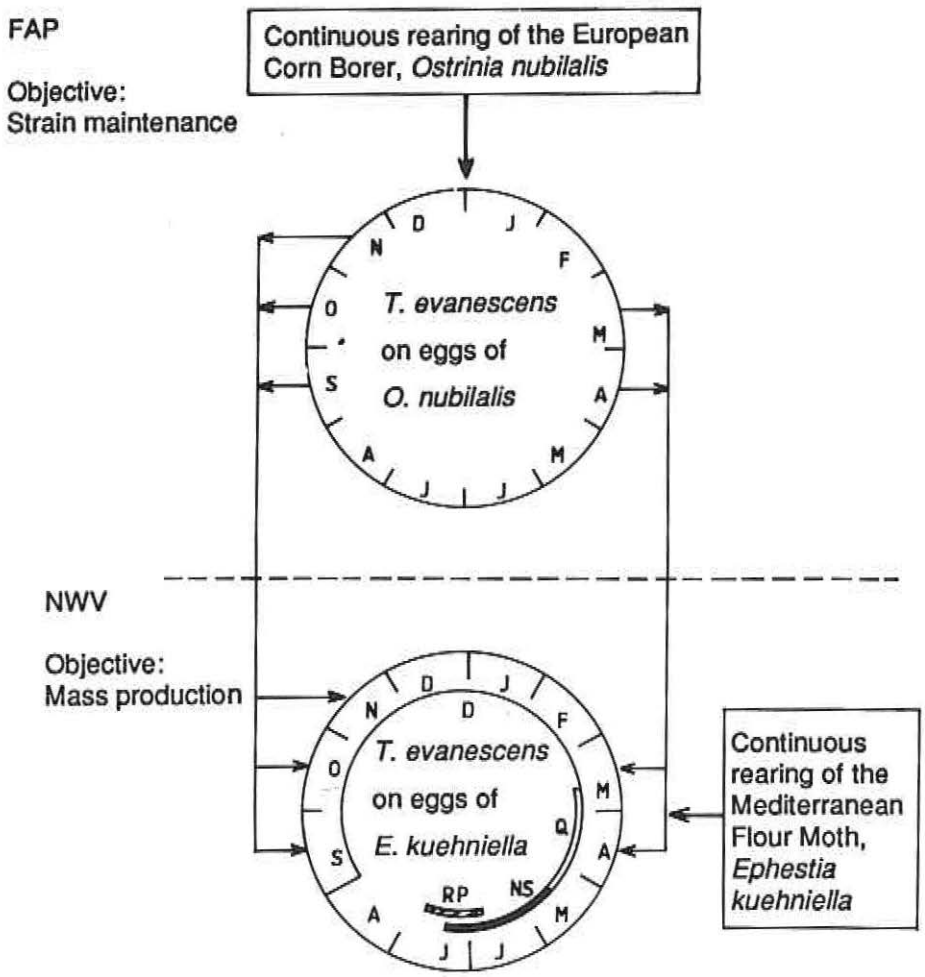

Fig. 1. Schematic presentation of the mass production system of *Trichogramma evanescens* in Switzerland (D: diapause production, Q: quiescence, NS: no storage, RP: release period)

the strain with the highest field performance. This *Trichogramma* population (OG) consisting of 0.1 – 0.5 million individuals is constantly reared under natural or semi-natural conditions on eggs of ECB (the rearing methods are discussed below in detail). Twice a year (March–April, September–November) a new strain, starting with OG, is developed on *Ephestia* eggs and shipped to the mass rearing plant (NWV). This production plant is run by a farmers cooperative society (a private organisation) which is producing *T. evanescens* for Switzerland. There, the OG strain is mass reared for 5 to 7 generations on eggs of *E. kuehniella* ($F_5 - F_7$) and then stored or shipped immediately to the farmers. Quality control is performed mainly by FAP and partly by NWV (see article on Quality control of *Trichogramma* in this volume).

Detailed Rearing Procedures

Strain Maintenance on Eggs of *Ostrinia nubilalis* (=OG)

Steps	Key points
1. Egg supply	• Daily collection of egg-masses of *Ostrinia nubilalis* oviposited on one side of a wet filter paper (45 x 45 cm), the number of egg-masses varies from 500 to 1,000 per paper, no chemical or physical treatments performed, when not used immediately the filter papers can be stored at 2–4°C, 80 ± 10% r.h. for 14 days without reduction of parasitism.
2. Stock colony	• Continuous rearing on egg-masses of *O. nubilalis*, if storage is needed it is done in the prepupal stage (eggs just turned black) at 12°C, 80 ± 10% r.h., light 16L:8D for 1 to 14 days. • Simultaneous emergence of the adults is advantageous for a better rearing management, thus, storage at 12°C is a suitable method for synchronisation of the development. • The development from egg to adult lasts 150 day degrees > 10°C, thus, 1 day at 25°C corresponds to 7.5 days at 12°C, or 1 day at 12°C is equal to 3.2 hours at 25°C.

Steps	Key points
3. Preparing egg-masses for parasitism	• Filter papers with egg-masses on one side are cut in strips of approximately 2 x 5 cm, each strip carries 5 to 20 egg-masses.
4. Rearing environment	• During the cold period, parasitism is performed in a greenhouse chamber 5 x 3 m at 25°C with 16 h light and at 16°C with 8 h dark, 70 ± 20% r.h. • During the summer season a fine meshed screen insectary in the open field (2.5 x 4 x 2 m) is used. • Twenty to forty maize plants of 0.5 to 1.5 m height are placed at one end of either the greenhouse chamber or the insectary.
5. Parasitism	• One to two filter paper strips are fastened to the lower side of each leaf of the plants. • The parasitoids, ready to emerge, are released on the opposite side, thus, they must fly to reach their hosts. • The parasitoids, ready for emergence, are held in transparent plastic containers (diameter 12 cm, height 20 cm) ventilated by 4 holes cut in the wall and covered with fine meshed screen. • After 140 to 150 day degrees development > 10°C the containers are placed to 25°C so that emergence starts after 1 to 3 hours, then the containers are moved to the greenhouse or insectary and opened to release the parasitoids. • The emergence period of one container lasts 2 to 3 days.

Steps	Key points
	• Egg-masses are withdrawn once or twice per day according to the number of adults present.
	• Observations of the number of adults per egg-mass are carried out 2 to 3 times a day.
	• If more than 2 females per egg-mass are counted, the number of egg-masses should be increased or the exposition period reduced, the optimal ratio of females to egg-masses is 1:1, the average number of eggs per egg-mass is 17, since *T. evanescens* lays two eggs per *Ostrinia* egg as an average, one female disposes of 34 "host units" which corresponds approximately to the average fecundity of our *T. evanescens* at the 1st day of its life.
6. Incubation of parasitised egg-masses	• When egg-masses are withdrawn, the females, still ovipositing, must be blown away, otherwise they will be transferred to the incubation cages.
	• The filter paper strips are transferred to trays (30 x 20 x 5 cm) which are placed above a tray (60 x 40 x 15 cm) filled with water.
	• After 4 to 5 days *Ostrinia* larvae hatch from unparasitised eggs, they are drowned when they move around before they start chewing egg-masses.
	• Incubation takes place under the same conditions as parasitism.
	• After all larvae are hatched and drowned, the paper strips are transferred to the plastic containers described above.

Mass production on eggs of *Ephestia kuehniella*

Steps	Key points
1. Egg irradiation	• *Ephestia* eggs are collected daily and irradiated by UV-light (Philips G30 T8 30W) for 60 minutes, one tube irradiates

Steps	Key points
	0.5 m², *Important:* for a 100% kill of the *Ephestia* embryos, the eggs must be exposed to direct irradiation, therefore, care for uniform spread and even, thin layers of eggs is important, optimal irradiation is performed if less than 40 ml of eggs per m² are spread (100 g = 160 ml, 1 ml = 22,000 eggs), hatched larvae will destroy eggs and cause silk-web when parasitised eggs are incubated.
2. Egg storage	• Storage of irradiated eggs is performed at 2–3°C, 80 ± 5% r.h. without light for max. 21 days in plastic trays (20 x 30 x 3 cm) with $^1/_3$ of the lid cut off and closed with a nylon gauze (mesh size 0.2 mm), to prevent the eggs to stick together, a good air circulation in the room is important and the egg-load per tray should not exceed 500 ml.
3. Rearing cages for *T.evanescens*	• The cage is constructed of 1.2 cm plywood and 0.5 cm Plexiglass® and consists of 10 independent (primary) shelves each measuring 20 x 33 x 0.3 cm (Fig. 2). The length-sides of the shelves tightly fit the walls to prevent adults to move

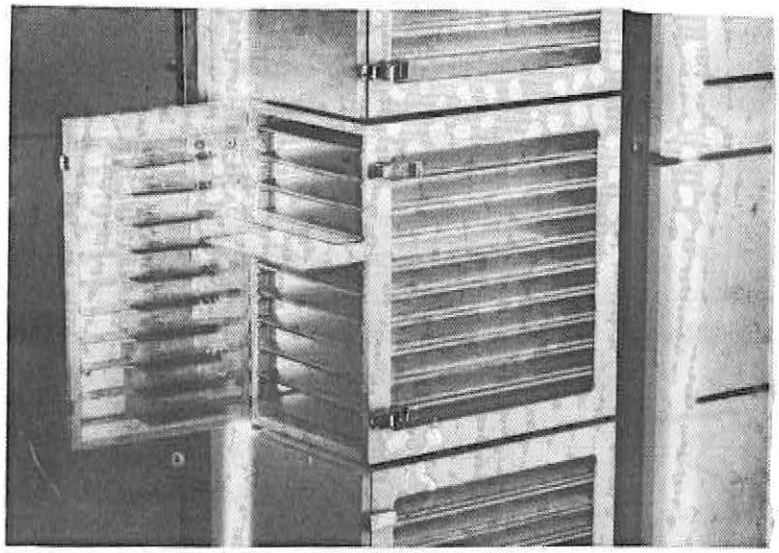

Fig. 2

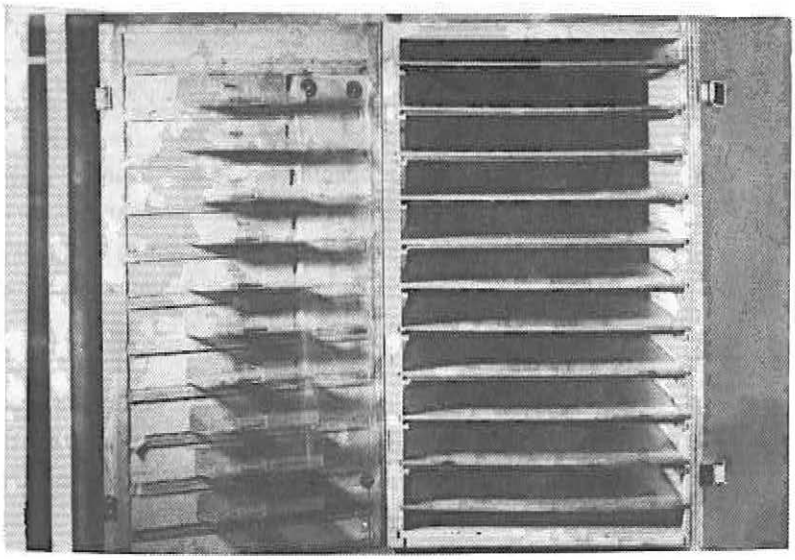

Fig. 3

Steps	Key points
	from one shelf to another. Both ends are closed with doors. Closed-cell, adhesive weatherstripping foam is added to the inside of the doors and tightly fits the shelves when the cage is closed. On the inside of the front door, between the foamstrips, are ledges (Fig. 3) which, by closing the door, fit between the shelves. The ledges are used to place small vials (2 x 2 x 1.5 cm) that contain parasitised eggs from which adults emerge (initial culture). The cage is divided in two halfs; one illuminated, the other dark. The walls of the illuminated part consist of clear, transparent Plexiglass®.
4. Rearing conditions	• Parasitisation is performed in a climatic chamber at 25 ± 1°C, 70 ± 5% r.h. and a light regime of 16L:8D.
	• Light is provided laterally by one tube (Philips TLD 58 W/33) on each side of the cages, the distance between wall and tube is 30 cm.

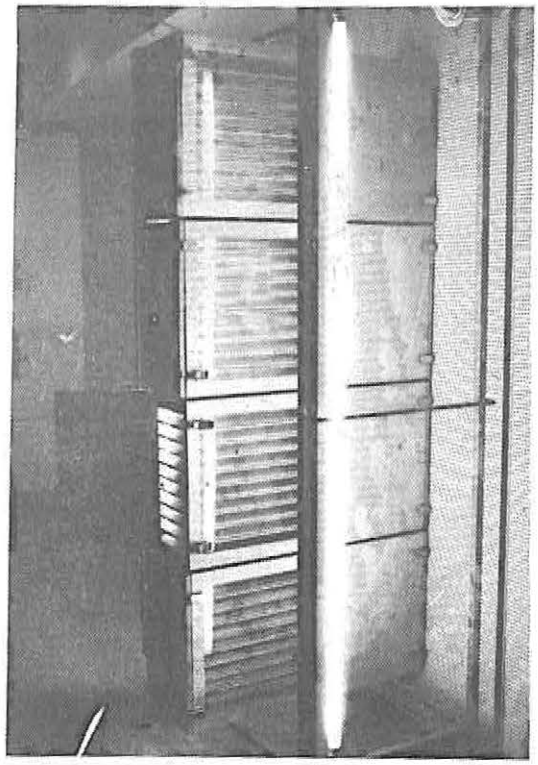

Fig. 4

Steps	Key points
	• 4 cages form a pile which is placed on a shelf on rolls that can be moved easily for handling (Fig. 4).
5. Daily parasitisation procedure	• The initial culture (parasitised *Ephestia* eggs containing adult *Trichogramma*) is distributed in small plastic vials (2 x 2 x 1.5 cm).
	• The amount of eggs in each vial depends on the parasitisation rate and the sex ratio of the initial culture, which have been determined before.
	• One vial is placed on each ledge of the front door.

Fig. 5

Steps	Key points
	• Secondary shelves with parasitised eggs from the previous day are removed from the dark part and brushed in a container.
	• 20 (secondary) shelves (20 x 33 x 0.2 cm) are distributed on a large table (3 x 1.5 x 0.8 m) and pushed tightly together to form an even surface (Fig. 5), 10 shelves (A) were stored previously at normal room temperature and 10 shelves (B) at 2–4°C for one day.
	• 60 ml *Ephestia*-eggs are filled in a cup, covered by a nylon gauze (0.5 mm mesh size) and evenly shaken over the shelves (3 ml per shelf).

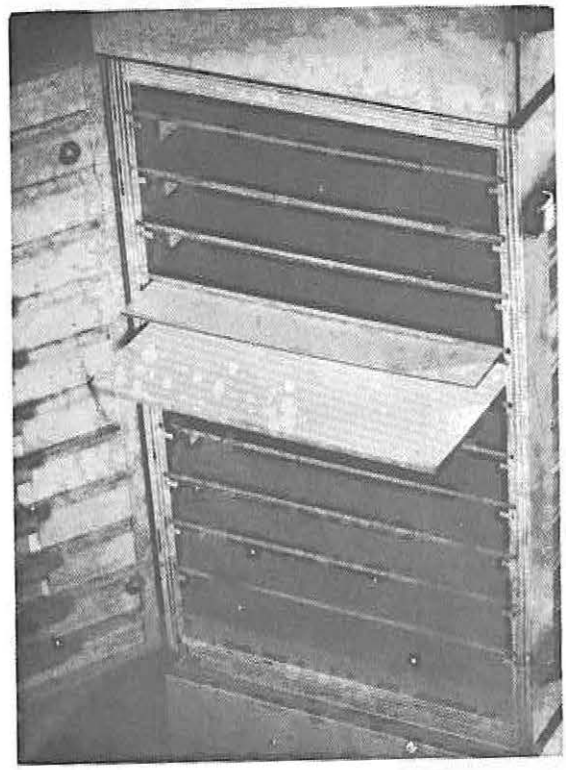

Fig. 6a

Steps	Key points
	• As soon as the shelves B are brought from the cool room to 25°C, 75% r.h., a thin and even layer of water is formed by condensation, this is just enough to stick the eggs slightly to the shelves, on shelves A, eggs are not adhered.
	• Shelves A are then placed on the bottom side of each compartment in one parasitisation cage, shelves B are turned up-side-down and placed on the ceiling held by ledges on the walls (Fig. 6a, 6b).
	• The shelves from the previous day are pushed from the illuminated to the dark part of the cage.

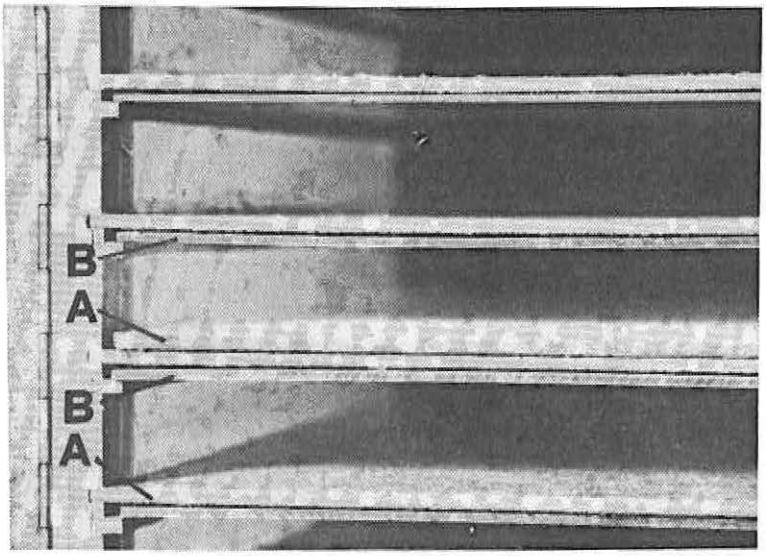

Fig. 6b

Steps	Key points
	• Still active adults are attracted within 30 to 60 minutes back to the lighted part.
	• If a high density of adults is observed (by experience!), the amount of the initial culture added is reduced.
	• The optimal ratio of females to *Ephestia*-eggs is 1:10.
	• A 30% honey-water solution is provided once a week on a plastic sheet (30 x 10 x 0.01 cm) pinned on the inside of the front door (Fig. 3).
	• Moisture is provided every day by treating the plastic sheet and the front space of the cage with a hand-sprayer (very small droplets are important).
6. Incubation and storage of parasitised eggs	• The collected eggs are incubated in different conditions depending on their use.

Fig. 7

Steps	Key points
	• Development without storage occurs either at constant 25°C, 70 ± 5% r.h., 16L:8D (total developmental time 9.0 days) or at fluctuating conditions:16h at 25°C, 70 ± 5% r.h., 8 h at 16°C, 80 ± 5% r.h. (total developmental time 10.5. days).
	• Storage in diapause lasts from 3 to 9 months at 3°C, 80 ± 5% r.h., no light is needed.
	• Storage in quiescence lasts from 1 to 30 days at 3°C, 80 ± 5% r.h., in a light regime of 8L:16D.
	• In order to prevent the eggs from sticking together and moulding, they are stored in special trays which consist of a wooden frame (60 x 40 x 3 cm) and a fine nylon gauze bottom (mesh size 0.15 mm), the egg-load should not exceed a layer of 1.5 cm.

Steps	Key points
7. Preparation for shipment	• When the time for field release has come (monitoring of *O. nubilalis* by the Swiss Federal Research Stations) parasitised eggs are removed from storage sites and further incubated at 25°C.
	• Batches of two different developmental stages (late pupae and early prepupae) are thoroughly mixed before they are glued on cardboard-strips (Fig. 7).
	• Cardboards which serve as carriers of the parasitised eggs are arranged in strips and placed on a large table.
	• The upper surface is moistened with a water-unsoluble, unpoisonous glue applied with a soft paintbrush.
	• Parasitised eggs are evenly distributed by shaking them from a beaker through a nylon gauze (mesh size 0.5 mm).
	• The cardboard strips are then filled in hard cardboard boxes for shipment.
	• For transporting the boxes to the farmer, fast mail during night is the normal procedure.

Conclusions

Trichogramma evanescens is being applied presently on 20,000 hectares in western Europe against the European corn borer in maize. In Switzerland 4,500 hectares are treated annually. An increasing demand for *T. evanescens* is observed in France, Germany and Switzerland. Thus, improvements of the mass rearing techniques will be of primary order in the future. Labour is very expensive in industrialised countries and must be minimised in the production of this parasitoid. At the same time, costs for insecticides are relatively low and many products are competitive with *Trichogramma*. Cost reduction in the mass production of the parasitoid is extremly important and will determine the future of *T. evanescens* to

a large extent. Mechanisation and automation of the mass rearing systems are two major issues to cope with the economic problems. Storage over long periods of either the unparasitised or parasitised host eggs is another possibility to make mass production more economic. Finally, the highest costs in the mass rearing of *Trichogramma* are caused by the production of the host eggs regardless of the host used. Artificial host eggs, filled with media adequate to the requirements of each species, will create new potentials in the cost reduction of mass rearing systems and in the competitiveness of *Trichogramma*.

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The Rearing of *Cotesia flavipes* for Classical Biological Control of *Chilo partellus*

William A. Overholt

International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772, Nairobi, Kenya

Abstract

Cotesia flavipes ***Cameron (Hymenoptera: Braconidae) is a gregarious endoparasitoid of gramineous stemborers that has been imported by ICIPE for a classical biological control programme against the exotic stemborer,*** **Chilo partellus** ***(Swinhoe) (Lepidoptera: Pyralidae). The life cycle of*** **C. flavipes** ***lasts approximately 22 days, and ca. 40 parasitoids develop in each host larva. The facilities and equipment necessary to rear*** **C. flavipes** ***are very simple and relatively inexpensive. A "hand-stinging" rearing procedure is described in detail, along with specifications on rearing facilities and equipment. Quality control through accurate record keeping and bioassays is discussed.***

Introduction

Chilo partellus, a pyralid stemborer of gramineous plants, is a major pest of maize and sorghum in East Africa. *C. partellus* is not native to Africa, but was accidentally introduced from Asia earlier this century. It was first reported in Malawi in 1932 (Tams 1932), and has now spread to most other countries in eastern and southern Africa. There is little doubt that the distribution of this pest will continue to expand to eventually include most of sub-Saharan Africa.

Although many indigenous natural enemies in Africa are able to attack and develop on *C. partellus*, they do not appear capable of regulating densities at levels acceptable to man. Therefore, a classical biological control programme against *C.*

partellus has been established at ICIPE in collaboration with Wageningen Agricultural University. This programme is currently in the process of identifying and introducing candidate exotic natural enemies of *C. partellus*.

The first natural enemy that has been imported is *Cotesia* (=*Apanteles*) *flavipes*, a gregarious braconid endoparasitoid of *C. partellus* which attacks medium and large instar larvae. The foraging strategy of *C. flavipes* involves entering holes in the plant stem and searching for larvae in the tunnels created by host feeding. *C. flavipes* occurs in many areas of Asia on several species of stemborers of maize, sorghum, rice, and sugarcane, and has been extensively used in classical biological control programmes against *C. partellus* and other related stemborer species. There is evidence of behavioural variation in the populations of this parasitoid originating from different regions and from different crop/stemborer complexes (Mohyuddin and Inayatullah 1981). The "strain" imported by ICIPE is from Pakistan where it attacks *C. partellus* in maize.

The first field releases of *C. flavipes* are scheduled to be made during the long rains of 1993 (March–June). In the interim, intensive laboratory studies on the taxonomy and biology of this parasitoid are being conducted. However, before studies could begin, ICIPE had to establish a viable laboratory colony to provide a constant supply of *C. flavipes*. Because of the extensive use of *C. flavipes* in stemborer biological control programmes, there is a considerable amount of literature available on rearing procedures (Gifford and Mann 1967; Kajida and Drake 1969; Wiedenmann *et al.* 1992). From these references, and our own experience, the laboratory rearing procedure presented in this paper has been developed.

A Brief Biology of *C. flavipes*

In order to fully understand the steps followed in the rearing of *C. flavipes*, it is essential to have a basic knowledge of its biology. As mentioned previously, *C. flavipes* is a gregarious endoparasitoid (Fig. 1). This means that females deposit multiple eggs in the host's body cavity. Our laboratory records indicate that about 40 eggs are laid in each host. First instar parasitoid larvae eclose after about 3 days and begin feeding internally. *C. flavipes* develops through three larval instars (Fig. 2) in the host body, and then emerges from the host by chewing through the integument. The egg/larval period lasts about 14 days at 25°C (Table 1). After emergence from the host, the last instar larvae spin cocoons and pupate. In nature,

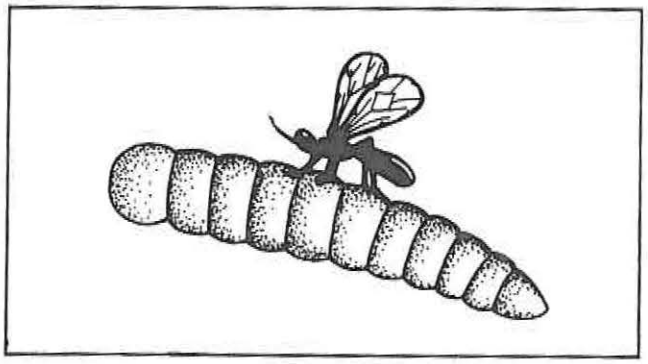

Fig. 1. Adult *Cotesia flavipes* preparing to oviposit in a stemborer host

the cocoons are found inside host feeding tunnels in gramineous plants. Pupation takes about 6 days at 25°C, after which adults emerge. The adults are small wasps ca. 3–4 mm in length. It is simple to differentiate the males from the females by the length of the antennae. The antennae of males are approximately twice the length of the female antennae.

Table 1. Developmental times for immature stages and adult longevity (fed) of *C. flavipes* at 3 temperatures

Temperature	Stage	Developmental time (days)
22°C	egg/larva	17.9 ± 1.7
	cocoon	9.1 ± 0.9
	adult	2.27 ± 0.8
25°C	egg/larva	14.0 ± 1.2
	cocoon	6.7 ± 0.9
	adult	2.13 ± 1.1
28°C	egg/larva	12.1 ± 2.9
	cocoon	5.9 ± 1.2
	adult	1.25 ± 0.6

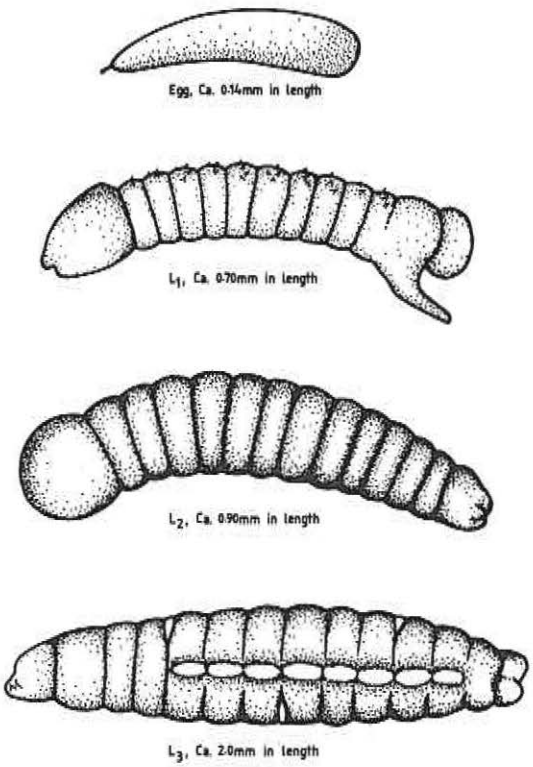

Fig. 2. Egg and first, second, and third instar larvae of *Cotesia flavipes*

The adult lifespan of *C. flavipes* is quite short, approximately 34 hours at 25°C if adults are not fed. Provision of a 20% honey/water solution prolongs the lifespan to about 51 hours. Because of the short lifespan, *C. flavipes* must quickly mate after emergence and begin searching for hosts. As with many species of Hymenoptera, a haplo-diploid system of sex determination operates. Fertilised eggs (diploid number of chromosomes) become females, and unfertilised eggs (haploid number of chromosomes) become males. Therefore unmated females, although completely capable of oviposition, are able to produce only male offspring. Mated females produce both male and female offspring. In laboratory rearing it is crucial to make sure that females are properly mated before exposing hosts. Strong lighting, incandescent or sunlight, appears to stimulate mating and oviposition in the laboratory.

The mechanisms of host finding by *C. flavipes* are not completely understood, but from previous works (e.g. Mohyuddin and Inayatullah 1981; van Leerdam *et al.* 1986), and the investigations now underway at ICIPE, it is clear that host frass is an important que in host finding. In our work we have shown that hosts from natural diet (maize or sorghum) are much more attractive to parasitoids than hosts from artificial diet, and this knowledge has been incorporated into our rearing procedure.

Rearing Facilities

Ultra modern rearing facilities are not necessary to maintain a viable colony of *C. flavipes*. All that is needed is a simple room. The size will vary depending on the size of the colony that is maintained. At ICIPE we are able to rear about 10,000 insects/week in an 8 m^2 room. If at all possible, the room should be dedicated to *C. flavipes* rearing, and not used for other activities. This will minimise the traffic moving in and out of the room, thereby decreasing the chances for contamination by microorganisms. If the room has windows, they should be kept closed at all times.

Climate controls are not necessary in most regions of the tropics since prevailing conditions are usually well within the range acceptable to *C. flavipes*. However, if available, regulating the room to a constant temperature is extremely useful because it allows for very accurate prediction of developmental times of the various stages. If the temperature frequently drops below 15°C, or surpasses 35°C, then some type of heating or cooling may be advised.

Rearing Procedure for *C. flavipes*

	Activity		
Day	Parasitoids		Hosts
1	(i)	When parasitoids are ready to emerge from cocoons (cocoons dark), place in large sleeve cage (35 cm^3)	Use medium to large sized host larvae (4th–6th instars). If *Chilo partellus* is reared on natural diet they can be used directly for exposures. However, if hosts are reared on artificial diet, they should be removed from artificial diet and all allowed to feed on natural diet (cut pieces of maize or sorghum stems) for 24 hours prior to exposures.
	(ii)	Provide 20% honey/distilled water solution on cotton wool in a petri dish in the bottom of the cage.	
	(iii)	When parasitoids begin to emerge, place cage under incandescent light for ca. 2 hours to stimulate mating.	
	(iv)	Mist cage with distilled water from a small hand-sprayer.	
2	(v)	Place cage under incandescent light and begin host exposures.	Remove hosts from natural diet and place in a petri dish or some other suitable container.
	(vi)	To expose hosts, offer individually to parasitoids by holding in soft forceps inside the cage (Fig. 3). Oviposition can be detected by closely watching the encounter between parasitoid and host. The parasitoid grasps the borer with her legs, curls the abdomen downward and forward, and inserts the ovipositor. At ovipositor insertion, the larvae reacts violently and then becomes quiescent. Oviposition generally occurs rapidly within ca. 5 seconds.	

Day	Activity		
	Parasitoids		Hosts
	(vii)	After oviposition, immediately remove the larvae from the cage. Avoid having a larvae stung by more than one parasitoid (superparasitism). If too many eggs are deposited in one host, the progeny will not receive enough nourishment for proper development.	After removing the larva from the cage, place in a small vial (ca. 7.5 cm x 2.5 cm) about one-third full of artificial diet and hold for parasitoid emergence. If artificial diet is not available, use cut maize or sorghum stems. If natural diet is used, it should be changed every two days to minimise the growth of fungi.
	(viii)	Continue exposing hosts until a sufficient number of parasitised or parasitoids are no longer interested in oviposition.	
	(ix)	Remove cage from incandescent light.	
	(x)	Mist the cage with distilled water. Repeat steps 5--10 each day until parasitoids are dead or no longer show interest in oviposition.	
3 10–18 days after exposure	(i)	Parasitoids exit the host larva and immediately begin spinning cocoons.	
	(ii)	Once cocoons are completely formed, remove the cocoon mass from the artificial diet and place in a clean vial.	Discard the host larva.
15–20 days after exposure	(iii)	Hold cocoons until they darken, and repeat all the operations for the next generation.	

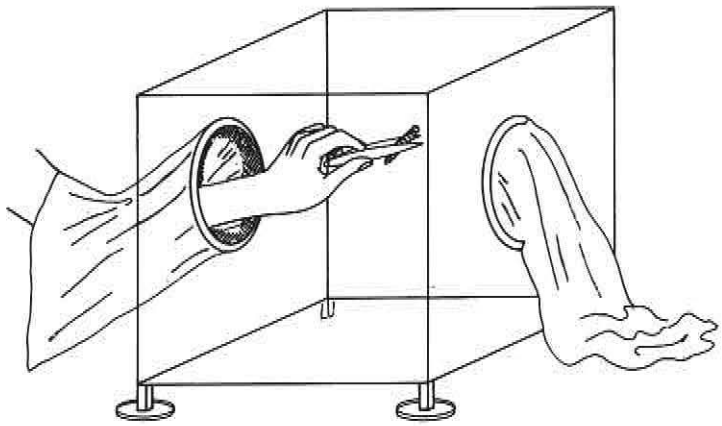

Fig. 3. Exposure of a stemborer to *Cotesia flavipes* for ovipostion using the"hand-stinging" method

The most important factor in the rearing facility is cleanliness. The room should be thoroughly cleaned with detergent and a weak bleach solution once a week. Walls, floors, shelves, and furniture must all be cleaned. Staff entering the room should wear laboratory coats at all times. Artificial diet is "cleaner" than natural diet since it usually contains one or more antimicrobial agents. If natural diet is used for rearing the stemborer hosts, diet needs to be changed regularly to avoid the buildup of harmful microorganisms. If predaceous ants are a problem in the rearing facility, and they often are in the tropics, all host larvae and parasitoids must be isolated from ants as they can rapidly destroy a culture. All cages, and vials or other containers housing hosts, parasitoids, or parasitised larvae should be elevated from floors, shelves, tables, etc. in water traps. Traps can be easily constructed from petri dishes or other suitable containers.

Materials and Equipment

No high technology equipment or materials are necessary for rearing *C. flavipes*. The basic elements include sleeve cages, glass vials or some other suitable small container, cotton wool, spray bottle, soft forceps, a desk lamp, and climate monitoring equipment.

Sleeve cages: Sleeve cages are used for mating and host exposures. Ideally, cages should be made from a clear plastic such as perspex (plexiglass). This will allow visual inspection of the insects, and it is easy to clean. Any size between 20 cm^3 and 50 cm^3 is appropriate. A solvent such as chloroform can be used to join the pieces of perspex. Cages should have two sleeves on opposite sides of the cage (Fig. 4). Sleeves should be approximately 16 cm in diameter and 60 cm long. They must be tightly glued to the cage with no gaps. Epoxy glue works well, but must be allowed to dry for several days before use until there is no perceptible odour. Wooden or metal cages with screen sides can also be used, but they are difficult to clean and hard to see through. Moreover, the screen must be a very fine mesh or the insects will escape.

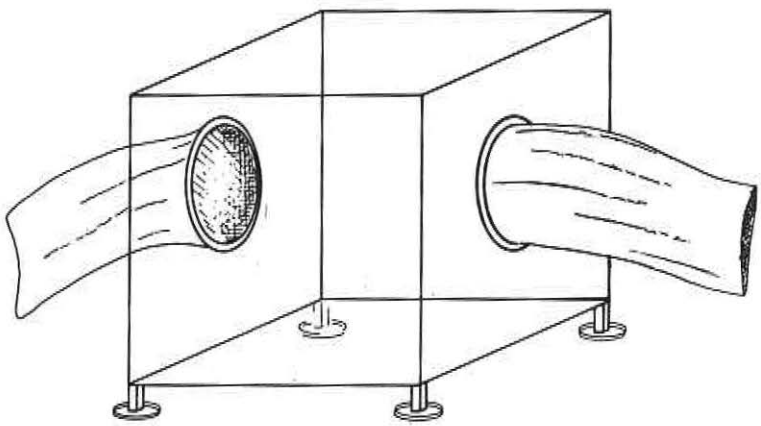

Fig. 4. Sleeve cage for maintaining adult *Cotesia flavipes* for oviposition

Vials: Vials are used to hold host larvae after they are parasitised, and to hold cocoons before they emerge. Again, size is not terribly important. The vials used for the colony at ICIPE are 7.5 cm long and 2.5 cm in diameter. The vial must hold sufficient diet so that the host larva can feed for 4–5 days. Vial racks are useful for organising the rearing room, e.g., all larvae exposed on the same day are held together in one rack. Racks can be procured through suppliers of scientific equipment, but it is simple to make them locally out of wood or metal. Although

at ICIPE larvae are held individually in vials, larger containers, such as jars, can be used to hold several larvae. The most important factor is to keep the containers clean. They must be thoroughly washed using detergent and bleach after each usage. Oven drying is ideal if available.

Spray bottle: The spray bottle creates a very fine mist of water which is used to maintain a high humidity and provide free water in the sleeve cages (Fig. 5a). Spray bottles in one-half or one litre sizes are available at plant shops and hardware stores. Never use a spray bottle that once contained a pesticide as it is nearly impossible to remove all the pesticide residue.

Cotton wool: Cotton wool is used as a medium for providing honey/water solution to adult parasitoids, and can also be used for stopping vials. Cotton wool soaked in honey/water solution should be held in a suitable container, e.g. a petri dish, on the floor of the sleeve cage.

Desk lamp: The desk lamp is used to stimulate mating and oviposition (Fig. 5b). Any small desk lamp with a 20–40 watt bulb will work. When using the desk lamp, keep the light source ca. 15 cm from the sleeve cage to avoid buildup of excessive heat.

Soft forceps: Soft forceps are used for holding the stemborer larvae during exposure (Fig. 5c). Soft forceps are very flexible so that the larvae are not injured during the process. Soft forceps are relatively inexpensive and available from any supplier of scientific equipment.

Climate monitoring equipment: This can be very simple and include only a room thermometer (Fig. 6), or be more elaborate and include tools for measurement of humidity, and barometric pressure. A thermo-hygrograph, which automatically records temperature and humidity is ideal, although they are moderately expensive. The records from climate monitoring equipment can be extremely useful in predicting duration of development and diagnosing problems in the insect colony.

Quality Control

Record keeping: Accurate record keeping is the only way to properly follow the performance of an insect colony. Staff must be required to keep daily records of room conditions and colony output. In a small colony, the number of hosts

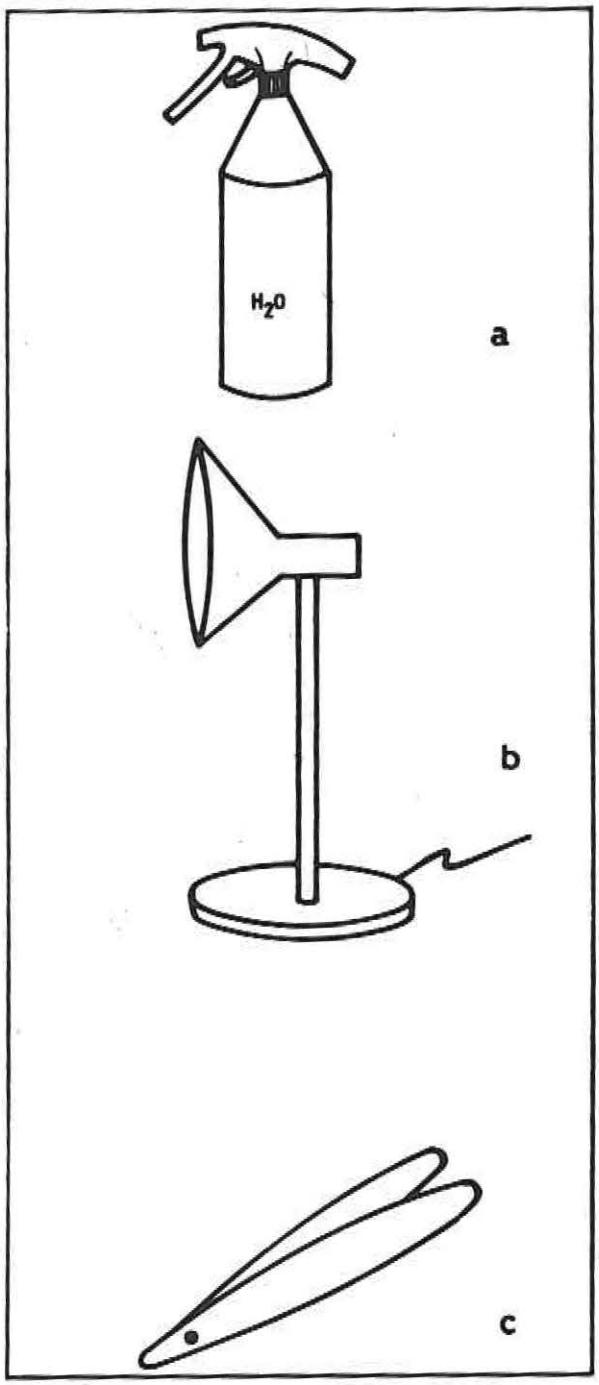

Fig. 5. Some equipment used for rearing *Cotesia flavipes*; (a) spray bottle, (b) desk lamp, and (c) soft forceps

exposed and the number producing parasitoid progeny, the number of progeny from each parasitised host, the sex ratio, and any particular comments on the progeny of each parasitised host should be recorded. Comments may include notations on the relative size of individual parasitoid progeny. In a larger colony, it may be impractical to count and sex the progeny from each parasitised host, but periodically (each week for example) a sub-sample of 10–20 cocoon masses should

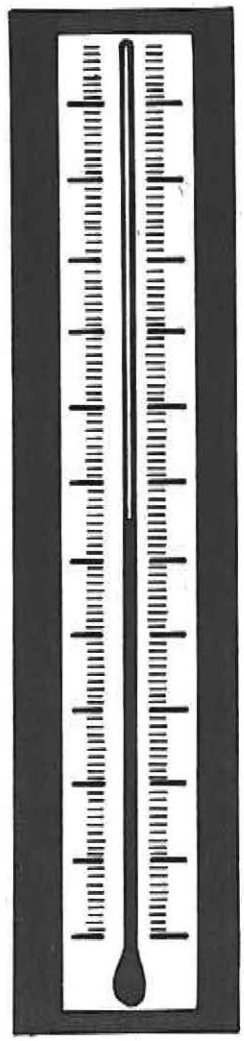

Fig. 6. Maximum/minimum thermometer for monitoring climatic conditions in the *Cotesia flavipes* rearing room

be randomly selected and examined. Accurate record keeping will allow the colony manager to detect problems in the colony before they become severe, and also to optimise production. For example, if records indicate that there is an increase of cases of all male progeny from hosts, there is likely a problem with mating, and more attention should be given to that step in the rearing procedure.

Bioassays to estimate field performance: As with all laboratory rearing of insects, there is a strong possibility of selecting a "laboratory ecotype" which is well adapted to the prevailing conditions in the laboratory, but less fit for the field. This becomes more likely the longer an insect remains in culture. If the colony is being used only to study the biology of the insect, this may not be so important, but if the objective of the colony is to rear insects for field releases, it is of paramount importance.

One obvious deficiency in the laboratory rearing procedure for *C. flavipes* which is described above, is that the parasitoids are not required to find the hosts. This is very different from the situation in the field where the parasitoid must first locate the host's habitat (maize, sorghum, wild host plant), and then locate the host within the habitat. Because of the danger of losing essential genetic traits involved in host finding, it is advisable to devise a bioassay which is conducted periodically to estimate host finding ability. One simple way would be to infest sorghum or maize stems with *Chilo partellus*, allow them to feed for 2–3 days, place the infested stems in a large sleeve cage, and release the parasitoids. After parasitoids have died, remove the stems and dissect out the stemborers. The stemborers can be held for parasitoid emergence to determine percent parasitism. Parasitoids emerging from the hosts could be infused back into the colony to maintain the host finding traits. An even better method to estimate host finding ability could be conducted in live plants in the field under large screen cages.

Conclusions

The rearing of *C. flavipes* for biological control of stemborer is not a difficult or expensive undertaking. A room, simple rearing equipment, a constant supply of hosts, and a committed staff is all that is necessary. The most important factor in a successful rearing programme is sanitation. Rooms must be kept very clean, and precautions must be taken against predaceous ants, to avoid disasters. If natural diet is used for host rearing, diet must be changed periodically.

The maintenance of colony statistics through accurate record keeping is essential for any insect colony. It is the only quantitative way of determining the colony's performance. The colony manager can review the data regularly to determine if there are any generation to generation trends that can be detected. If these trends are negative (e.g. unfavourable sex ratio), he can take steps to correct the problem before it becomes too serious.

Finally, the staff rearing *C. flavipes* must always keep the ultimate objective of the laboratory colony in mind. Maintaining the colony for the sake of having a colony serves no purpose. If the goal is field release and establishment for classical biological control, the insects must be adaptable to field conditions. The best way to insure that the colony does not deteriorate genetically, is to minimise the number of generations reared in the laboratory. If *C. flavipes* is to be reared through several generations, bioassays should be developed to estimate fitness under field conditions.

Another factor which must be considered is mating. As mentioned previously, unmated females will only produce male offspring. A colony with reduced mating will rapidly decline to extinction. It is essential to keep accurate colony records so that problems with mating can be detected early and corrected.

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F. Rearing of Phytophagous Insects

Rearing the Pink Stalk Borer, *Sesamia calamistis* and the Sugarcane Stalk Borer, *Eldana saccharina*

Zbigniew T. Dabrowski

International Centre of Insect Physiology and Ecology,
P.O. Box 30772, Nairobi, Kenya

Stemborers are considered a major pest of graminaceous crops worldwide. In the temperate regions, relatively few species of important stemborers are known. In the tropics, these pest species are more numerous and reduce considerably the yield of maize, and other crops, often the very subsistence crops most widely grown in the developing countries. It is estimated that the stemborers are responsible for 15–78% (but often 100%) of yield losses on cereals in Africa.

Stemborers attacking maize and sorghum in the tropics caused typical "dead hearts" in young plants, in older plants the upper part of the stem usually dies due to the boring of the larvae in the stem pith. The cobs are also attacked.

There are six major stemborer species attacking maize and sorghum in Africa: *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae); *Sesamia calamistis* Hamps (Lepidoptera: Noctuidae); *Sesamia botanephaga* Tams and Bowden (Lepidoptera: Noctuidae); *Chilo partellus* (Swinhoe); *Chilo orichalcociliellus* (Strand) (Lepidoptera: Pyralidae) and *Eldana saccharina* Wlk. (Lepidoptera: Pyralidae).

Sesamia calamistis, Eldana saccharina and *Busseola fusca* occur in all countries South of the Sahara. *S. botanephaga* and *C. ignefusalis* are mainly West African species.

In Eastern and Southern Africa, the predominant maize borers are: *Busseola fusca, Sesamia calamistis, Eldana saccharina* and both *Chilo* species. In this region, *B. fusca* occurs in the cooler high lying inland areas while *S. calamistis* occurs in the warmer coastal regions.

In West Africa, *S. calamistis* occurs in the humid lowland areas and *B. fusca* in the Tree Savanna area. In Nigeria and Ghana *S. botanephaga* occurs in the lowland rain forests (Harris 1962).

It is, however, common for different species of borers to occur together in the same ecological region. Girling (1978) noted that the different borer species occur together in the same locality, they occupy different ecological niches so that there is no interspecific competition among them. He reported that in East Africa, the peak of attack of the four species of stemborers occurred at different times. *C. partellus* attacked the two month old plants. *B. fusca* reached its peak of attack in the three months old plants and *S. calamistis* in the four month old mature stems while the numbers of *E. saccharina* went on increasing after maturity in the dry stem. Presently, *S. calamistis* attack is most serious during the second season (July–November), therefore, most of the farmers do not grow maize in this season, even if rains are sufficient. Peaks of *Eldana* population coincide with the pre- and post-harvest period of maize.

Sesamia calamistis

Female moths deposit egg batches of up to 100 eggs between the leaf sheath and the plant stem. A total of about 300 eggs is laid by each female. Eggs hatch on the 6–7th day and the larvae bore into the stem. Larval development takes 30–50 days depending upon the season. Population takes place in the stem or under the leaf sheaths. The pupal period is from 7–13 days. The life cycle is completed in 43–70 days with continuous generations throughout the year.

Larvae bore into the stems under the leaf sheath on plants. Their stem boring may kill young plants, make older plants more susceptible to lodging and seriously reduce yield. The experiment on the effect of maize growth stage on a yield reduction by *Sesamia* larvae released artificially on plants at 3–7 and 11-leaf stage showed that yield reduction is 94.4% and 30.8% respectively (Dabrowski *et al.* 1984). Later generations of *Sesamia* are developing on maize cobs.

The name "Pink Borers of Maize" which is often applied to *Sesamia* larvae are rather unfortunate since larvae of *B. fusca* also are frequently decidedly pink (Tams and Bowden 1953). *Sesamia* larvae are separable from both *Poeonama* and *Busseola* by the fact that setae S0 2 of abdominal segment 0 is practically in line with S0 1 and L1, not distinctly set forward. Setae S0 1 and S0 2 of abdominal segment 8 are either anterior to the spiracle (nonaerioides group) or antero-dorsal to it. The colour markings and size of adult specimens of *S. calamistis* are so variable that although typical specimens may be distinguished by their external features, the only sure way of distinguishing the species is by examination of the genitalia.

S. calamistis Hmps. males have genitalia with juxta short and broad valve as broaders along the coastal spine short, little curved and almost bifid at apex.

S. botanephaga Tams and Bowden has genitalia with juxta flask-shaped with a long neck, the valve longer than broad, and coastal spine long curved with a strong sub-apical tooth.

According to Tams and Bowden (1953) *S. calamistis* has been confused in the past with *S. botanephaga*. They stated that, in West Africa *S. calamistis* is more common in savanna areas which have a well-marked dry season, whereas *S. botanephaga* is dominant in forest and forest areas. They also recorded a similar distinction between the distribution in Ghana of *S. penniseti*, which was founded only in wet forest areas, and *S. neophaga* which occurred in the Southern savanna and transition zone of Southern Togoland.

Moths of *S. calamistis* have brownish forewings with a darker border before the lighter fringed tip and numerous small brown spots. Hind wings are whitish and fringed. The wing spread is about 27.35 mm. Larvae are pinkish as they mature with a darker stripe down each side of the body. The head capsule and thoracic shield are brown. Matured larvae are about 30 mm long.

Rearing of *Sesamia*

Techniques of rearing *Sesamia calamistis* on artificial diet under insectary conditions have been described by Jackai and Raulston (1982); Dabrowski and Jackai (1983) and Bosque-Perez and Dabrowski (1989) as follows:

Starting a Colony

Collect large numbers of larvae and pupae from maize or sorghum plants from various ecological zones and keep individually in glass vials capped with plugs of cotton wool.

Vials for larvae contain diet. When the larvae pupate, they are transferred to sterilised empty vials until adults emerge. Only healthy adults are used to establish new colonies.

Preparation of a Diet

The diet composition is shown in Table 1 and the preparation procedure is as follows:

(i) add 55 g of agar to 1,800 ml of distilled water, bring to a boil and then cool to 60°C before adding vitamins;

(ii) mix 1,800 ml of water with soy flour, boil to prevent contamination with microorganisms and cool to 60°C;

(iii) to this mixture (ii) add a salt mixture, wheat germ, sugar, ascorbic acid, aureomycin, benlate and Fumidil-B, blend for one minute, add the agar portion (i), and blend for another minute;

(iv) add the mixture of vitamins, KOH, chlorine chloride, acetic acid, methyl p-hydroxy-benzoate, phosphoric acid with propionic acid and formaldehyde and blend for three minutes; and finally

(v) pour 500-ml portions into plastic containers (or glass vials in small portion), allow them to cool to room temperature for 12 hours and store at 5°C before elimination of excess water.

Rearing of Larvae

1. Two types of rearing containers are used for larval rearing. For rearing small colonies, small glass vials (with 15 ml of diet) capped with sterilised cotton wool plugs are used (Fig. 1).

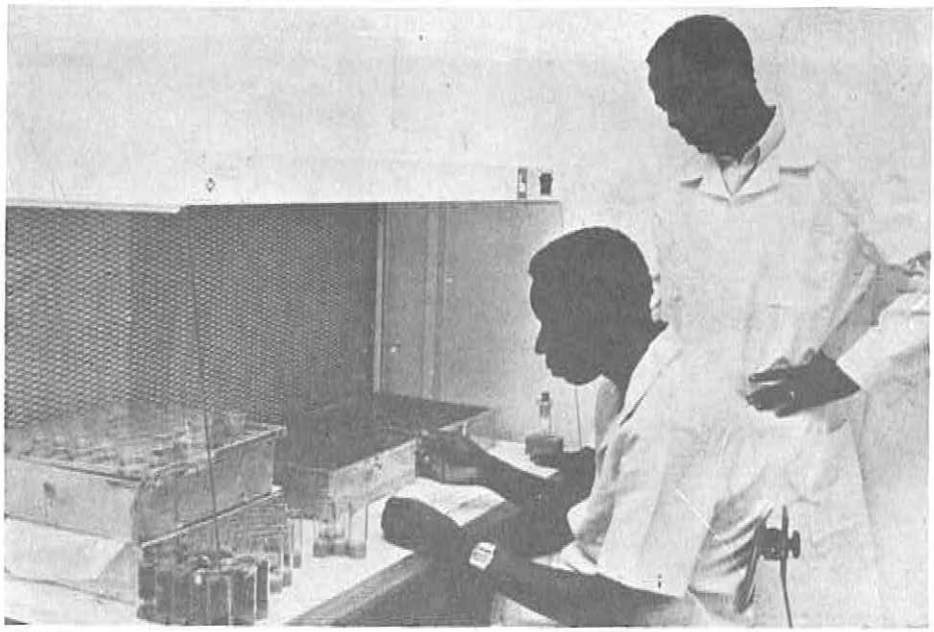

Fig. 1. Diet poured into vials is inoculated with 2–3 *Sesamia* larvae under a micro-flow hood to ensure a sterilised environment

For larger colonies round plastic dishes (18 cm in diameter and 7.5 cm deep) covered by tight-fitting lids are used. Each container holds 500 ml of diet. The lid has a 9 x 9 opening covered with a brass fine screen mesh (80 gauge, Fig. 2).

Before the diet is added, glass vials and the plastic containers are washed in soapy water, sterilised in a bleach solution, and rinsed in sterilised water. Glass vials are sterilised in steam and dried in an oven.

2. Before *Sesamia* egg masses are placed on the diet, they are surface sterilised. 224 ml of water and 27 ml of the 37% stock solution of formaldehyde is poured in the plastic sandwich box and the egg masses laid on tissue paper and held in ice-cream cups are exposed for surface sterilisation for 20 minutes. After that the sterilised mounting pins are used for mounting a small piece of tissue paper with egg mass in the middle of diet (there is no direct contact between the paper holding egg mass and the surface of the diet).

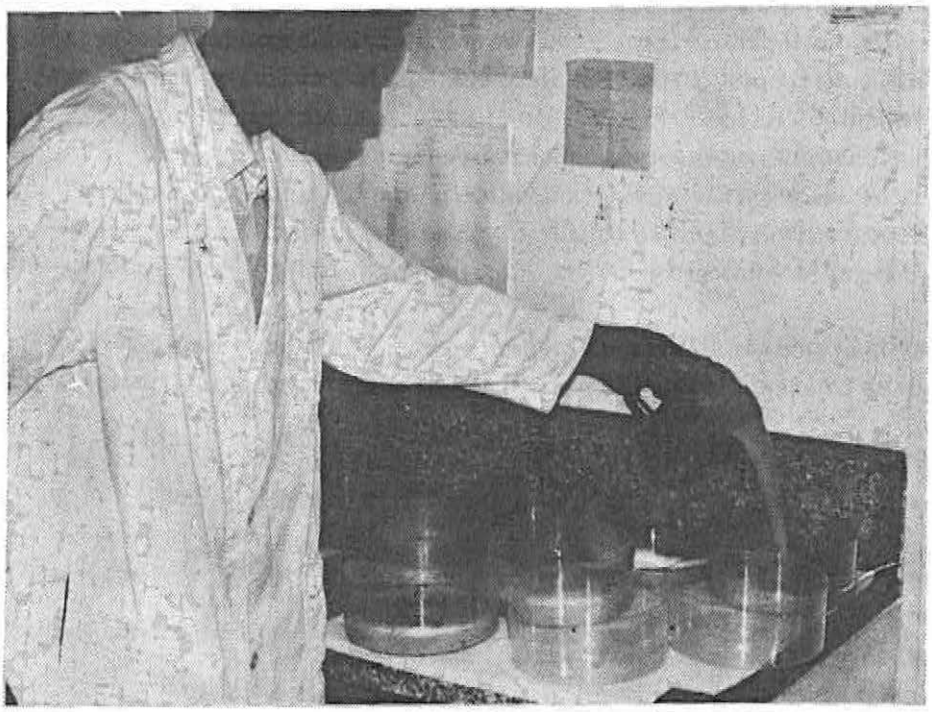

Fig. 2. Round plastic dishes are used for rearing *Sesamia* and *Eldana* larvae

When the small glass vials are used for larval rearing, larvae are allowed to hatch in the sandwich boxes maintaining high humidity and 3–4 larvae are later transferred by a fine brush into vials (Fig. 1).

3. The diet is poured in while hot into vials and containers. Infestation with egg masses or larvae takes place after the diet has cooled and its surface is scarified with a knife or a fork to ease larval penetration. All infestation is done under a micro-flow which ensures air sterilisation (Fig. 1).

4. A double lining of paper toweling is placed over the container before the cover is placed on. The paper towel absorbs some moisture and later serves as a pupating substrate for some pupae (Fig. 3).

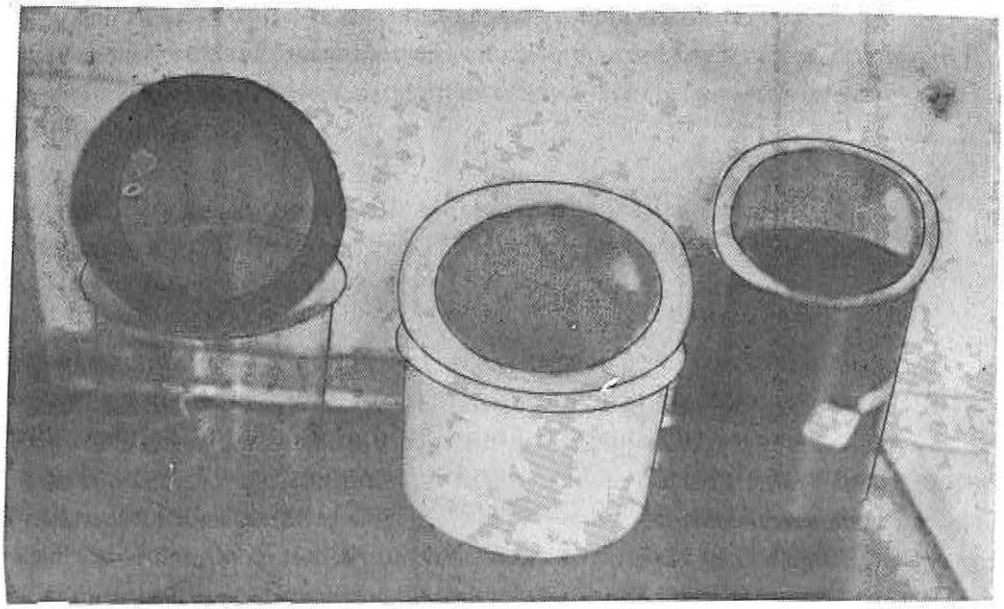

Fig. 3. Use of locally available containers in the insectaries of national research systems should be encouraged

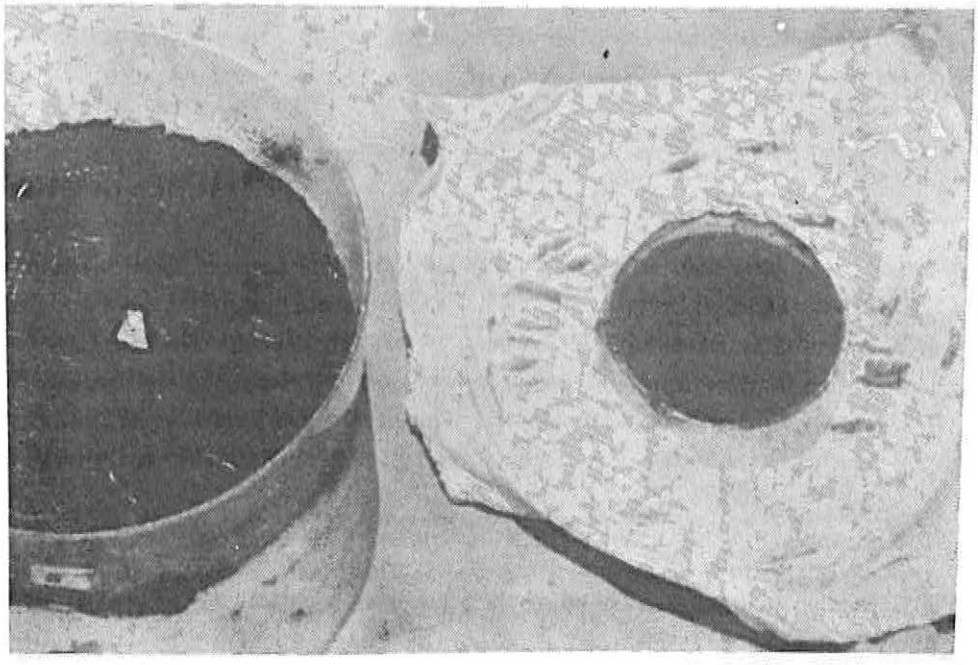

Fig. 4. *Sesamia* pupates on the surface of dried diet and between tissue papers and a plastic top

Fig. 5. Rearing containers should be regularly inspected to detect diet contamination and/or diseases affecting the insects

5. Rearing containers and glass vials are kept in the larval holding room, maintaining 25–27 degrees centigrade, 50–60 per cent relative humidity and using 12:12 hours light dark period. The higher humidity increases diet

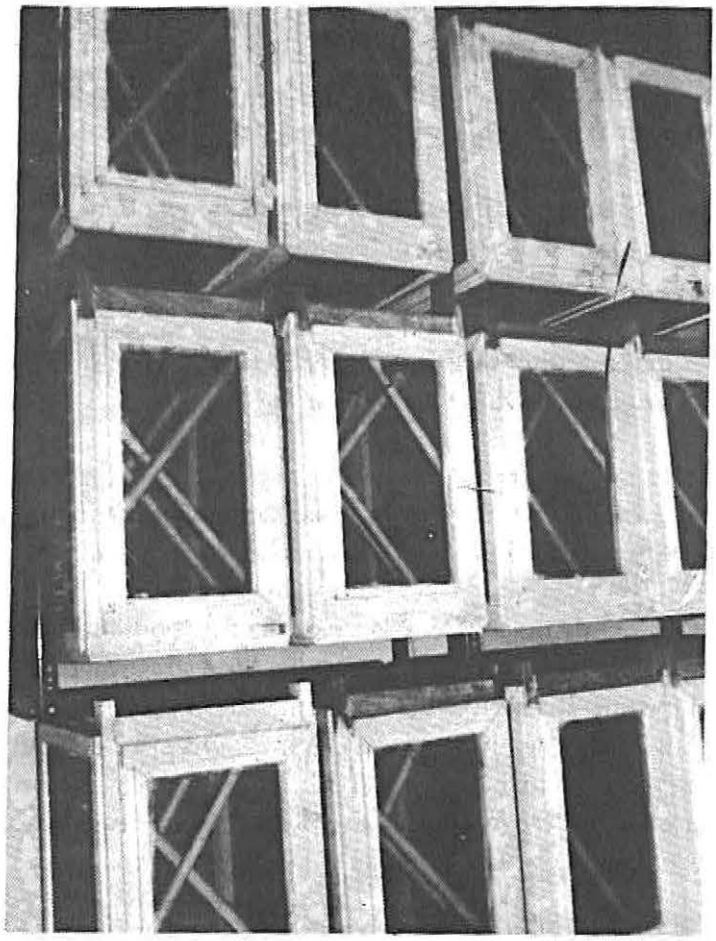

Fig. 6. Cages with waxed paper wrapped around wooden brush handles for *Sesamia* oviposition

contamination with microorganisms (moulds etc.). The average developmental time is 26–29 days (Fig. 4).

Oviposition and Egg Collection

1. Pupae are collected manually from larval rearing containers (vials), sterilised by immersion in a 5% bleach solution for 2 min, then rinsed and placed in cages for emergence of the adults. Approximately 200 pupae are placed in a cage. The cages are made of a metal frame (31 x 31 x 42 cm) and wire mesh. Pupae are kept at 22°C to 24°C and 80–90% relative humidity. Male adults emerge after 10 to 12 days and females after.

2. After adults emerge, 25 pairs are transferred to wooden or metal cages (41 x 50 x 71 cm) for mating and oviposition. The cages are covered with fine metal or plastic netting; the bottoms are lined with paper towel (Fig. 5).

   The sex of the adult insects is determined from the dimorphism shown by their antenna and by their abdominal shape.

3. The adult oviposition cages are kept at the same temperature and high humidity conditions as the pupal cages, but photoperiod is not controlled. The lights are turned off in the oviposition rooms when no one is working there.

4. For oviposition, *Sesamia* females require a smooth surface with closely overlapping edges. The overlapping effect appears to meet requirements of a tight passage through which the adult can insert its ovipositor to lay its eggs. *Sesamia* lay egg masses on wax paper wrapped around cut wooden sticks, equivalent to maize stem size in circumference. The wrapped sticks are placed in each cage. Four small vials with 5% water-sugar solution with a piece of paper towel for adult feeding are also placed in the cages (Fig. 6).

5. The egg masses are removed daily by cutting small pieces of waxed paper each with one or two egg masses. The wax paper is changed every 3 or 4 days on the sticks.

Eggs Incubation

1. Egg masses collected are sterilised and stored in petri dishes or plastic containers, the bottom of which are lined with a moist paper towel for incubation and kept at 20°C to 21°C under high humidity. The eggs are sprayed with sterilised water every morning.

2. Most of the larvae hatch after six days and it takes about 5 days to reach black head stage. *Sesamia* larvae have a tendency to aggregate after hatching. The young larvae can survive about 2 days without food when the containers are moist.

3. Egg masses can be stored at 10°C for up to 5 days to delay development.

Eldana saccharina

First recorded outbreak of *Eldana saccharina* in Africa was observed on sugarcane in Tanzania in 1959, and in Uganda in 1967. Since that time, it has been an important pest of maize in most countries in Africa (Girling 1978).

E. saccharina larvae first appear shortly before the host plant begins to bloom. The number increases steadily toward the period of cob ripening and reaches its peak after the harvest, if the plant is allowed to remain standing in the field. Since *E. saccharina* is a dry season oriented species, the population is generally larger during the period.

The adult male has a wingspan of 28–30 mm, and the female 39–40 mm. They have pale brown forewings, each with two small spots in the centre, and whitish hindwings with a short fringe. The eggs are oval, yellow and laid in batches on the soil surface, although some may be laid on the leaf bases or in cracks on mature stalks. On average 200 (100–500) eggs are laid per female in batches of 10–15 (3–20 have been recorded). The female starts egg-laying the second night after emergence. The egg incubation period is 5–6 days at 25°C. The larval period is 30–35 days. When burrowing in the stem, the larvae characteristically push their faecal pellets outside.

The newly emerged larvae can feed on decaying trash and on the leaves; when they reach about 1 cm in length, they bore into the stem just above a node. Seedlings or young plants of pre-flower stage are seldom attacked, although when reared on these materials in the lab, larvae developed normally. In the field, larvae also fed on growing cobs. The feeding sites within the maize plant consist of 60% in stems (32% upper, 23% central and 5% lower parts) and 40% in cobs. They also bore down into the root stock, but in heavy infestations they can be found in any internode along the stem, and often bore through the nodes into other joints.

The only external sign of attack on the host plant by *E. saccharina* is the moth exit hole cut by the larva before pupation. If the hole has a large amount of frass hanging from it then the damage is recent. When the stalk is split upon the borer tunnels can be seen in the joints and there is usually discoloration and fungal attack as well.

Rearing Procedure

The composition of the diet used for rearing *Eldana* larvae used by the author is similar as those used for *Sesamia* (Table 1). The differences are only as follows:

1. The *Eldana* diet does not contain Benlate and Fumidil B, which were found to cause a deformation and affected survival of *Eldana* pupae (Bosque-Perez and Dabrowski 1989).

   Streptomycin and Aureomycin are added to the *Eldana* diet since there are usually bacterial diseases affecting this insect.

2. Maize tassel powder is also included in this diet, increasing adults' fecundity and shortening the larval development period. Green tassels are collected before pollen is shed, washed, sterilised, ground, and added to the diet.

3. *Eldana* often pupate on the paper towel and the pupae are covered by a silk cocoon. Pupae are removed from the silk cocoon either with fine scissors or by immersing them in a mild bleach solution for 5 min. Removing the cocoon increases adult emergence.

4. Oviposition cages are the same as for *Sesamia* only with different type of artificial surface for laying eggs. *Eldana* requires a rough surface with closely overlapping edges. Paper towels folded diagonally (so as to have overlapping edges) size 23 x 2 cm were found preferable for oviposition (Jackai and Raulston 1982).

   Twenty papers are provided per cage. Eggs are collected daily and new paper towels provided for oviposition.

5. *Eldana* eggs are glued in batches so firmly that removing them manually may destroy them. Therefore, in order to collect the eggs, it is necessary to moisten the paper towels by spraying them with water, since the eggs are

Table 1. Composition of the diet used for rearing *Sesamia* and *Eldana* larvae (modified after Dabrowski and Jackal 1983)

Components	Amount	
	Sesamia	*Eldana*
Water	3,600 ml	3,600 ml
Agar	559 g	559 g
Soy flour	256 g	256 g
Wheat germ	114 g	114 g
Salt mixture W	38 g	38 g
Sugar (sucrose)	46 g	46 g
Maize tassel powder	–	50 g
Ascorbic acid	40 g	40 g
Aureomycin	10 g	10 g
Benlate	2 g	–
Fumidil B	4 g	–
Streptomycin	–	0.1 g
Vitamin mixture[a]	26 ml	26 ml
KOH	20 ml	20 ml
Chlorine Chloride	26 ml	26 ml
Acetic acid	42 ml	42 ml
Sorbic acid[b]	10.5 ml	10.5 ml
Methyl p-hydroxybenzoate[c]	20 ml	20 ml
Phosphoric acid and propionic acid[d]	23 ml	23 ml
Formaldehyde[e]	2.5 ml	2.5 ml

[a]Mixture of 100 ml of distilled water with 1.2 g of calcium penthatenate, 0.6 g of niacin, 0.3 g of riboflavin, 0.3 g of folic acid, 0.15 g of thiamine NCl, pyridoxine, 0.15 g of HCl, 0.12 g of biotin and 0.0006 g of B12.

[b]Solution of 100 g of sorbic acid in 50 ml of 95% ethyl alcohol.

[c]Solution of 280 g of methyl p-hydroxybenzoate in 1,000 ml of 95% ethyl alcohol.

[d]Mixture of 836 ml of propionic acid with 84 ml of phosphoric acid and 1,080 ml of distilled water.

[e]37% solution of formaldehyde.

glued to the paper and break easily. Eggs do not stick so firmly to the moist paper. After the paper towels are opened and the eggs exposed, the egg masses are cut off with scissors. Eggs are placed in plastic sandwich boxes (17 x 12 x 7 cm) lined with moistened paper towels. The boxes have tight-fitting lids with a 5 x 5 cm opening to which a fine wire mesh is affixed.

6. The author as well as other entomologists have tested suitability of a number of diets, including mediums used for rearing the European corn borer. Most of them provided a good growth of larvae and high fecundity (350–450 eggs/female) of females. As an example the medium used for Betbeder-Matibet *et al.* (1977) is given in Table 2.

Table 2. Composition of *Eldana saccharina* diet used by Betbeder-Matibet *et al.* (1977, modified)

Components	Amount
Water	3,600 ml
Agar	84.0 g
Wheat germ	168.0 g
Yeast	180.0 g
Ascorbic acid	60.0 g
Benzoic acid	7.2 g
Napagine	6.0 g
Aureomycin	1.2 g

Control of Mould

Mould control is the most troublesome problem in insectaries which do not have a proper ventilation system and adequate number of micro-flow hoods in a diet preparation and diet inoculation rooms.

Mould must be controlled under nearly ideal growth conditions of humidity and nutrition for extended periods, without significant harm to the reared insects. *Aspergilus niger* is the most commonly mentioned and probably the most resistant to control. However *Aspergilus flavus*, *Penicillium* spp., and *Rhizopus* spp. have also been identified.

There are a number of chemicals in addition to antibiotics used to control the mould development in insect diets. Their efficacy, however, depends largely on pH of a diet.

Sorbic acid or its much more soluble salt is the most efficient under 5–6.5 pH. It is active against yeasts and moulds and has some activity against many bacteria.

Propionic acid is most commonly used as either the calcium or sodium salt. The optimum pH of the inhibitory action of sodium propionate is given as 3.5 to 5.0. Propionates are relatively ineffective against yeasts and bacteria.

Benzoates are generally applied as the soluble salt of benzoic acid, sodium benzoate. The optimum pH has been given as 3–4.5. The mode of action is due to damage to fungal cell membranes.

The alkyl esters of para-hydroxybenzoic acid are mainly used in pharmaceutical products and cosmetics. They appear in the literature under many different brand names and an assortment of systems of chemical nomenclature. The effectiveness of parabens is somewhat better at lower pH levels but covers a very wide pH range of about 4 to 8. They are effective against a wide spectrum of microorganisms including many bacteria as well as fungi.

Another compound widely used as an antimicrobial agent in insect rearing is formaldehyde, normally as the aqueous solution Formalin. It is almost universally antimicrobial, particularly against bacteria. Fungi are more resistant, but a concentration of about 1 part in 1,000 is usually effective for their control. Its mode of action is due to denaturation of protein. Because formaldehyde reacts with many components of media, the effective residual concentration remaining for control may vary considerably with the medium. It has the advantage of being antiviral.

In addition to those most commonly used antimicrobial agents, a large number of other chemicals have been tested. Several fungicides have been shown to be effective but are seldom used. Some of them can be, however, detrimental to the growth of young larvae (as observed for Benomyl used in the *Eldana saccharina* diet). The following fungicides were successfully used for controlling moulds in insect diets: captan, folcid, folpet, benomyl, thiram, TCMTB (Busan 72A), manzeb, dinocap and maneb.

The control of bacteria is, generally speaking, not as frequent a problem as the control of moulds. Methods of selection of antibiotics effective against troublesome bacteria are outlined by Childress and Williams (1973). Most diet recipes include antibiotics of the broad-spectrum type that are effective against a wide range of both Gram-positive and Gram-negative bacteria. The most common are chlortetracycline, streptomycin, and kanamycin.

In summary, the control of moulds in insect-rearing medium by antimicrobial chemicals remains at least an occasionally troublesome problem. The amounts necessary for effective control are usually high enough to cause some adverse effects on larval development. Before introducing new antimicrobial compounds into an insect diet, a bioassay should be run to compare larval development and females fecundity under a new composition.

Microorganisms can be expected to develop resistance to frequently used chemicals. Some antifungal agents and particularly of the agricultural type, might be held in reserve and used to control mould outbreaks by such resistant strains. Good sanitation practices to minimise accumulation of airborne mould spores in laboratory environment are certainly a useful precaution.

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Standard Rearing Procedures for the Spotted Sorghum Stemborer, *Chilo partellus*

F. O. Onyango and M. D. O. Bungu

Insect and Animal Breeding Unit (IABU),
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Abstract

Chilo partellus *is the most important stemborer of sorghum and maize in Asia and many parts of Africa. The rearing of this insect has been possible in many laboratories particularly in India. Several artificial diets have evolved over the years with emphasis on easy preparation technique, low cost, and indigenously available ingredients for formulating artificial diets for the mass rearing of* **C. partellus.**

At the ICIPE, **C. partellus** *is the largest single species reared and used for research. The standard rearing procedures are explained. Seasonal infusion with the wild type is undertaken regularly for the restoration of vigour and behaviour of the insect over successive generations of laboratory rearing.*

Introduction

Chilo partellus is the most important stemborer of sorghum and maize in Asia (Jotwani and Young 1977) and many parts of Africa (Ingram 1957; Seshu Reddy 1989). It also infests sugarcane, millets, wheat and rice (Harris 1989). Alternate hosts comprise various species of wild grasses (Harris 1989; Seshu Reddy 1989).

The rearing of *Chilo partellus* on artificial diet has been possible in many laboratories in India. Earlier attempts on the compounding of artificial diet

containing dry leaves of maize was somehow adequate for rearing *C. partellus* (Pant *et al.* 1960). Chatterji *et al.* (1968) used the wheat germ diet formulated after Kaester and Harrendorf (1965) for the South-Western corn borer, *Zeadiatraea grandiosella* (Dyar) to rear *C. partellus*. Siddiqui and Chatterji (1972) replaced the wheat germ with locally available kidney bean *(Phaseolus vulgaris)* as a base ingredient and added vitamin E to increase fecundity of *C. partellus*.

Further efforts to use locally available ingredients were attempted by Siddiqui *et al.* (1977) who formulated artificial diets composed of different pulses of the genus *Vigna* as principal base-ingredients. Sharma and Sarup (1978) included lentil *(Lens culinaris)* in place of bean or *Vigna* spp. A bean and dry sorghum leaf powder-based diet for rearing *C. partellus* was developed by Seshu Reddy and Davies (1978). More recently, Singh and Sarup (1985) compounded an improved alternative diet by adding sorghum leaf factor with green gram and dew gram as base ingredients.

At the ICIPE, Ochieng' *et al.* (1985) have reared *C. partellus* on a large scale using an artificial diet similar to the one developed by Seshu Reddy and Davies (1978) but with minor modification. The diet has since been modified further as described in this paper. Seasonal infusion with the wild type from the neighbourhood is undertaken regularly for the restoration of vigour and behaviour of the insect.

Rearing Methods

Chilo partellus can be reared on both artificial and natural food sources. Both methods are described.

Steps	Key points		
1. Preparing artificial diet	• Prepare the following ingredients. The quantity listed is sufficient for 3.6 litres of diet.		
	A.	Distilled water	2,000.0 ml
		Bean powder	439.2 g
		Brewers' yeast	32.0 g
		Sorbic acid	4.0 g

Steps	Key points		
		Ascorbic acid	6.4 g
		Vit. E capsules (250 – 300 i.u.)	0.9 g
		Benlate (benomyl)	4.0 g
		Sorghum leaf powder	160.0 g
	B.	Methyl p-hydroxybenzoate	6.4 g
		Ethanol absolute	20.0 ml
	C.	Distilled water	1,600.0 ml
		Agar powder	48.0 g
	D.	Formaldehyde 40%	8.0 ml
	• Boil distilled water for blending and cool to 60–70°C.		
	• Weigh the ingredients of fraction A and pre-mix in a sauce pan.		
	• Weigh methyl p-hydroxybenzoate in a clean beaker, add ethanol absolute, and stir until completely dissolved.		
	• Weigh agar separately.		
	• Pour distilled water for blending into the blender and add pre-mixed ingredients of fraction A.		
	• Blend the concoction of fraction A in a blender for 3 minutes.		
	• In the meantime, put agar in a clean sauce pan containing water for agar and stir the suspension periodically on a hot plate until it boils, then cool to 60–70°C.		

Steps	Key points
	• Pour the warm agar into the blender and mix together with ingredients of fraction A for another 3 minutes.
	• Add solution of fraction B and fraction D to the blender and mix with the rest of the ingredients for a further 1 minute.
	• Dispense the diet in glass jars (19 cm H x 7.5 cm dia.), sterilised in the oven at 150°C for at least 1 hour, each jar containing approximately 200 ml of diet.
	• Cover the glass jars containing the diet with a sheet of white cloth or paper towel and allow to set at room temperature overnight.
	• Proportionate quantities of the diet may be prepared as desired.
2. Preparing sorghum leaf	• Harvest a *Chilo*- susceptible variety of sorghum (e.g. Serena) leaves 6 – 10 weeks old.
	• Wash leaves with running tap water and drip-dry.
	• Place leaves in a thin layer in the oven at 60°C for 12–24 hours until brittle.
	• Chop leaves to small pieces approximately 1.0 cm^2 using a pair of secateurs.
	• Grind the leaves immediately to a fine powder using electric grinder.
	• Store leaf powder in a plastic container with lid in a cool dry place for use as and when required.

Steps	Key points
3. Preparing beans powder	• Obtain bean (*Phaseolus vulgaris)* from the local market.
	• Wash the beans thoroughly with tap water.
	• Drip-dry the beans in wire mesh trays.
	• Place beans in the oven at 60°C for 12–24 hours until thoroughly dry.
	• Grind the beans immediately in an electric grinder to a fine powder.
	• Store the beans powder in a plastic container with lid in a cool dry place for use as and when required.
4. Adult management	• Use group oviposition unit made of a rectangular wire frame mesh cage (40 cm L x 30 cm W x 40 cm H).
	• Clean the cage thoroughly with disinfectant and detergent and allow to dry.
	• Place a petri dish containing tissue paper soaked in 10% sucrose solution in the cage for adult diet daily.
	• Line the interior of the cage with pleated butter paper for the moths to oviposit.
	• Collect adults from the rearing jars using glass vials (7.5 cm L x 2.5 cm dia) or a battery operated aspirator.
	• Determine the sex of the adults at this stage. Females are pale in colour and bigger in size while males are darker and smaller.

Steps	Key points
	• Replace new pleated butter paper and sucrose solution in the oviposition cage daily.
	• Spray the oviposition paper in the cage daily with distilled water from a hand sprayer.
	• Introduce 200 adult pairs per cage.
	• Label each cage accurately with date of adults emergence and generation number.
	• Cull adults on the 6th day after emergence.
5. Egg management	• Remove the butter paper from the oviposition cage daily and separate egg sheets for supply and those for colony maintenance.
	• Incubate egg sheets in the incubator at 27 ± 1°C and 70 – 80% r.h.
	• Eggs will take 4 days to turn to black-heads under the above conditions.
	• Cut eggs in small batches as required for supply at 3 days when they are brown-heads.
	• Cut eggs in batches of 50 eggs at brown-heads if they are for colony maintenance.
	• Surface sterilise eggs for colony maintenance at black-head stage using 10% formaldehyde for 20 minutes, then rinse in 5 washings of distilled water.
	• Dry the eggs between two filter papers.

Steps	Key points
	• Place the cut egg batches for supply in well ventilated lunch boxes (15 cm L x 9 cm W x 6 cm H) lined with moist filter paper.
6. Larval management on artificial diet	• Inoculate one batch of sterilised black-head stage eggs, each batch containing approximately 50 eggs/jar in the 200 ml diet contained in glass jars 19 cm L x 7.5 cm diameter. • Cover each jar with paper towel first, then fasten the ventilated metal screw cap. • Arrange the jars in rearing units on the shelves. • Label each rearing unit with date of inoculation, generation number, number of jars, and diet batch number. • Allow the larvae to feed undisturbed upto pupation and eventual adult emergence. • If the larvae are to be retrieved for supply at 2nd or 3rd instar stage, inoculate upto 500 black-head eggs per rearing jar.
7. Larval management on natural food source	• Inoculate first instar larvae in glass vials 7.5 cm L x 2.5 cm diameter at a density of 200 larvae per vial containing 4 weeks old sorghum leaves. • Change the leaves every second day and provide fresh leaves. • Introduce larvae to young sorghum stems 6–8 weeks old in petri dishes (10 cm diameter) when they are 3rd instars, 5 larvae per petri dish. • Change the larvae to fresh stems every second day until pupation.

Steps	Key points
8. Pupal management on artificial diet	• Allow the larvae to pupate in the rearing jars. • Start expecting pupae 28 days after inoculation of black-heads eggs. • If the pupae are for supply, provide the whole rearing jar to the recipient for extraction of pupae. • If the pupae are for colony maintenance, allow the adults to emerge in the rearing jars.
9. Pupal management on natural food source	• Remove pupae from the petri dishes daily. • Put the pupae in an adult emergence perspex cage (30 cm L x 30 cm W x 30 cm H) whose floor is lined with moist paper towel.

Conclusion

The rearing of *C. partellus* at the ICIPE, Mbita Point Field Station has been simplified, using ambient laboratory conditions to supply over 40 million insects annually to the various research units and programmes of Crop Pests Research. The method can easily be adopted by the resource-limited National Agricultural Research Centres in Africa. The use of cheaper and locally available ingredients is a major priority of future focus. For instance, there are high hopes that fruit jelly will replace the use of expensive agar in the near future. Similarly corn starch, (Maizena) is being tried as a part or whole replacement to agar.

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Rearing of *Cicadulina* Leafhoppers

Zbigniew T. Dabrowski

International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772, Nairobi, Kenya .

Abstract

Two species of* Cicadulina *leafhoppers (Hemiptera: Cicadellidae) are presently used for mass rearing:* Cicadulina mbila *and* C. storeyi *(Syn.* C. triangula*). The critical step in developing a large colony of* Cicadulina *for maize resistance screening of maize streak virus (MSV) and the maize mottle/chlorotic stunt virus (MMCSV) is a proper selection of suitable species. Inaccurate identification of* Cicadulina *spp. for MSV resistance screening can be very costly and wasteful as all subsequent inputs such as collection and rearing are unproductive if the species is unsuitable from the start. There are large differences in the suitability of various* Cicadulina *species for mass rearing.

Introduction

Recent successes in maize resistance breeding to maize streak virus (MSV) by the IITA and CIMMYT breeders has largely depended on the development of reliable screening methods under uniform artificial infestation of maize seedlings with viruliferous *Cicadulina* leafhoppers (Efron *et al.* 1989; and Wedderburn *et al.* 1989). The continuous improvements in rearing and screening techniques were possible after conducting detailed studies on the effect of temperature and host plants on leafhoppers development and fecundity and the factors affecting MSV transmission by various species of *Cicadulina* (Dabrowski, 1985).

Developing and maintaining large *Cicadulina* colonies for MSV resistance screening is possible only if the following procedure is developed:

(i) *Cicadulina* species suitable for mass rearing are collected and correctly identified from the numerous populations of leafhopper and plant-hopper species always found on grasses and cereals in Africa;

(ii) a large number of live individuals of the proper species are collected to start colonies;

(iii) the initial small populations of these species are properly handled;

(iv) the large colony is correctly managed to produce enough insects for field infestation; and

(v) viruliferous leafhoppers that have been selected for their capability for high and uniform infestation of plants with MSV are released in the field under optimal conditions.

Establishment of a Colony

Two species of *Cicadulina* leafhoppers are presently used for mass rearing in Africa: *C. storeyi* was found to be better adapted than *C. mbila* for mass rearing in temperatures higher than 26°C that often occur in the glasshouses in a humid tropical zone of West Africa (Dabrowski 1985).

On the other hand, at the CIMMYT Maize Station in Harare, Zimbabwe and at Mt. Makulu Central Agricultural Research Station, Zambia, where the temperatures are lower, particularly during the dry season, *C. mbila* leafhoppers were better adapted in mass rearing under lower temperature conditions at a mid-altitude ecology (Wedderburn *et al.* 1989; Okech and Chinsembu, 1991).

In some cases, the development of large colonies of leafhoppers for MSV resistance screening has not been successful because the vector species was incorrectly identified or two–three different species were mixed up. Proper identification procedures are provided by Webb (1987); Dabrowski and Cwikla's (1991) publications.

The last two authors cautioned that care should also be taken to distinguish African species of leafhoppers from the externally similar *Afrosteles distans* (Liunavuori). *A. distans* is slightly larger than specimens of *Cicadulina* and lacks the dark apex of the ovipositor present in *Cicadulina*.

Collecting Live Leafhoppers

Live leafhoppers are collected from green grasses by using a trapping net cage (Dabrowski 1983) as described below:

(i) To construct a framework, take four 150 cm pieces of construction steel rod, 8 to 15 mm in diameter, sharpened at the lower end and bent at the top into a short 20-cm arm (corner support). Drive these rods into the ground to frame 1 to 1.25 m^2 of young grasses, preferably close to a maize field or between maize plants (Fig. 1).

Fig. 1. The most practical means of collecting live *Cicadulina* adults: metal supports, dark cloth, and fine netting

(ii) Quickly place a dark cotton cover, one side of which is made of fine netting, over the supports, forming a cage with three sides and the top darkened and one side transparent netting.

(iii) Enter the cage and shake the grasses to disturb all insects and force them to move to the light. Collect *Cicadulina* individuals landing on the netting with an aspirator.

Young grasses of the genera *Digitaria, Brachiaria, Eleusine, Chloris, Paspalum,* and *Pennisetum polystrachion,* and *P. purpureum* are preferred for feeding and oviposition by *Cicadulina* and should be selected as sampling sites (Okoth and Dabrowski 1987).

Because *Cicadulina* spp. are scarce on grasses and maize during most of the growing season, and their populations increase during the growing period, the optimal period for collecting live *Cicadulina* adults is at the end of the growing season. Large numbers of the leafhoppers always migrate from older plants to young grasses or maize plants of the second crop.

Starting a *Cicadulina* Colony

Approximately 200 collected females should be caged singly on maize seedlings growing on plastic tubes (Fig. 2). Some national programmes were using cut pieces of plastic bottles from mineral water. The top of tube is covered with a fine netting.

F_1 progeny of individual females are reared to the adult stage and some males from each rearing tube are used for species identification. The females and remaining males of the same species are then bulked together in larger cages to form a new mixed population of individuals of the same species originating from various locations (Fig. 2). Wooden or metal frame cages 20 to 50 cm wide and 70 to 100 cm high covered with fine netting may be used for the rearing of the initial 2–3 leafhopper populations (Fig. 3).

During the initial stage of rearing, fecundity, egg and nymphal development periods, and adult longevity of various *Cicadulina* species maintained on selected host plants such as maize, sorghum, pearl millet and wheat are compared (Dabrowski 1989). Various techniques used for studying the effect of temperature

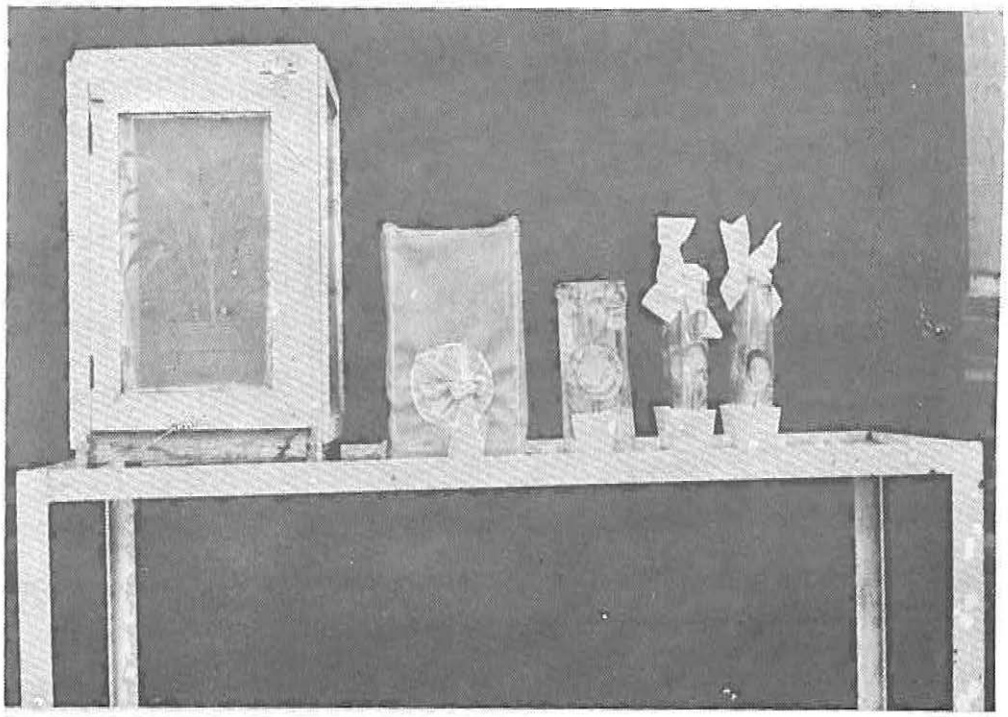

Fig. 2. Individual insects and small *Cicadulina* colonies are kept in various types of cages

on biology and fecundity of both species; *C. mbila* and *C. storeyi* (*triangula*) are given in papers published by Rose (1973), van Rensburg (1982), Dabrowski (1985) and Okoth *et al.* (1987).

Preference for mass rearing should be given to species that are highly efficient in virus transmission and with high reproductive potential under mass rearing conditions.

Large-Scale Rearing Procedures

Wooden or metal frame cages 1.25 x 1.25 x 1.50 m, covered by fine mesh and containing potted pearl millet or maize seedlings, are used as oviposition and nymphal rearing cages (Fig. 4). At IITA, potted 14 day old millet or maize plants are exposed to *C. storeyi* oviposition on an open table. However, the *C. storeyi* population used at IITA since 1976, shows high adaptation to artificial, caged conditions, and probably some genetic selection led to the increment of less mobile

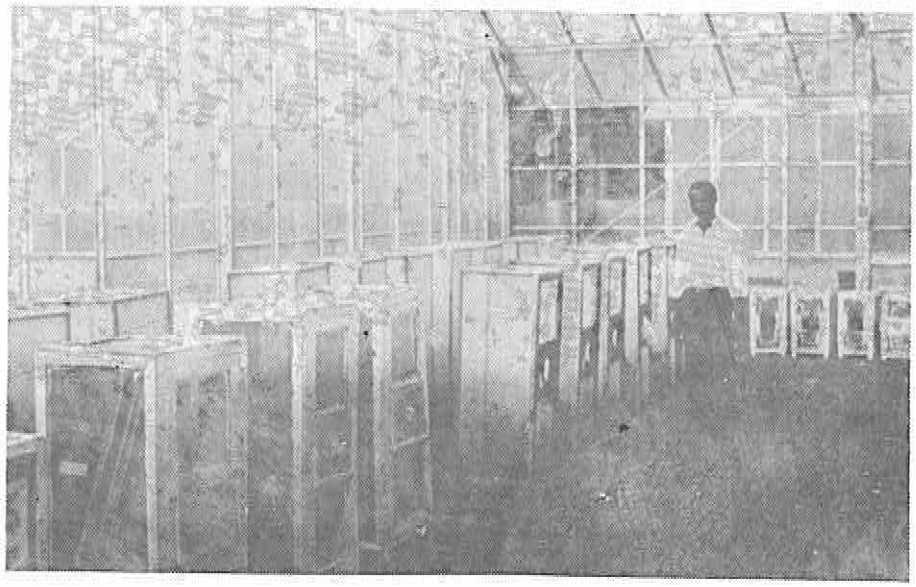

Fig. 3. First 2–3 generation of mixed F_2 progenies of individual females of the same species are kept in larger cages

individuals in the population (Dabrowski 1985). When disturbed, those insects always return to host plants after short, 2 to 4 sec. flights.

Different greenhouses are used for *Cicadulina* mass rearing under humid tropic conditions, extra large fans are installed to keep temperature lower. Under mid altitude conditions in Harare, the greenhouses have additional coal heating system to increase temperature during the dry season, especially during nights (Fig. 5).

Pearl millet (*Pennisetum americanum*) was found more suitable for rearing *Cicadulina* leafhoppers than maize plants. Millet plants are more tolerant of extensive feeding and oviposition, and are better host plants nutritionally than maize for some species of *Cicadulina* (Dabrowski 1985; Okoth *et al.* 1987). Similar relations were observed by van Rensburg (1982) for *C. mbila* and host plants.

On the 7th day of *C. storeyi* oviposition at IITA, the plants are transferred to nymphal rearing cages. For other species and different rearing conditions, the exposure period for oviposition should be established based on the species biological parameters, especially egg incubation period. Nymphal development

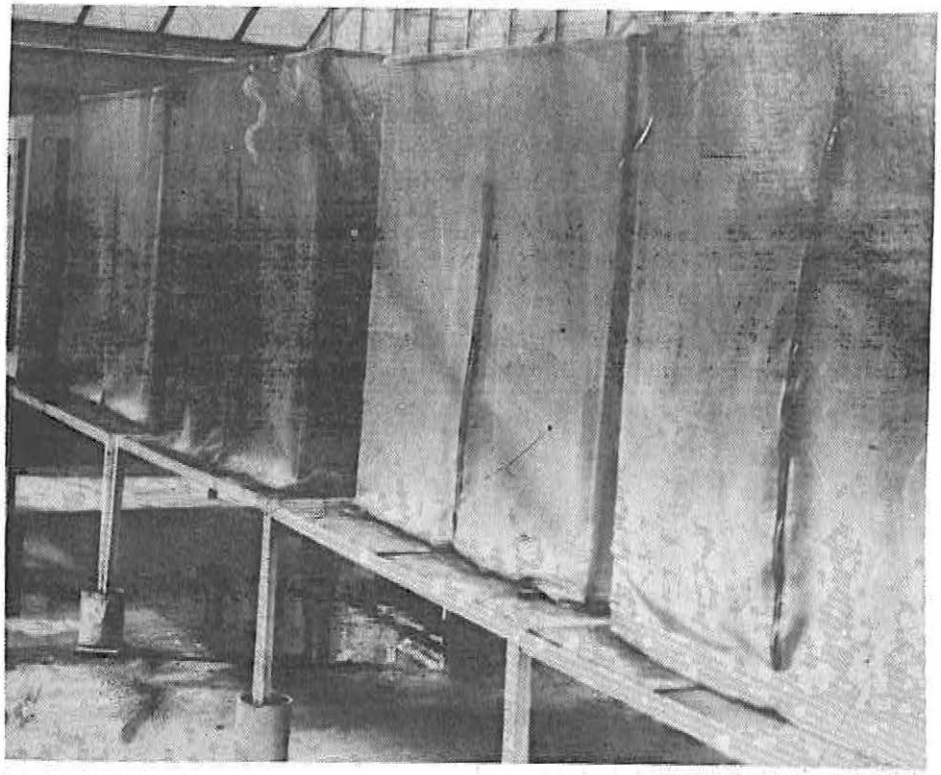

Fig. 4. Cages used for oviposition and nymphal rearing

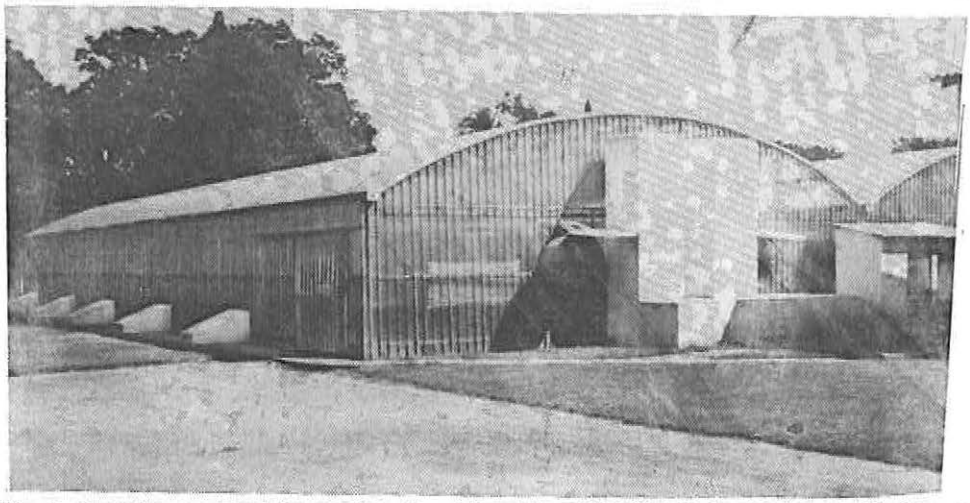

Fig. 5. Greenhouse used for rearing *C. mbila* at the CIMMYT Maize Research Station, Mt. Pleasant, Harare (Zimbabwe)

period varies between 14 and 21 days in the screenhouse at IITA. For other conditions and different species, nymphal development may vary between 14 and 35 days (van Rensburg 1982).

Newly emerged adults are collected from the nymphal rearing cages by covering the cage with a dark cotton cloth, leaving a small section of fine transparent mesh uncovered (Fig. 6). The insects, attracted to light entering through the mesh portion, aggregate on the inside of the net, where it is not covered by dark cotton. From there, they are collected with a modified vacuum cleaner (400–600 W). A plastic vial 5 cm in diameter and 10 cm long with one end covered by fine mesh is held tightly in the vacuum's thick rubber tube, which is 6 cm in diameter and 5 cm long (Fig. 7).

Fig. 6. Rearing cages are covered by a dark cloth before collecting leafhoppers aggregating on the transparent side of the cage

Fig. 7. Vacuum attachment for collecting leafhoppers from the nymphal rearing cage

Virus Acquisition Feeding

The methodology of maize streak virus acquisition feeding is well established based on detailed studies conducted by various authors on the relationships between different species of *Cicadulina* spp. and the virus. For the maize mottle/chlorotic stunt virus (MMCSV), only preliminary experiments on MMCSV transmission using *C. mbila* and *C. storeyi* (*triangula*) were conducted by Dabrowski (unpublished) in Nigeria in 1986–87 and recently by Pham (personal communication) in Zimbabwe.

The basic requirement in maintaining an efficient *Cicadulina* species and population for MSV resistance screening is regularly testing their efficiency for MSV transmissions. 30 to 50 females and males of each species are released on caged maize plant with severe streak symptoms for 48 hr to acquire the virus. They are then transferred into individual poly-vinyl chloride cages with individual

seedlings of a maize cultivar susceptible to maize streak (Fig. 8). On the 10th and 14th days, percentages of maize plants showing maize streak symptoms are calculated. Infestation percentages indicate the portion of *Cicadulina* leafhoppers actively transmitting maize streak virus.

Fig. 8. A simple bioassay used for determining the percentage of *Cicadulina* specimens actively transmitting maize streak virus

CIMMYT Station in Harare is using a different bioassay by caging single viruliferous *C. mbila* in small vials and attached to a leaf (Fig. 9). A special plastic device holds the vial at the leaf surface.

Virus Acquisition Feeding for Field Release

A few thousand viruliferous leafhoppers should be released in short periods for maize resistance screening under field conditions. A large cage with plants showing severe symptoms of MSV infection is used for acquisition feeding. Adults collected in the dark cages are released into cages containing plants infected with streak virus for 48 hr for acquisition. The length of the optimal virus acquisition feeding period should be based on a bioassay (previously described) and on the length of time maize plants with severe symptoms of maize streak virus infection can withstand the feeding of several thousand leafhoppers.

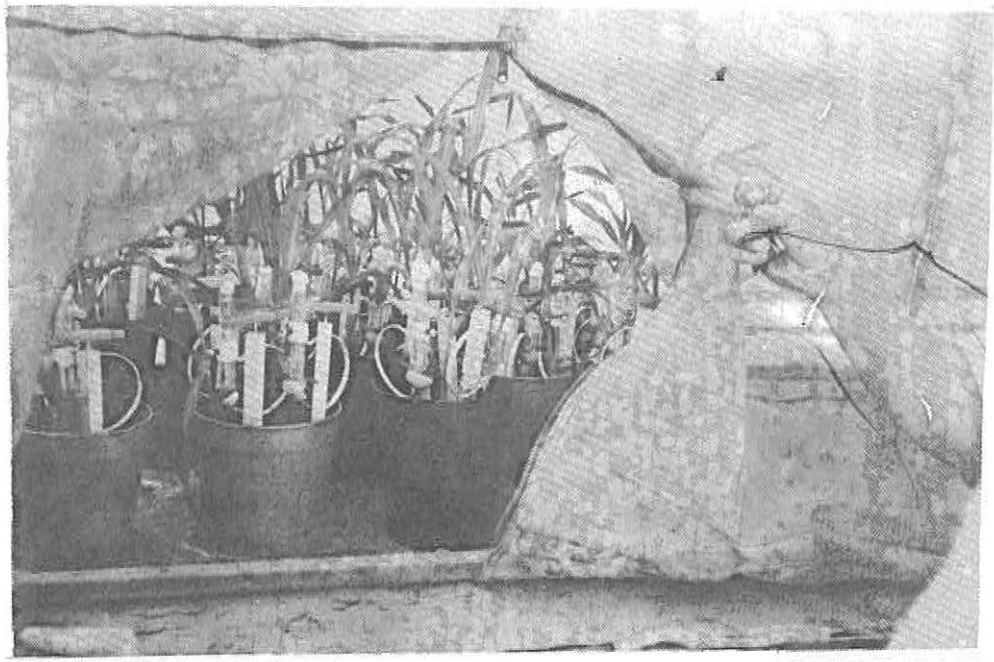

Fig. 9. Caging single viruliferous *C. mbila* in vials attached to leaves at the CIMMYT Maize Research Station, Harare (Zimbabwe)

At the end of the acquisition feeding period, the viruliferous leafhoppers are again collected into plastic vials (5 x 10 cm) with vacuum cleaner and transported to the field for release. If the field is located some distance from the rearing facility, the insects should be transported in a cooler. Under high humidity, they can be stored up to 12 hr at 10 to 12°C, and for 36 hr at 5 to 7°C without reducing survival. It is important to place a piece of filter paper around the inner wall of the plastic vials to prevent the insects from sticking to the condensed water that collects there.

Field Release of Viruliferous Leafhoppers

Immediately before infestation, the leafhoppers in the vials are treated with carbon dioxide to anaesthetise and immobilise them so they will fall into the leaf whorl and not fly away. The carbon dioxide is released from a portable anaesthetising and dispensing unit consisting of a rubber inner tube (filled from a standard commercial CO_2 tank) with an attached rubber hose with a valve (Fig. 10).

Fig. 10. Viruliferous *Cicadulina* specimens (a few thousand per vial) are anaesthetised with carbon dioxide for 3 to 4 sec. directly before releasing

Approximately four *Cicadulina* adults are shaken out through a 3 mm hole in the lid of the vial into the leaf of each young maize plant. Shortly after being released, the leafhoppers revive and protected by the leaf whorl, begin to feed (Fig. 11).

Infesting three-leaf-stage plants produces clear maize streak virus symptoms after 5 to 7 days, allowing reinfestation if necessary without substantially increasing the number of leafhoppers/plant. Older plants require more *Cicadulina* adults than younger plants to produce symptoms (Dabrowski 1985). A scale of 0 to 5 is used for evaluating resistance, where 0 = no symptoms, and 5 = severe streaking on 75% or more of leaf area and plants severely stunted or dead (Fajemisin 1986; Bjarnason 1986).

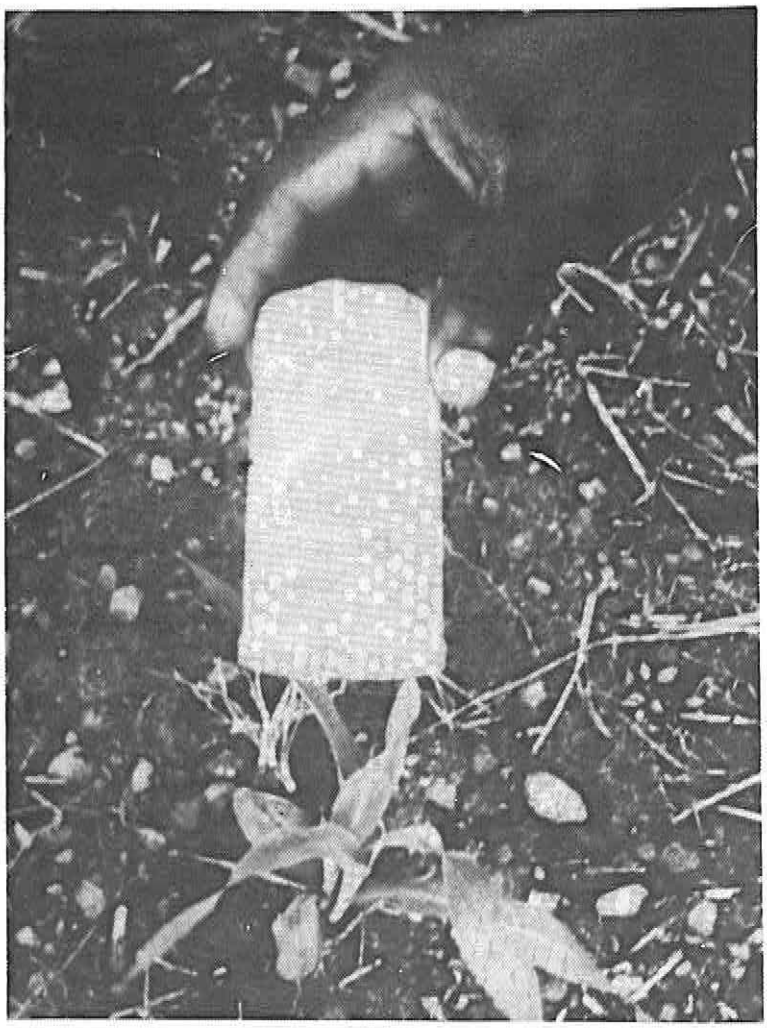

Fig. 11. Releasing 3–4 viruliferous leafhoppers into young maize seedlings for MSV resistance screening

Conclusions

The suitability of the techniques presented above for other environments has already been checked and confirmed by: IITA (Ibadan, Nigeria); CIMMYT Mid-Altitude Maize Program at Harare (Zimbabwe); Mount Makulu Research Station (Zambia), and national maize research programmes in Burundi, Togo and Cameroon.

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Standard Rearing Procedures for the Pod Borer, *Maruca testulalis* on Artificial Diet

F. O. Onyango and P. O. Wagara

Insect and Animal Breeding Unit (IABU),
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772 Nairobi, Kenya

Abstract

The legume pod borer, **Maruca testulalis** ***is a major pest of several tropical and sub-tropical grain legumes. Reports on the rearing of*** **M. testulalis** ***on both natural and artificial food sources are fairly recent. The standard rearing procedures used for rearing on artificial medium NMD2/89 at the ICIPE are explained.***

Introduction

The legume pod borer, *Maruca testulalis* is a major pest of several tropical and sub-tropical grain legumes. The larvae cause damage by feeding on stems, peduncles, flowers, and pods, which results in characteristic feeding symptoms of frass and webbing. This leads to destruction of major stages of growth, flowering and seed production. Singh and Allen (1980) estimated the yield losses to range between 20–60%.

There were no previous reports on the rearing of *M. testulalis* either on natural or artificial food sources until Ochieng' *et al.* (1981) reported for the first time, a detailed methodology for mass rearing on natural food source comprising fresh cowpea flowers. However, since rearing on natural diet is often laborious, time consuming, less efficient, and expensive on a large scale, rearing on artificial diet is preferred.

In this endeavour, Jackai and Raulston (1982) made the first successful attempt to rear the pod borer on a soy bean - based artificial medium which was essentially a modification of the wheat germ diet due to Raulston and Lindgren (1972). Ochieng' and Bungu (1983) developed a chick pea (kabuli gram) *Cicer arietinum* based diet. Jackai and Raulston (1988) further modified their earlier diet, substituting soybean flour with cowpea flour.

The artificial medium NMD2/89 described in this paper is a modification of the diets reported by Jackai and Raulston (1982, 1988) and that of Ochieng' and Bungu (1983). However, the rearing technique differs from both and has been found to be simple, reliable, and with high performance.

Rearing Procedure on Artificial Diet

Steps	Key points		
1. Preparing the artificial diet NMD2/89	• Prepare the following ingredients. The quantity listed is sufficient for 3.2 litres of diet.		
	A.	Distilled water	1,200.0 ml
		Brewers' yeast	40.0 g
		Ascorbic acid	11.0 g
		Sorbic acid	2.5 g
		Methyl p-hydroxy-benzoate	5.0 g
		Vit E capsules (250–300 i.u.)	1.5 g
		Soybean (*Glycine max*) flour	350.0 g
		Cowpea flower powder	50.0 g
	B.	Distilled water	2,000.0 ml
		Agar powder	50.0 g
	C.	Formaldehyde 40%	1.0 ml

Steps	Key points
	• Boil distilled water for blending and cool to 60–70°C.
	• Weigh the powdered, crystallised, and solid ingredients of fraction A and pre-mix in a saucepan.
	• Weigh agar separately.
	• Pour distilled water for blending into the blender, add the pre-mixed ingredients of fraction A and mix for 3 minutes.
	• In the meantime boil agar with distilled water of fraction B and cool to 60–70°C.
	• Pour the warm agar into the blender and mix together with other ingredients for 3 minutes.
	• Lastly, add formaldehyde 40% to the mixture and blend with the rest of the ingredients for a further 1 minute.
	• Dispense the diet to a depth of 3.0 cm per vial using a ketchup dispenser in sterilised glass vials (7.5 cm L x 2.5 cm dia) at 150°C for 1 hour. Alternatively, dispense the diet in lunch boxes to a depth of 3.0 cm.
	• Cover the glass vials or the lunch boxes containing the diet with a clean white cloth or paper towels and allow to set at room temperature for at least 12 hours.
	• Proportionate quantities of the diet may be prepared as desired.
2. Preparing cowpea flower powder	• Harvest cowpea flowers of a *Maruca* -susceptible variety (e.g. Ex-Lwanda, Vita 1 or TVX 66-2H) every morning.
	• Wash the flowers with running tap water and drip-dry.

Steps	Key points
	• Place the flowers in a thin layer in the oven at 60°C for 12–24 hours until the flowers are brittle.
	• Grind the flowers immediately in an electric grinder to a fine powder.
	• Store the flower powder in a plastic container with lid in a cool dry place for use as and when required.
3. Preparing soybean flour	• Obtain soybeans from the local market, a farmer or grow a *Maruca* susceptible variety e.g. TGX 536-02D.
	• Wash the soybean under running tap water and drip-dry in wire mesh tray.
	• Place soybean in the oven at 60°C for 12 – 24 hours until they are thoroughly dry.
	• Grind the soybean immediately in an electric grinder to a fine powder.
	• Store the soybean powder in plastic container with lid in a cool dry place for use as and when required.
4. Adult management	• Use group oviposition unit (30 cm L x 30 cm W x 45 cm H) made of metal frames, whose sides and top are covered with wire mesh 5 squares per linear cm with door on one side and a window at the top to allow for manipulation inside the cage.
	• Clean and disinfect the cage with detergent and disinfectant respectively.
	• Place a filter paper wick dipped in 10% sucrose solution in a 25 ml plastic vial in the cage to provide diet for the moths.

Steps	Key points
	Alternatively, the sucrose solution may be soaked in cotton wool in a petri dish.
	• Change the sucrose solution daily.
	• Collect adults using a vial 7.5 cm L x 2.5 cm dia. from the adult emergence cage made of perspex (40 cm L x 40 cm W x 45 cm H) each morning.
	• Determine the sex of the moths by examining the tip of the abdomen. Males have darker abdominal tip, tapering to a sharp point whereas females have a flat abdominal tip with a lighter complexion.
	• Pair the moths in the oviposition cages at a density of 50 pairs per cage.
	• Place clean potted young cowpea plants 2–3 weeks old on the metal base of the oviposition cage. One pot per cage, each pot containing 5 young cowpea plants is sufficient.
	• Label each cage with date of adult emergence and generation number.
5. Egg management	• Start checking for eggs beginning 2 days after adult emergence.
	• Remove the potted young cowpea plants from the oviposition cage.
	• *Maruca* eggs are found scattered on both surfaces of the leaf and stem.
	• Excise the leaves and stem parts containing the translucent eggs with a razor blade or a pair of scissors.

Steps	Key points
	• Place the eggs collected each day in a separate lunch box (15 cm L x 9 cm W x 6 cm H) on a moist filter paper and cover with a well ventilated top.
	• Incubate the eggs at laboratory temperature and relative humidity of 27 ± 1°C and 70–80% r.h.
	• Moisten the filter paper with distilled water every day to prevent desiccation of eggs.
	• Place a fresh solution of sucrose 10% daily in the oviposition cage.
6. Larval management	• Expect neonate larvae to hatch 3 days after oviposition.
	• If the artificial medium was dispensed in the lunch boxes then:
	– Using forefinger and thumb, grip the bottom of sterilised glass vial (7.5 cm x 2.5 cm dia.) and push the open end of the glass vial into the lunch box.
	– Withdraw the vial with a slice of diet and repeat with as many vials as desired.
	– Push the diet to the bottom of the glass vial with sterilised glass rod (15 cm L x 0.6 cm dia.).
	• If the artificial medium was dispensed directly in glass vials using a ketchup dispenser, then:
	– Move to the next step.
	• Wipe the working bench top with 70% ethanol and allow to dry.

Steps	Key points
	• Dip camel hair brush in 70% ethanol, rinse with distilled water then mop on a clean paper towel.
	• Transfer 5 neonate larvae per vial very carefully onto the diet using camel hair brush.
	• Plug each vial with a tight-fitting cotton wool.
	• Place the vials upright in rearing trays.
	• Label each tray with generation number, number of vials, diet batch number, and generation number.
	• Place the trays in rearing units on the shelves at 27 ± 1°C and 60–70% r.h.
7. Pupal management	• Start checking for pupae 10 days after inoculation of neonate larvae.
	• Remove cotton wool plugs from the rearing vials 5 days after commencement of pupation or 15 days after inoculation of neonate larvae.
	• Clean the adult emergence cage (40 cm L x 40 cm W x 45 cm H) with detergent and disinfectant and allow to dry.
	• Place the open vials from the rearing unit in one adult emergence cage and wait for the adults to emerge.
	• Collect adults each morning and determine their sex as described earlier.

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Techniques for the Rearing of the Pod Borer, *Maruca testulalis* (Geyer) on Host Plant

Raphael Sumba Ochieng'

Department of Zoology,
Moi University,
P.O. Box 3900, Eldoret

Abstract

The procedure described allows production of over 100,000 eggs per month of the legume pod borer, Maruca testulalis ***(Geyer). More than 170 eggs per moth were obtained. The optimal number of moths placed in the mass oviposition cage having potted cowpea plants was 30 pairs. The average life-span of the moths was 7.7 and 9.5 days for females and males respectively. The optimal number of larvae in the rearing box was 50; larval survival declined sharply above a density of 50 per box.***

Introduction

The legume pod borer *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) is an important pest of grain legumes in tropical and sub-tropical regions. Both young and advanced instar larvae concentrate their attack on the reproductive parts of the flowers and first consume the anthers, filaments, style, stigma and ovaries before limited feeding occurs on the internal compound of the corolla of the leguminous flower. The larvae usually migrate from one flower to another as soon as the reproductive parts are consumed, with the result that four to six flowers may be damaged by one larva before development is completed. The larvae are also capable of boring into and damaging the peduncle and stem.

Rearing Methods

Steps	Key points
1. Starting an initial culture	• Collect infested flowers and pods from the field. • Provide fresh flowers for the field collected larvae to carry them through to full development. • Provide pupation medium (sand or corrugated paper). • Collect adults on emergence after pupation. • Put adults in oviposition cage and provide young potted cowpea plants.
2. Collecting eggs	• Collect leaves with eggs from the young potted plant placed in the oviposition cages. • Carefully observe for eggs, often found along leaf veins and on tender stems. • Pluck leaflets off the main leaf and place in a petri dish to incubate. • The translucent eggs turn dark and hatch within 2–3 days.
3. Rearing of larvae	• Use transparent polyvinyl sandwich boxes 6.0 x 17.5 cm in size. Prepare the sandwich boxes by lining the floor with 18.5 cm filter paper and place a piece of nylon netting (15 mesh/ linear inch) with several holes (5 mm dia.) opened at regular intervals. • The filter paper absorbs some of the moisture from the flowers, thus preventing the flowers from sticking to the bottom of the box.

Steps	Key points
	• Place fifty flowers, each containing a larva on the netting.
	• Securely cover the top of the rearing box with fine white cotton cloth. This provides adequate aeration and completely eliminates the accumulation of moisture in the box.
	• Provide fresh food at 2-day intervals. This is done by lifting the larvae in the used food by holding the sides of the nylon mesh and placing them on top of the fresh food without touching them individually.
	• With minimum of turning of old flowers, 100% larval transfer from the old to the fresh food can be achieved.
	• Change the food 4 times before transferring the larvae in their pre-pupal stage to the pupation medium.
	• In absence of flowers, larvae can be fed on flower buds and pods.
4. Pupation and pupation medium (i) Sterile sand	• After 8 to 14 days larvae reach pre-pupal stage.
	• Place a layer of sterile sand about 2.5 cm in the rearing box.
	• Place the last food change on this wire mesh.
	• Slightly disturb the screen containing the old food and the larvae will move to the pupation medium.
	• Pre-pupal larvae will bury themselves in the sand and make cocoons about 0.5 cm under surface.
	• Collect pupae every 2 days by lifting the wire mesh from the sand and searching for the cocoons, which are normally covered with sand and easy to collect.

Steps	Key points
(ii) Corrugated paper	• When corrugated paper is used, the wire mesh is again placed 0.4 cm on the top of the paper, to avoid getting the medium mixed up with frass from the food. • To collect the pupae from the corrugated paper, cut off the insects from the paper. • After collection, transfer the pupae singly into sterile glass vials for adult emergence. • Pupation lasts between 5 and 14 days.
5. Oviposition cage and management	• Oviposition cage is a box 50 cm high, 35 cm wide and 45 cm long. • Three of the sides are covered with nylon mesh, while the fourth side with plywood, with a hole in the centre through which plant material or insects are introduced into the cage. • Moths emerging in the glass vials during the night are released into the rearing cage in the morning. • Each cage should hold about 30 pairs of adults. Crowding results in high mortality. • Newly emerged moths should be mated within 24 to 48 hours. • Adults are provided with 10% sucrose solution offered on cotton wool in petri dishes.
6. Sterilisation of laboratory and rearing apparatus	• At the end of every working day swab the laboratory floor and working tables with 0.5% sodium hypochlorite solution. • All the glassware are sterilised in the oven at 150°C; or steamed over moist sand on gas cooker.

Steps	Key points
	The apparatus that cannot be steamed is sterilised by immersing in 5% sodium hydroxide overnight and then rinsed several times in clean distilled water or boiled water.

Conclusion

This technique, though laborious, is sure to produce the required results. If labour is available, large numbers of insects can be produced as required. When using the natural food for the insect, there is virtually no fear of behavioural changes in the insect. However, as a precaution, it is recommended that from time to time there should be introduction of new genetic materials from the wild. This avoids loss of vigour due to inbreeding in the laboratory.

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Standard Rearing Procedures for the Maize Stemborer *Busseola fusca*

F. O. Onyango and A. G. Nyangwara

Insect and Animal Breeding Unit (IABU),
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Abstract

Busseola fusca ***has been found to be a widespread and important pest of sorghum and maize in many countries of tropical Africa. However, little detailed research has been done on this species in the recent past. The intervening larval diapause and the lack of artificial diet have been major constraints in the laboratory rearing. These have hindered the much needed progress in*** **B. fusca** ***research.***

Methodology for the continuous and sustained rearing of **B. fusca** ***on artificial diet is described. A total of 15 successive generations have been reared using this method and diet without loss of vigour or reproductivity. Rearing of more advanced generations continues.***

Detailed step-wise description of preparation of maize and sorghum leaf powder, bean powder, artificial medium, and management of various metamorphic stages are provided.

Introduction

Busseola fusca has been recognised as a major pest of maize and sorghum in many countries of tropical Africa (Harris 1989). Young larvae of the maize stemborer cause damage to the leaves, while older larvae bore in the main stem and cob (de Pury 1968). In severe attack, the central shoot withers, causing a typical "dead heart". These lead to reduced yields (Swaine 1957).

Rearing *Busseola fusca*

Although *B. fusca* is a widespread and important pest, little detailed research has been done on this species in the recent years (Harris 1989). The intervening facultative larval diapause and the lack of rearing technique have been major constraints of laboratory rearing of this insect continuously.

The off-season development and productive biology of *B. fusca* have been reported by Unnithan, (1987). Five successive generations were reared on sorghum shoot without any intervening larval diapause. However, rearing on host plant on a large scale was found to be laborious, time consuming, inefficient, and largely impractical.

Therefore, it was vital to develop a suitable medium and rearing technique for continuous and sustained production of *B. fusca* in the laboratory. A total of 15 successive generations have been reared using the method and the diet without loss of vigour or reproductive fitness. Rearing of more advanced generations continues.

Less than 5% of the larvae diapause on this diet per generation. The detailed step-wise procedures for medium preparation, processing of plant factors, management of adults, eggs, larvae, and pupae, are described. Research on the improvement of the artificial medium and more efficient rearing techniques are in progress.

Artificial Diet

Steps	Key points		
1. Preparing the artificial diet	•	Prepare the following ingredients. The quantity listed is sufficient for 1.6 litres of diet.	
	A.	Distilled water	800.0 ml
		Beans powder	175.0 g
		Brewers' yeast	45.0 g
		Sorbic acid	1.3 g
		Methyl p-hydroxy-benzoate	2.0 g

Steps	Key points		
		Ascorbic acid	5.0 g
		Vit.E capsules (250–300 i.u.)	4.2 g
		Maize or sorghum leaf powder	50.0 g
		Sucrose	140.0 g
	B.	Distilled water	800.0 ml
		Agar powder	25.0 g
	C.	Formaldehyde 40%	2.0 ml
	• Boil distilled water for blending in a saucepan and cool to 60–70°C.		
	• Weigh the ingredients of fraction A and pre-mix in a saucepan.		
	• Weigh agar separately.		
	• Pour distilled water for blending into the blender and add pre-mixed ingredients of fraction A.		
	• Blend the concoction for 3 minutes.		
	• In the meantime, put agar in a clean saucepan containing water for agar and stir the suspension periodically on a hot plate until it boils, then cool to 60–70°C.		
	• Pour the warm agar into the blender and mix together with the ingredients of fraction A for another 3 minutes.		
	• Lastly, add formaldehyde 40% to the mixture and blend with the rest of the ingredients for a further 1 minute.		

Steps	Key points
	• Dispense the diet to a depth of 3.0 cm per vial using a ketchup dispenser or alternatively, dispense the diet in lunch boxes to a depth of 3 cm.
	• Cover the vials or lunch boxes containing the diet with clean cloth or tissue paper and allow the diet to set at room temperature for at least 12 hours.
2. Preparing sorghum/maize leaf powder	• Harvest maize (Inbred A) or sorghum (Serena) leaves 6 weeks after planting.
	• Wash leaves with running tap water and drip-dry.
	• Place leaves in thin layer in the oven at 60°C for 12–24 hours until brittle.
	• Chop leaves to small pieces approximately 1.0 cm^2 using a pair of secateurs.
	• Grind the leaves immediately to a very fine powder using an electric grinder.
	• Store leaf powder in a plastic container with lid in a cool dry place for use as and when required.
3. Preparing bean powder	• Obtain beans (*Phaseolus vulgaris*) from the local market.
	• Wash the beans thoroughly with tap water.
	• Drip-dry the beans in wire mesh tray.
	• Place beans in oven at 60°C for 12–24 hours until thoroughly dry.
	• Grind beans immediately in an electric grinder to a fine powder.

Steps	Key points
	• Store bean powder in a plastic container with lid in a cool dry place for use as and when required.
4. Adult management	• Use group oviposition units made of metal frames 30 cm L x 30 cm W x 45 cm H.
	• Clean and disinfect the cage with detergent and bleach solution respectively.
	• Place four mature sorghum stems with intact leaf sheaths diagonally in the cage, each stem approximately 45 cm long.
	• Place a filter paper wick dipped in 10% sucrose solution in a 25 ml plastic cup in the cage to provide the diet for the moths. Alternatively, use cotton wool soaked in sucrose solution in a petri dish.
	• Change the sucrose solution daily.
	• Collect adults each morning from the cage and determine their sex by sexual dimorphism in antennae. Females are filiform (threadlike) while males are pectinate (comblike).
	• Label the cages accurately with the date of adult emergence and generation number.
5. Egg management	• Remove the sorghum stems from the oviposition cage and check for eggs daily.
	• *B. fusca* eggs are found sandwiched between the leaf sheath and the main stem laid singly in vertical rows.
	• Carefully cut out the portion of the leaf sheath containing the egg cluster with razor or scalpel blade.

Steps	Key points
	• Dislodge the eggs from the leaf sheath into a beaker.
	• Sterilise the eggs using 1% sodium hypochlorite solution for 10 minutes. Add a few drops of Teepol detergent to allow the eggs to sink to the bottom of the solution.
	• Rinse the eggs with 5 washings of distilled water.
	• Dry the eggs between two filter papers.
	• Place the eggs in a glass vial 7.5 cm long x 3.5 cm diameter whose bottom is lined with a moist filter paper.
	• Cover the vial with tight fitting cotton wool wrapped in tissue paper.
	• Incubate the eggs at 27±1°C and 70–80% r.h.
	• Replace fresh sorghum stems with sheaths into the oviposition cage daily after collecting eggs.
	• Replace a fresh set of 10% sucrose solution daily after collecting eggs.
	• Moisten the filter paper at the base of the incubation vial with just enough moisture daily to prevent desiccation of the eggs.
6. Larval management	• Expect eggs to start hatching 6 days after oviposition.
	• If the artificial diet was dispensed in lunch boxes then:
	– Using forefinger and thumb, grip the bottom of the sterilised rearing glass vial 7.5 cm x 3.5 cm diameter and push the open end into the diet contained in the lunch box.

Steps	Key points
	– Withdraw the vial with a plug of diet and repeat with as many vials. – Push the diet to the bottom of the glass vial with a sterilised glass rod 15 cm long x 0.6 cm diameter. • If the diet was dispensed directly in the glass vials using a ketchup dispenser, then: – Move to the next step. • Wipe the working bench top with 70% alcohol and allow to dry. • Dip camel hair brush in 70% alcohol, rinse in distilled water, then mop the brush on a clean paper towel. • Transfer one neonate larva per vial carefully onto the diet, care being taken to avoid the contact of the brush with diet. • Cover each vial with a tight-fitting cotton wool immediately and place upright in rearing trays. • Label each tray with date of inoculation and generation number. • Place the trays with vials on shelves at 27 ± 1°C and 60–70% r.h. and wait for the larvae to pupate. • The diet should never be allowed to dry. In case they do, change the larvae to fresh diet. • Any contaminated vial should be discarded.
7. Pupal management	• Start checking for pupae 30 days after inoculation of neonate larvae.

Steps	Key points
	• Collect pupae on a weekly basis and place in adult emergence cage made of perspex (30 cm L x 30 cm W x 30 cm H) whose floor is lined with moist paper towel.
	• Moisten the paper towel regularly to prevent desiccation of pupae.
	• Expect adults to emerge approximately 15 days from the date of pupation.
	• Approximately 5% or less of the larvae enter diapause on this artificial medium per generation. Diapausing larvae appear dirty white in colour while non-diapausing larvae are pale grey or pink.

Conclusion

The method described has been used to rear *B. fusca* continuously in the laboratory for 15 successive generations even during the off season when the wild population are on diapause in the field. Less than 5% of the larvae would normally enter diapause per generation.

The colonisation and rearing of *B. fusca* has facilitated a regular supply of larvae and pupae throughout the year for studies on host-plant resistance, efficacy of natural enemies, and for the elucidation of the nature and causes of induction and termination of diapause in *B. fusca*.

The individual vial rearing technique, diet infestation using neonate larvae, and egg collection methods are rather laborious and time consuming. Better and more efficient procedures for carrying out these operations need to be developed.

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Rearing the Cotton Bollworm, *Heliothis armigera*

Rami Kfir

Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa

Abstract

A simple method of rearing the polyphagous bollworm,* Heliothis armigera, *on artificial diet is described.

Introduction

The bollworm, *Heliothis armigera* (Hubner) (Lepidoptera : Noctuidae), is distributed all over Africa, southern Europe, the Middle East, India, Central and Southeast Asia, Japan, the Philippines, Indonesia, New Guinea, Australia, New Zealand and some Pacific islands (Annecke and Moran 1982). *H. armigera* is probably the most polyphagous and injurious pest of agriculture and home gardens in South Africa. It prefers flower buds, flowers and developing fruit and attacks peas, beans, cotton, maize, grain sorghum, wheat, oats, barley, sunflower, tobacco, citrus, cucurbits, potato, tomato, lucerne, gooseberry, chickpea, groundnuts and many garden ornaments (Annecke and Moran 1982). Because of its importance as an agricultural pest *H. armigera* was the subject of intensive studies in South Africa and a reliable supply of insects was required. Hence, a laboratory culture was established.

If a new culture has to be started from field collected material, the preferred stage is gravid females which can be collected with a light trap. In this way there is a better chance of avoiding disease and parasitoids than if eggs or larvae are used. The culture has been continuously maintained in the laboratory in Pretoria without augmenting the colony, for 16 years.

Several rearing methods have been used successfully to rear *H. armigera*. The method described here is simple and can be used by other laboratories wishing to rear about 2,000 insects per week. If more insects are required it is recommended that they look at an automatic medium pouring machine and the use of multicell rearing units.

Rearing Procedure

The rearing insectary is kept under natural photoperiod and at 26 ± 1°C and 50 ± 10% r.h.

About 15 male and 15 female pupae or the same number of moths are placed in 5 litre perspex oviposition jars. The separation of the sexes in the pupae can be done using Paul *et al.* (1979) criteria, and the brownish female moths can be easily separated from the greenish males. The oviposition jars contain a layer of vermiculite in which the pupae are buried and a petri dish with cotton wool soaked with 7% sugar sucrose solution to feed the moths. After mating the female moths deposit their eggs at night and early morning on the cloth-netting lids of the cages which are collected daily. The netting with the eggs are disinfected by soaking them for 10 minutes in 2% formaldehyde. The netting is then rinsed in running tap water

Table 1. Composition of an artificial diet for mass rearing *Heliothis armigera* larvae in the laboratory

Ingredient	Quantity
White kidney beans*	720.0 g
Brewers' yeast	96.0 g
Methyl 4-hydroxybenzoate	6.0 g
Ascorbic acid	9.6 g
Sorbic acid	4.2 g
Formaldehyde	2.2 ml
Distilled water**	2,000.0 ml

* Powdered *Phaseolus vulgaris* L.

** The water is boiled with all ingredients stirred in it then left to cool for about 30 minutes and it is ready to use.

for about 15 min., drip-dried and placed in lidded glass jars for the eggs to hatch. The first instars are transferred singly to glass vials (10 x 2.5 cm) containing a modified Shorey and Hale (1965) larval diet (see Table 1). About 10 g of diet is placed into glass vials with a domestic cake syringe (13 mm nozzle) and plugged with cotton wool. The larvae develop normally and pupate without further attention. The larvae are highly cannibalistic; only one larvae can survive in a vial. This of course causes limitations on production and increases costs. The pupae are harvested daily and disinfected in 0.2% sodium hypochlorite (Van Der Walt 1988).

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Methods for the Rearing of the Banana Weevil, *Cosmopolites sordidus* (Germar 1824)

Albrecht M. Koppenhoefer

International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30, Mbita, South Nyanza, Kenya

Abstract

Banana weevils can be trapped from infested plantations and kept in cages on spent pseudostem or rhizomes. They need shelter and moisture and should be protected from high temperatures and predators. From one sucker exposed to 100 adults after one week more than 100 eggs can be collected within two hours. If left for six or eight weeks a similar amount of pupae or young weevils can be obtained within one hour. Per metre pseudostem exposed to 100 weevils after six weeks 50–70 larvae and 10 pupae can be collected in one to two hours.

Introduction

The banana weevil *Cosmopolites sordidus* (Germar) originates from South East Asia (Stover and Simmonds 1987) but has spread to almost all banana growing areas in the tropics and subtropics (Feakin 1975; Hill 1987). Although adult weevils can be kept on other plants e.g. potatoes, the development is restricted to the genera *Musa* and *Ensete* as host plants. The tunnelling of the larvae in the rhizome interferes with root and sucker initiation, the water and nutrient transport in the sucker and if the growing point is damaged, the whole sucker will die. Extensive tunnelling can cause snapping of the rhizome at ground level due to wind or the weight of the bunch.

The eggs are laid singly in the rhizome and the base of the pseudostem in 2 mm deep holes eaten with the rostrum or directly in cracks and depressions. The larvae

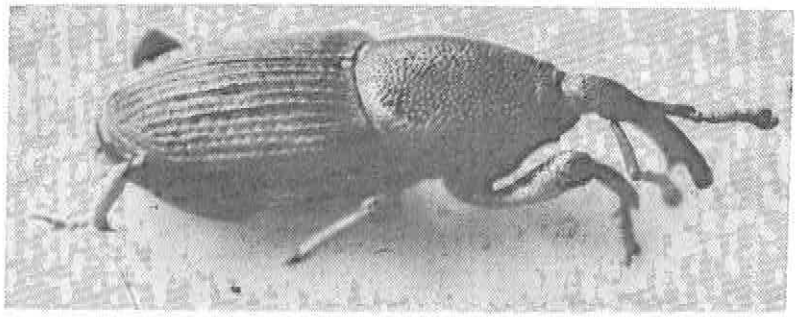

Fig. 1. Banana weevil

hatch after 4–7 days and tunnel preferably through the cortical tissue of the rhizome. After usually 6 larval instars and 3–6 weeks, the pupation occurs in a pupal chamber eaten by the last instar larva near the rhizomes surface. The 8–16 mm long adults emerge after 4–7 days but stay in the chamber for 3 to 6 more weeks until their cuticle is fully sclerotised. 1–2 weeks after emerging from the pupal chamber, the females start laying eggs. Though the adults can live longer than two years, the females are said to lay not more than 100 eggs in total (Frogatt 1924; Beccari 1967; Cuillé 1950). The weevil is active between 15°C and 30°C, but its temperature optimum is 23–26°C. Temperatures above 40–45°C are lethal (Cuillé and Vilardebo 1963). Until now no artificial diet has been developed for the banana weevil but large quantities of all life stages can be reared on its natural diet, the rhizome and pseudostem.

Rearing Methods

The rearing should be done at average temperatures between 23°C and 28°C with night temperatures not lower than 20°C. Lower temperatures will decrease the number of eggs/female and increase the length of the developmental period.

A. Establishment of a Colony

1. Collecting Initial Weevil Population

- Set traps in an infested plantation at the foot of the stools. Traps are made from spent pseudostem: 1. 40–60 cm long piece split lengthwise through the centre with the cut surface on the ground (split-pseudostem-trap), 2. disc put on top of a pseudostem cut near ground level (disc-on-stump-trap).

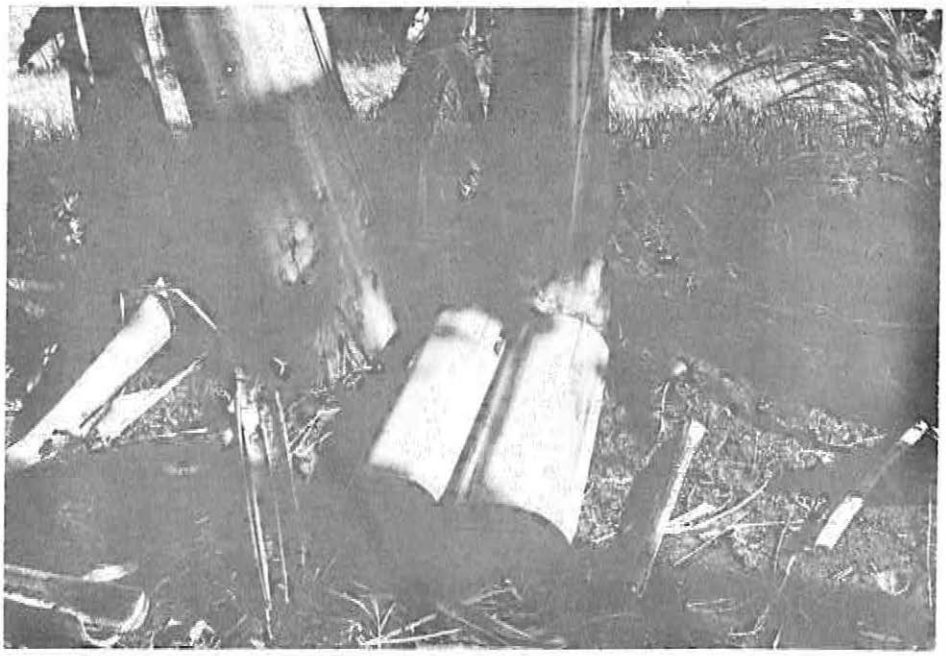

Fig. 2. Split-pseudostem-trap

Traps are most attractive during 1st week but weevils can still be collected after several weeks. Pieces of rhizome are more attractive but dry out faster.

2. Colony on Pseudostem

- Put pseudostem pieces (0.5–1 m) in cages covered with a mesh. Bigger cages (2 x 2 x 0.5 m) are more convenient because old rotten pseudostems can be left until they are no more attractive to weevils while fresh ones can already be added. This will save time when collecting the adults from the removed material.

- Apply superficial cuts to pseudostems and add banana mulch and/or moistened soil to provide weevils with shelter and moisture. Make sure that cages are not too much exposed to direct sunlight and rains. They are very susceptible to high temperatures.

- Release 50–100 weevils per metre pseudostem.

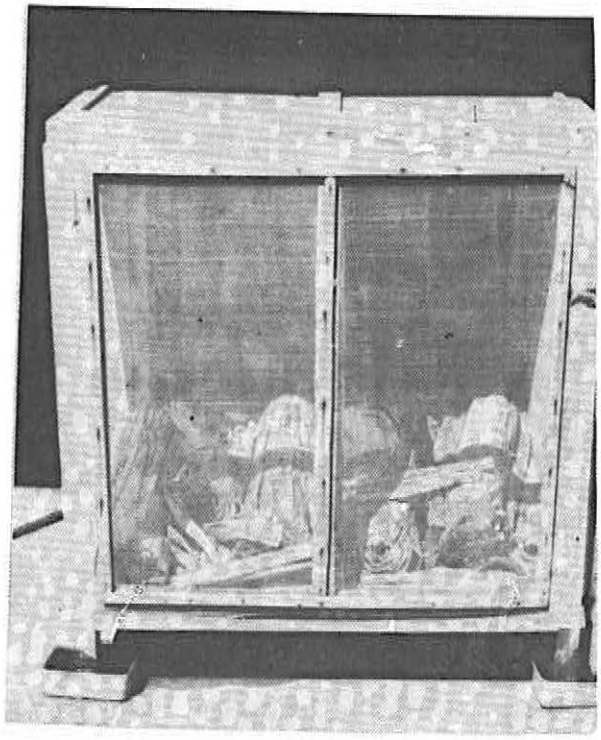

Fig. 3. Rearing cage

- Protect cages from ants.
- Remove old pseudostems when they are completely rotten or dry (approx. 10 weeks) and add fresh ones accordingly.

3. Colony on Rhizomes

- Choose a susceptible cultivar and uproot rhizomes of harvested or younger suckers and clear them from soil and roots. Remove the aerial part of the young suckers including the growing point.
- Place the rhizomes in buckets on a 1–2 cm layer of moistened soil. As under point 2, bigger containers taking more rhizomes are more convenient.
- Release 10–20 weevils per 1 kg rhizome and cover containers with a mosquito net.

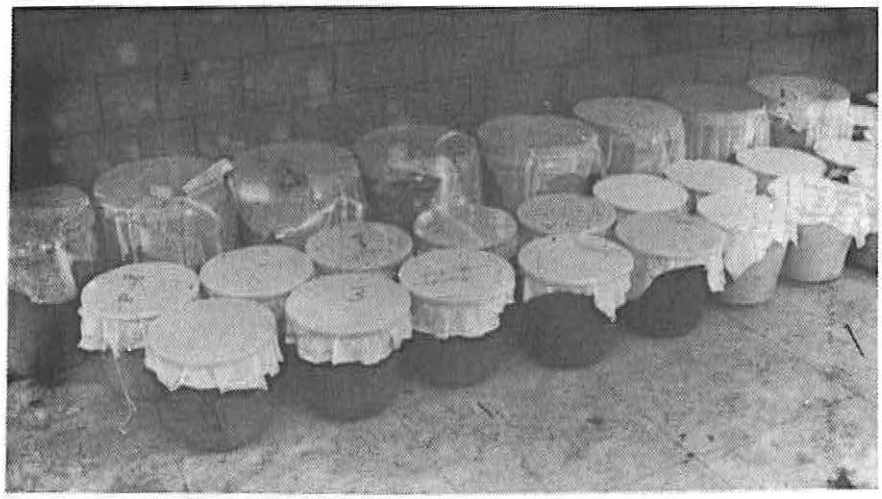

Fig. 4. Rearing buckets with young suckers (front) and spent rhizomes (background)

- Protect containers from direct sunlight and rain but make sure that soil is always moist.

- Replace exhausted rhizomes with fresh ones but remove weevils from the old material.

B. Production of the Various Life Stages

1. Collecting Weevils from Colonies

- Put traps (fresh pieces of pseudostem or rhizome) overnight or longer and collect the adults. To collect the weevils more thoroughly the old material has to be dissected.

2. Sexing Adult Weevils

- The inclination angle of the last (9th) abdominal segment is usually bigger in males. In addition the ventral surface of this segment is rather straight in females while exhibiting a downward curve in males.

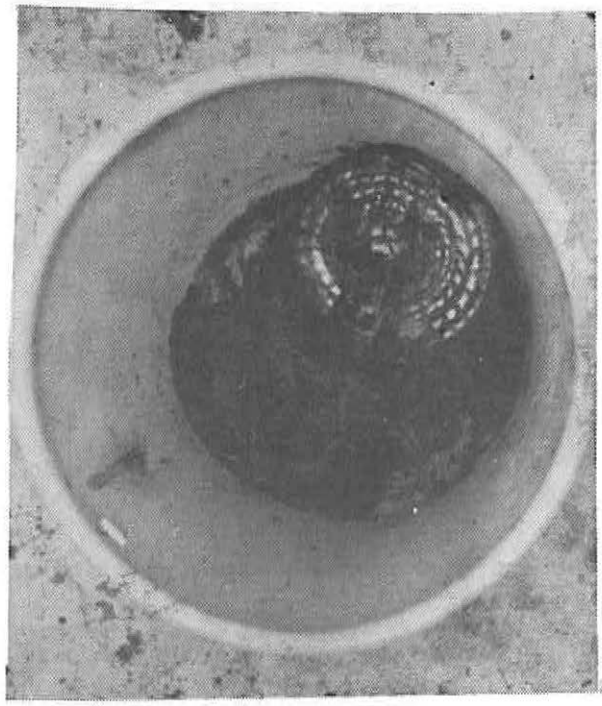

Fig. 5. Rhizome portion, a sucker on moist layer of soil inside rearing bucket

- The punctuation of the cuticle on the dorsal side of the rostrum in males extends downwards at least to the rostrum's middle (between the point of insertion of the antennae to its tip). In females the punctuation usually does not reach the middle (Longoria 1968).

3. *Production of Eggs*

- Put the rhizome of a sucker (prepared as under point a.3.) on a 1–2 cm layer of moistened soil in a 20-litre bucket. The rhizome should weigh 2.5–4 kg.
- Release 50 male and 50 female weevils and cover with a mosquito net.
- After one week remove rhizome and peel off its surface not thicker than 1 mm with a very sharp, preferably round-tipped knife (pen-knife).

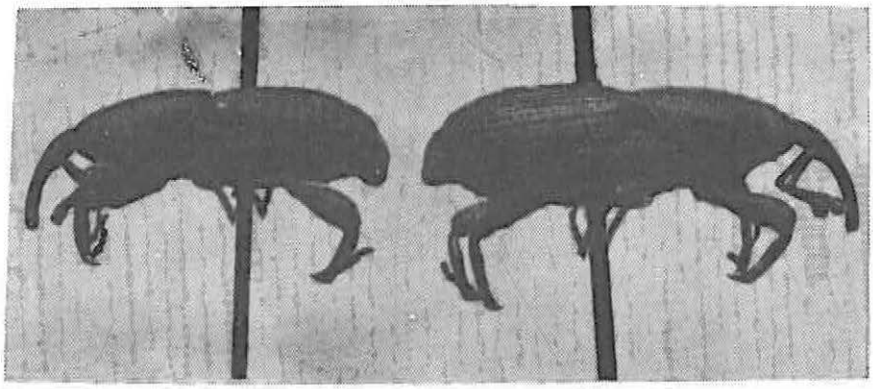

Fig. 6. Sexing of adults: Last abdominal segment: male left, female right

- Collect the exposed eggs with a camel hair brush on wet filter paper.
- Dissection takes about two hours and 1–4 eggs/female released can be collected (mean: 2.5 eggs/female).
- The rhizome can be used a second time.

4. *Production of Pupae or Adults*

- The procedure is the same as for egg production but the weevils are removed after one week.
- Leave rhizome for 6–7 weeks if prepupae and pupae are to be obtained.
- Leave rhizome for 8–9 weeks if adults are needed.
- Dissect carefully with a knife or use hands if material is very exhausted and soft.
- Dissection takes one hour and on average 100 weevils can be collected per sucker.

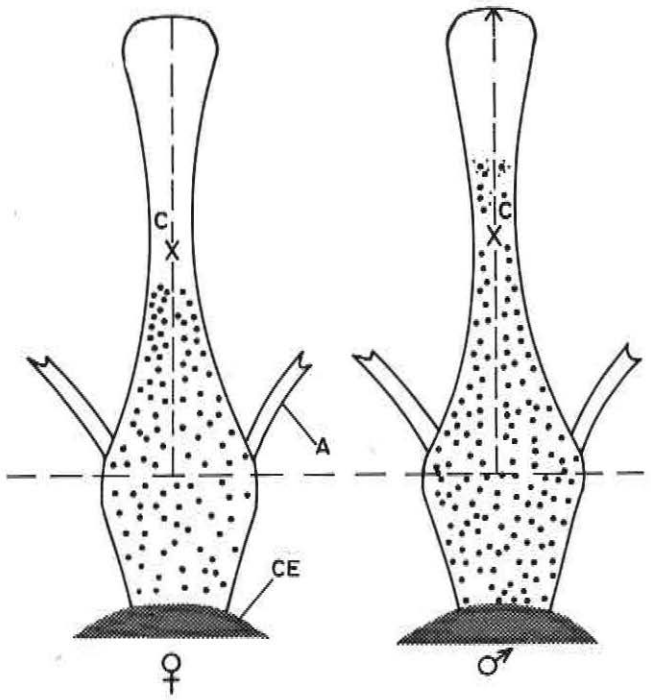

Fig. 7. Sexing of adults: punctuation of rostrum: female left, male right

5. Production of Larvae

- Prepare pseudostems as under point a.2. and release 100 weevils per metre pseudostem.
- After 6 weeks the pseudostems are dissected with a knife splitting the leaf sheaths parallel to their surfaces thus exposing the larvae in their tunnels.
- To prevent cannibalism they are collected singly in 2.5 cm long pieces of hose pipe glued on trays. Add some rhizome or pseudostem material.
- Dissection takes 1–2 hours per metre pseudostem and gives 50–70 larvae of all stages and about 10 pupae.

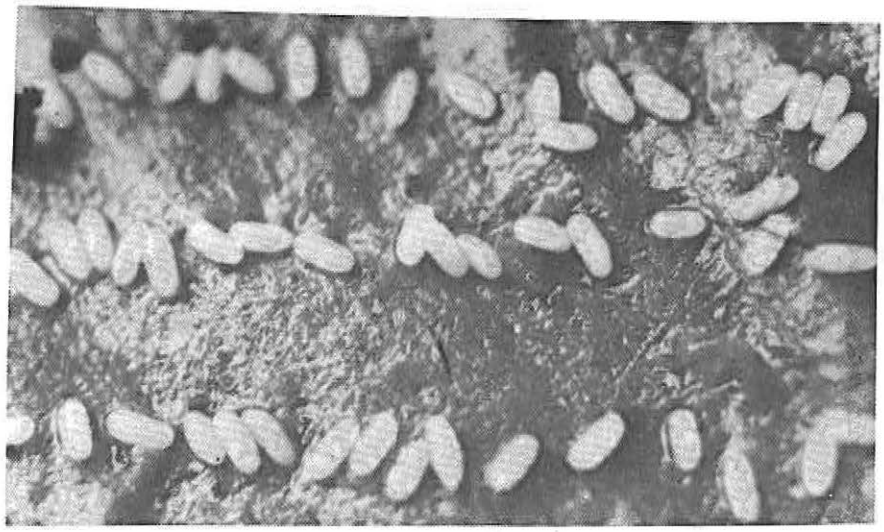

Fig. 8. Banana weevil eggs collected on wet filter paper

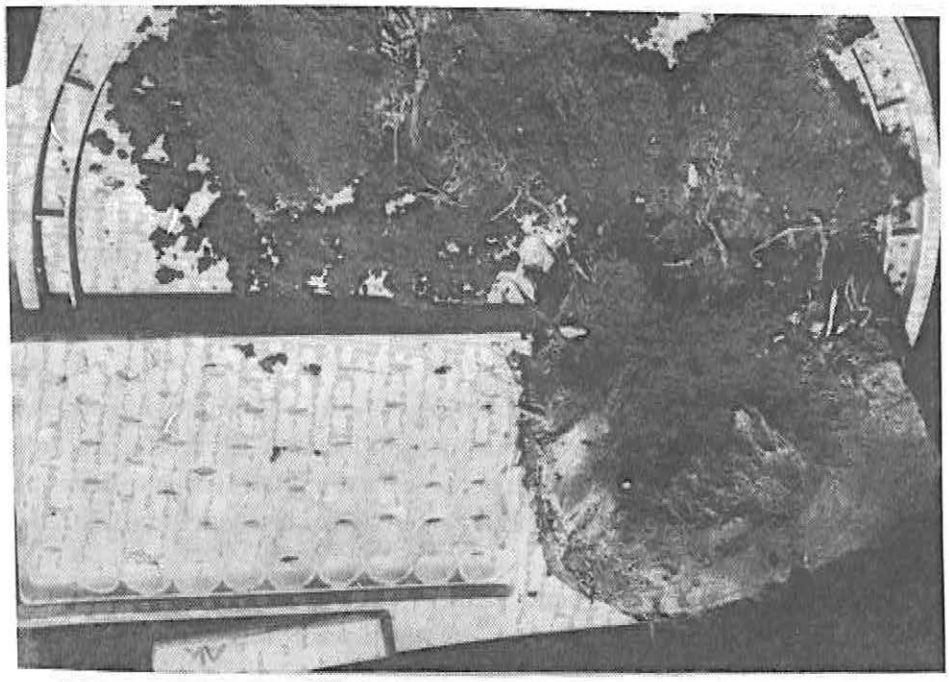

Fig. 9. Dissecting a rhizome 6 weeks after infestation. Tray with hosepipe pieces for collection of larvae

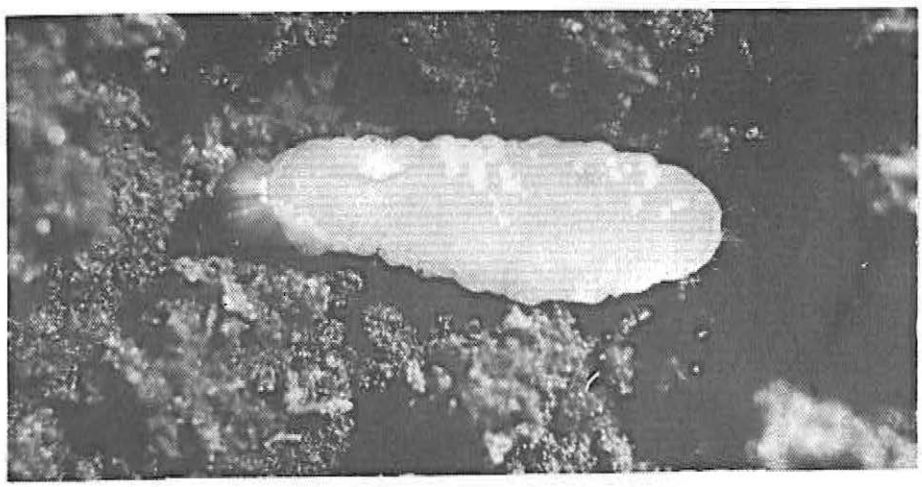

Fig. 10. Banana weevil larva

Conclusions

The rearing of the banana weevil and the production of its various life stages on their natural diet is rather simple because the rearing cannot easily be affected by diseases or parasites. Yet the containers have to be sealed to prevent predators like ants or hydrophilid beetles (e.g. *Dactylosternum abdominale*) from invading them. For artificial infestation experiments, eggs are more reliable than larvae, especially 1st instars because the latter are far more delicate and likely to succumb due to the handling. The hatchability is higher in the more developed eggs (where both ends turn transparent) with >90% while being 75% in eggs which are still of a homogeneous ivory colour.

For multiplication purposes the rearing should be done on rhizomes, which is far more effective than the method using pseudostems. Yet for the production of larvae, the latter is more convenient because the material is easier to dissect. When dissecting the tough rhizomes a high percentage of larvae will be injured.

Under point b.5. the number of adult weevils released per metre pseudostem as well as the time of exposure can be changed according to the purpose. If e.g. only younger larvae are to be produced, the number of adults can be increased and the material has to be dissected accordingly earlier. If only older larvae are needed the higher number of adults should be removed from the material using traps. In the methods using rhizomes for the production of eggs (point b.3.), pupae or

adults (point b.4.) the number of adults released cannot be increased much because a crowding effect will decrease the number of eggs laid per female.

Acknowledgements

The various methods presented were developed during research done on different aspects of the banana weevil in the ICIPE-Banana Project which was funded by the Federal Ministry for Economic Collaboration (BMZ) of the Federal Republic of Germany.

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Techniques for Rearing the Desert Locust, *Schistocerca gregaria* (Forsk.)

S. M. Ndugo

Insect and Animal Breeding Unit,
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Abstract

Techniques used in the ICIPE for rearing the desert locust are described in this paper. It is necessary to bear in mind that many other rearing techniques have been used worldwide for rearing the same. Techniques used for rearing the isolated locust have also been described.

To rear the gregarious desert locust a suitable cage is used and the insects are reared in crowded conditions, i.e., more than one insect per cage. On the other hand solitary locusts are reared in individual cages whereby each locust is isolated from the rest from visual and body contact.

The locust's phase is judged by its morphometrics for the time being.

Introduction

The desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) is widely distributed covering the area between Iran in the north to Kenya in the south and between West Africa and India to the east during outbreak periods. It exhibits phase polymorphism existing as gregarious phase in high densities and solitary phase when in low densities.

Rearing the Desert Locust

Due to its polyphagous nature, the desert locust has been easily reared on different diets consisting of green feed (shoots) and dry feed e.g. wheat shoots and wheat bran, sorghum shoots and wheat bran, lettuce and wheat bran, cabbages and wheat bran, other grasses and wheat bran etc.

The three very crucial factors in locust rearing include: adequate and controlled temperature, correct feed and general hygiene. Temperature on its part determines the duration of life cycle, i.e., can hasten or slow development. The correct feed means feeding the right type of plant at the right stage because some plants and some stages of certain plants might have some antifeedant or repellant chemicals which might affect growth and development of the locust. Hygiene on the other hand might determine the health of a colony e.g. high infections can bring about the following: increased mortality, reduced fecundity and can slow down a colony or wipe it out.

Rearing Method

Steps	Key points
1. Starting an initial population	• Catching gregarious or solitary individuals in the field and putting them in a portable cage (Fig. 1) ready for transportation or • Getting egg pods from an established colony then packing well for transportation. • If adult individual is from the field, set up a rearing cage and introduce them into it. • Provide them with feed. • After mating, provide them with laying sand for egg collection. • Collect as many egg pods as possible and incubate at 30–32°C to start the 1st generation. • If egg pods are from an established colony, then incubate the eggs at 30–32°C.

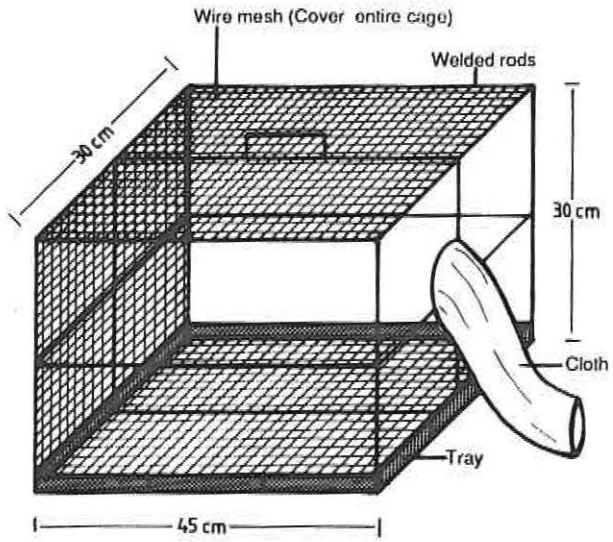

Fig. 1. A portable cage

Steps	Key points
	• The hatchlings should be set up in clean cages placed in equally clean rooms.
2. Rearing room setting conditions	• Good air circulation = 10 air changes per hour (ducting system). • Low relative humidity = 40–50%. • Temperature—28°C–37°C. • Photoperiod—12 hrs light, 12 hrs dark. • Artificial lighting—40 watt bulbs for crowded and 25 watt bulbs for solitary. • Thermostatically controlled heaters to maintain required temperature.

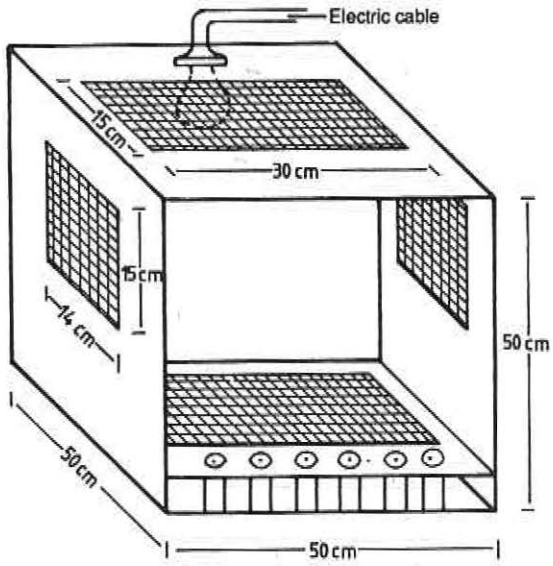

Fig. 2. Crowded locust cage

Steps	Key points
	• Humidifier to boost humidity when very low. • Thermohygrograph for temperature and r.h. records. • Max. and min. thermometer.
3. Rearing cage for crowded colony	• An aluminium cage of 50 x 50 x 50 cm (Fig. 2). • A removable front perspex glass panel. • A fixed roof with hole for light bulb fitting. • A sliding door on side of cage. • A perforated false floor.

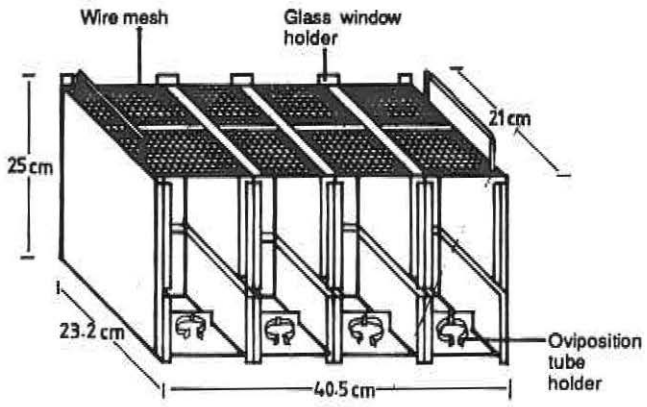

Fig. 3. Solitary locust cage

Steps	Key points
	• 3.5 cm diameter holes in the false floor for holding egg tubes.
	• A swinging metal strip to cover the holes while not in use.
	• Perforated ventilation panels on one side and roof.
	• A false bottom for droppings collection.
	• A perch.
4. Rearing cage for isolated colony	• Compartments of 10 x 10 x 24 cm held in a bank of 8 (Fig. 3).
	• A trolley with lamp holders (Fig. 4).
	• A removable glass front for each compartment.
	• A false perspex floor with a hole (3.5 cm) for oviposition purpose.

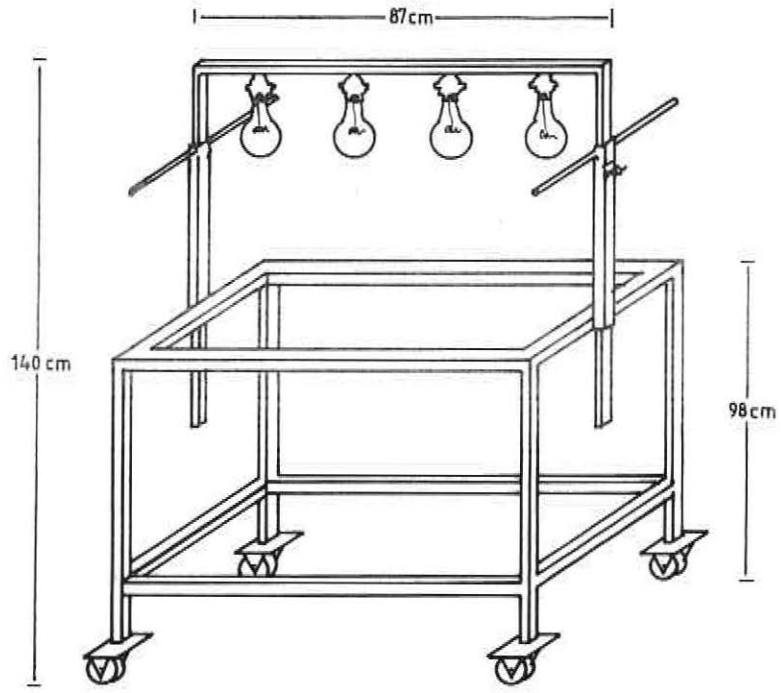

Fig. 4. Trolley for solitary locust cage

Steps	Key points
	• A feed vial holder.
	• An egg tube holder below the floor.
	• A tray for holding the banks onto trolleys.
	• Perforated roof for ventilation.
	• Curtain to prevent individuals seeing one another.
	• A perch (Fig. 5).
	• Isolated locusts can also be reared in jars.

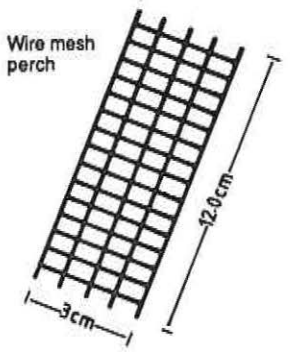

Fig. 5. Solitary locust perch

Steps	Key points
5. Setting up hatchlings in a crowded culture	• The cage should be thoroughly cleaned with a detergent and a suitable disinfectant e.g. sodium hypochlorite solution. • All spaces between joints to be blocked with cotton wool to avoid escape. • Provide a perch. • Provide fresh green shoots of sorghum held in a pot. • Provide wheat bran in a petri dish. • Provide a water swab to boost the humidity in cage. • Put the tubes with hatchlings in the cage and uncover the tubes. • Leave the tubes in the cage till all nymphs have emerged.
6. Setting up hatchlings in an isolated culture	• The cages should be thoroughly cleaned and sterilised in the oven (Fig. 6).

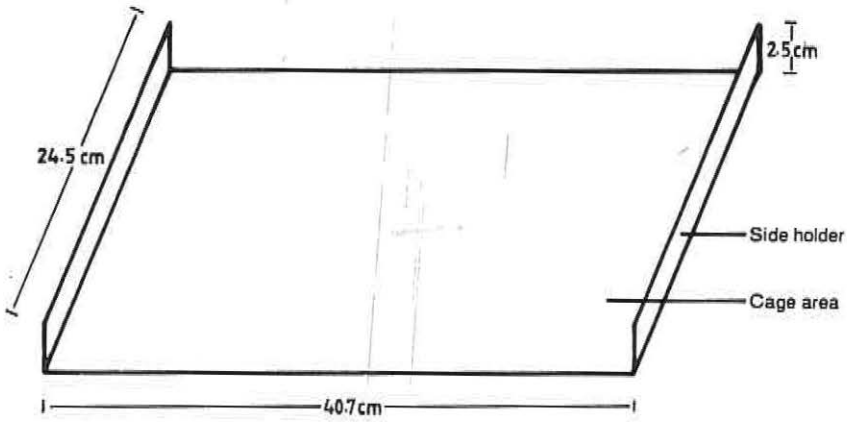

Fig. 6. Solitary locust cage tray

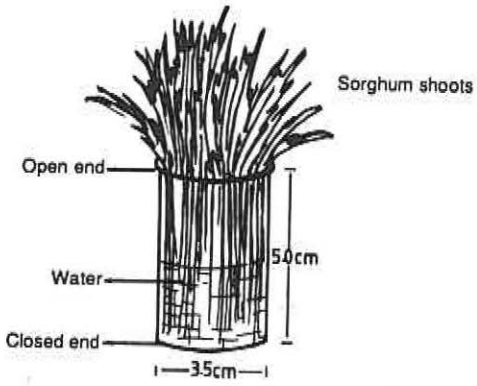

Fig. 7. Solitary locust green feed vial

Steps	Key points
	• Green feed is provided in a feed vial (Fig. 7).
	• Dry feed is provided in small containers.
	• All escape routes are blocked with cotton wool.

Steps		Key points
		• The hatchlings should be separated as soon as possible after hatching and each introduced to its own compartment.
7.	Feed preparation for the crowded culture	• Sorghum is sown in the garden or in pots and is used when over 3 weeks old. • The sorghum is uprooted and the roots cut using a knife or scissors. • The shoots are put in a pot and offered in the cage. • The green feed is changed every day. • Wheat bran is provided in a petri dish.
8.	Feed preparation for the isolated culture	• Young sorghum shoots (3 weeks old) are cut from the garden. • The sorghum is cleaned in water to remove any soil. • The shoots are dipped in feed vial containing water. • The feed vial with the shoots is clipped in the feed-vial holder in the compartment. This feed is enough to last for two days.
9.	Daily cage cleaning process	• All dead locusts are removed from cages. • A hand held hoover is needed to clean up all the feed debris and faecal material. • A small brush can help in the cleaning process.
10.	Major cage cleaning	• This is done when the cage is empty (Figs 8,9). • Solitary locust rearing cages are first soaked for a number of days in water with detergent and hypochlorite solution.

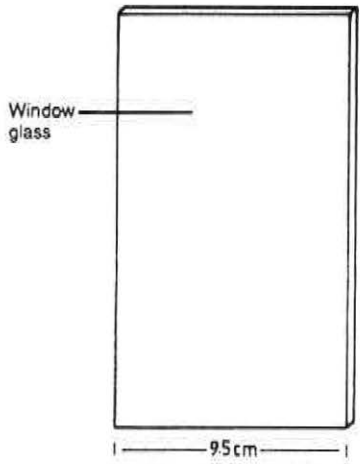

Fig. 8. Solitary locust cage front

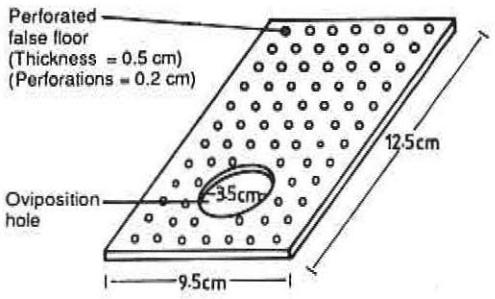

Fig. 9. Solitary locust cage floor

Steps	Key points
	• Major cleaning is done using a machine releasing pressurised steam through a nozzle.
	• Hand cleaning can also be done.

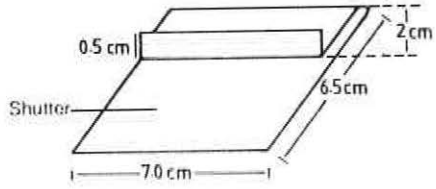

Fig. 10. Oviposition hole shutter

Steps	Key points
	• The crowded locust rearing cages are left to dry in the sun whereas the solitary rearing cages are sterilised at 150°C in the oven, including the front glasses and bottom trays.
11. Room cleaning	• The floor is swept daily after cage cleaning. • Benches are mopped with a wet cloth with some sodium hypochlorite solution. • The floor is washed with water and detergent at least twice a week.
12. Mating	• For the crowded culture mating occurs when adults reach maturity. • As for the isolated culture the male and female are brought together in one cage for 24 hours (when they are mature = 10 days after final moult) and then separated.
13. Egg collection	• Females should be supplied with sand to oviposit in after mating (Fig. 10). • Builder's sand is sieved to remove large particles.

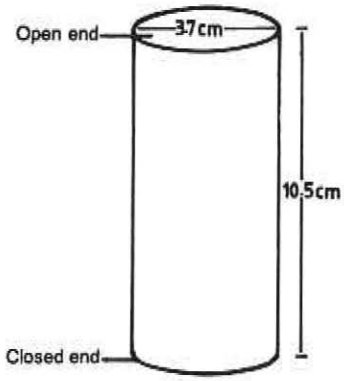

Fig. 11. Egg collection tube

Steps	Key points
	• The sand is cleaned with water to remove very fine dust particles. • The sand is dried and sterilised in oven at 150°C for over 2 hours. • The sand is allowed to cool to room temperature. • The sand is moistened at 15% water by volume (distilled water). • Sand is packed in clean and heat sterilised egg tubes (Fig. 11). • The sand should be packed to firmness. • The tubes are placed underneath the oviposition holes in the false floor.

Steps	Key points
14. Egg incubation	• Egg tubes underneath the false floor should be checked daily for any egg pods. • If a tube has egg pods, then the top 1 inch of sand is removed. • If the sand is too dry then a few drops of water is added. • The top of the tube is covered with a polythene paper fastened using a rubber band. • The covering paper should be perforated with a pin to allow for air exchange. • The tubes are put in a tray and incubated at 30°C – 32°C • After 12 days the tubes should be checked for any hatchlings. • When hatchlings are sighted, then a cage is set up as in 5 and 6 above.
15. Disease minimisation	• Restrict the entry to the insectary. • Quarantine any new locusts from the field or other insectaries. • Maintain a high degree of hygiene. • Treat the 2nd instars using a suitable bactericide drug e.g. Septrin. • 2.5% solution of Septrin is prepared and the green feed dipped in it before being fed to the locusts. A powder of the drug can also be administered through the wheat bran.

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G. Rearing Haematophagous Arthropods

Guidelines for the Rearing of Tsetse Flies Using the Membrane Feeding Technique

Udo Feldmann

Entomology Unit, Joint FAO/IAEA Programme
Agency's Agriculture Laboratory Seibersdorf
International Atomic Energy Agency, P.O. Box 100, A-1400 Vienna

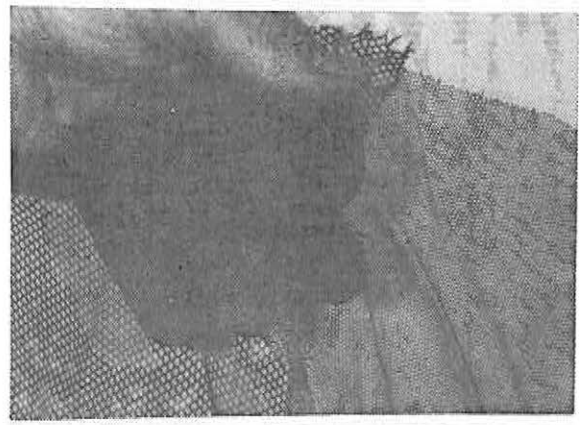
The laboratory rearing of tsetse flies originally depended on the availability of host animals for *in vivo* feeding (Nash *et al.* 1966, 1968; Jordan *et al.* 1967; Itard and Maillot 1970; Van der Vloedt 1982; Oladunmade *et al.* 1990). However, many factors associated with the maintenance of a host animal colony have an impact on fly colony performance. The frequent use of host animals as blood donors imposes the risk of over-challenging them. Some host animals develop adverse skin reactions from tsetse fly bites (Parker 1978); this limits the use of host animals considerably. Special care has to be taken to ensure that the animal feed is not deficient in proteins or essential amino acids. Drugs in the host animal feed, such as coccidiostats or antibiotics may adversely affect the fecundity of the female fly (Nogge 1976). Furthermore, there is a pertinent risk that host animal feed components may contain toxins, e.g. aflatoxin, if ground nut cake is used in the feed; or insecticides, if, for example the host diet contains cotton seed. In view of the above risks, some researchers in Africa import host animal feed from overseas, thus increasing the cost of tsetse fly rearing.

There is considerable demand for tsetse fly material of constant quality for basic and applied research as well as for sterile insect releases in the field. Appropriate use of the membrane (also called *in vitro*) feeding technique provides a means to produce tsetse flies more economically and with less risk.

1. Preparation of a Silicone Membrane

Silicone membranes used for the *in vitro* feeding of tsetse flies were first described by Bauer and Wetzel (1976). Todate the slightly modified process of preparation is as follows:

- Boil a 60 cm by 60 cm piece of terylene bobbinet to remove chemical residues of impregnation and dye. The netting determines the thickness of the membrane and provides reinforcement during later use. For tsetse fly species such as *G. tachinoides* or *G. austeni* thin netting of 130 – 150 µm thread diameter (62 holes per cm^2) is used; for other species such as *G. palpalis* ssp., *G. morsitans* ssp., *G. brevipalpis* , *G. pallidipes* or *G. fuscipes fuscipes*, a thicker bobbinet of 250–300 µm thread diameter (16 holes per cm^2) is recommended (Fig. 1)



- For the thicker membrane mix thoroughly (Fig. 2) 180 g silicone caoutchouc and 9 ml hardener (e.g. Wacker silicone RTV 3500 and hardener T35). For the thinner membrane use 150 g silicone and 7.5 ml hardener. Proper mixing is essential to ensure uniform consistency of the membrane.

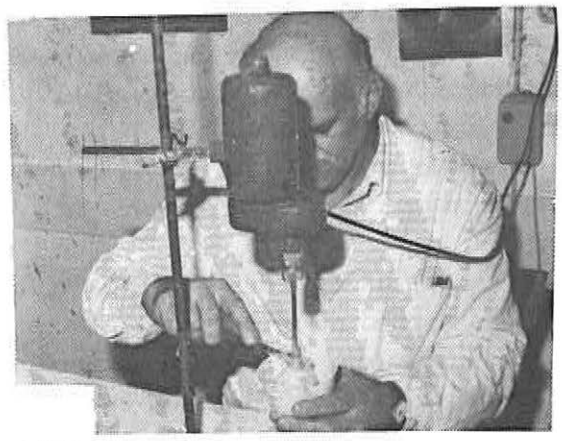


Fig. 2

- Place the dried netting onto a clean and smooth perspex matrix (Fig. 3). Add the silicone and hardener mixture and cover it with at least 0.3 mm

thick transparent polyethylene foil (Fig. 4).

- With a smooth plastic rod (4 – 7 cm thick and 70–80 cm long) evenly distribute the silicone mixture on the matrix by rolling the rod over the PE foil (Fig. 5). It takes some time and physical effort to achieve the desired result.

- Allow the silicone to harden at room temperature for 24 hours.

- Gently pull off the PE foil and then the membrane from the matrix (Fig. 6).

- Cut off surplus silicone and netting around the thick edge of the membrane (Fig. 7) and wash the membrane in hot water. Should the membrane not be used within the next few days, place the dry membrane between two layers of tissue paper and loosely roll it up for storage.

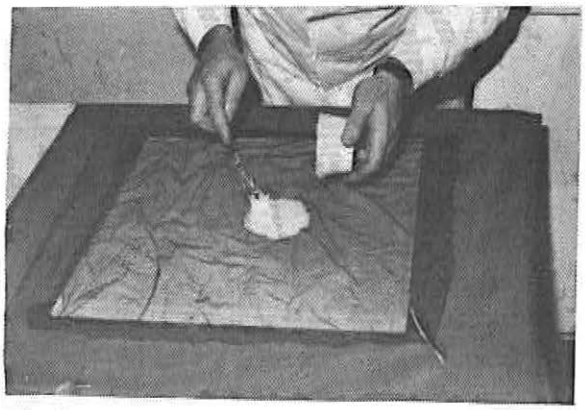


Fig. 3

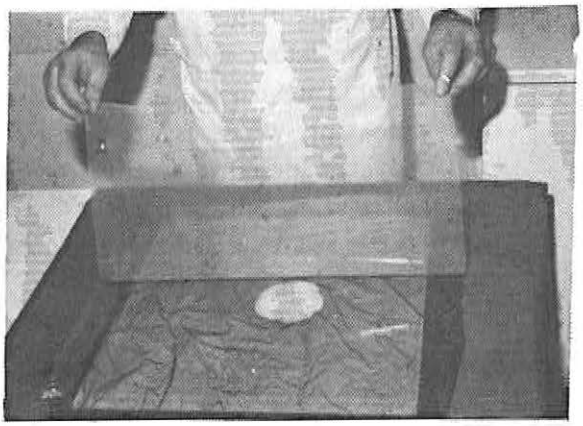


Fig. 4

Fig. 5

2. *In vitro* Feeding Unit

- It is necessary to warm up blood to body temperature before feeding to flies. Heating may be achieved with the aid of a 5–8 mm thick aluminum cover plate, at least of the size of the silicone membrane. This plate may be either heated from below by light bulbs in a metal box or by heating wires. Alternatively a specially designed PVC heating mat may be used for one or several feeding trays and membranes (Fig. 8). The heat production by all systems must be adjustable.

- An anodised aluminium feeding tray (1.2 mm thick, 48 x 48 cm^2, 3 mm "diamond"-shape surface (Fig. 9) or corrugated sheets, bent up 1 cm at all sides) is placed onto the heating unit to hold the blood that will be covered by the silicone membrane as artificial skin.

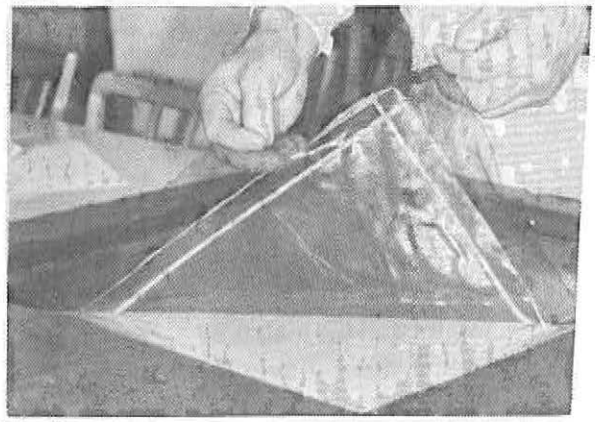


Fig. 6

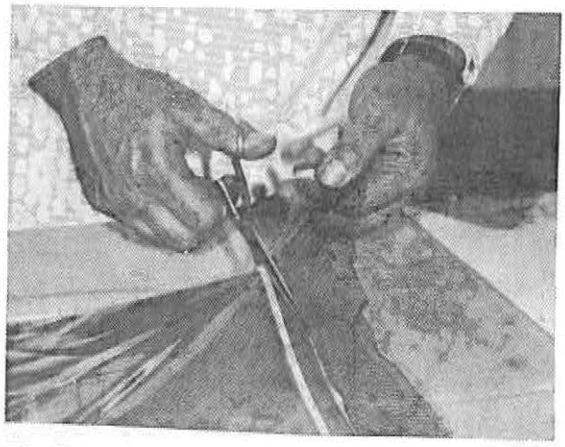


Fig. 7

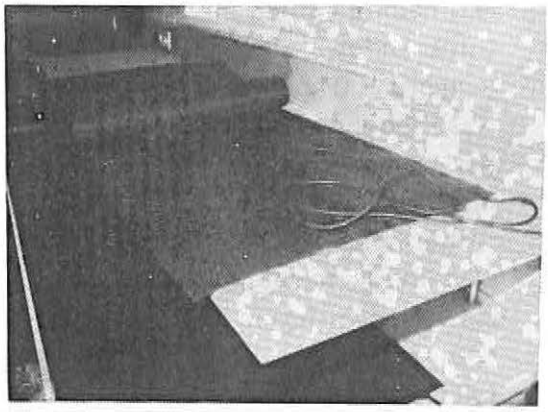


Fig. 8

- The feeding tray and the silicone membrane have to be thoroughly washed. Detergents must be removed by washing all materials and rinsing with distilled water. The membrane is placed onto a feeding tray. Overnight this tray-membrane set is sterilised in a dry heat of 120°C for use next day (Fig. 10). The feeding trays and the membranes are reusable. The silicone membranes last about one year if used daily.

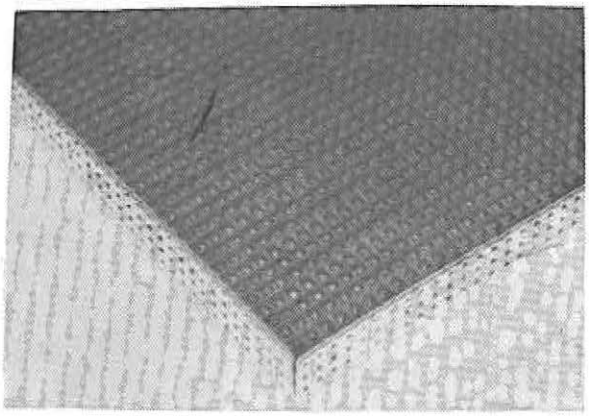


Fig. 9

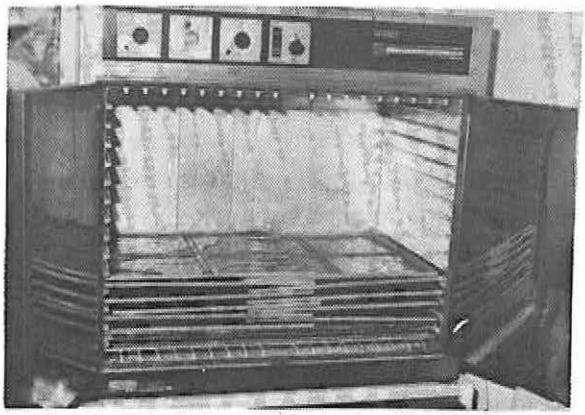


Fig. 10

3. Blood Collection and Diet Processing

The materials recommended for blood collection depend on blood consumption (colony size) and on a variety of local conditions, including access to a γ-radiation source. All records on blood collection, blood processing, etc. should be noted down in bound record books.

To ensure sterile collection of blood from cattle for a small group of experimental flies:

- Autoclave: (a) 500 ml blood transfusion bottles or a conical flask, covered with aluminum foil, and filled to one fourth with glass beads (5–8 mm diameter) (Fig. 11); (b) a 1 m long and 4 mm thick silicone hose, with hypodermic needles on both ends and a roll-clamp in the middle; (c) several 100–200 ml glass bottles. Furthermore autoclave standard nutrient agar and petri dishes.

- Select healthy looking cattle and use a tourniquet, a chain or fingers to block the flow of blood in the vena jugularis.

- Close the roll-clamp at the silicone hose and clean the skin at the blocked vena with 70% ethanol.

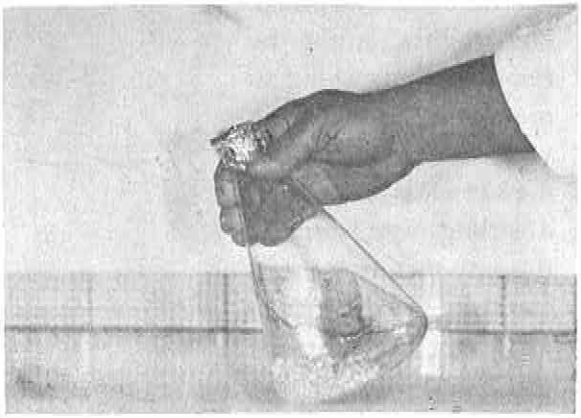


Fig. 11

- Insert one needle into the vena (Fig. 12), the other into the sterile glass flask and open the roll-clamp. Close the clamp when the bottle is filled and remove the needle from the bottle. If blood is still flowing, insert a needle into another evacuated bottle and open the roll-clamp again, etc. Do not collect too much blood from one animal and ensure that the wound stops bleeding after removal of the needle.

- Remove the needle from the flask immediately and shake or gently swirl the bottle with the blood and the glass-beads for 5 minutes to defibrinate the blood.

- Transfer the blood bottles into a sterile room or chamber or to a sterile laminar air flow bench (Fig. 13) and pour the blood under sterile conditions (flame the bottles, Fig. 14) into the smaller (100–200 ml) storage bottles. Half-fill the bottles, mark the collection date and bottle number and transfer about 1 ml of blood from each bottle to a sterile petri dish (Fig. 15). Mark the petri dish accordingly.

Fig. 12

- The closed blood bottles can be stored frozen until use.

- Take the autoclaved nutrient agar, still hot and liquid, and allow it to cool down to 35°C. Pour agar (shortly before hardening) into each of the petri dishes (Fig. 16). Then gently mix the blood with the agar (Fig. 17). In addition two bacteriological controls are run: one with 1 ml sterile distilled water instead of 1 ml blood, and the other with a plate of nutrient agar, which is left open under the flow bench for 1 minute to confirm the sterility of the working environment. Incubate the plates at 25–30°C for 48–72 hrs and count the colony forming units (CFU). Discard storage bottles with contaminated blood (Fig. 18).

If there is access to a γ-radiation source and a local abattoir is in the vicinity, larger amounts of blood can be collected. In some areas religious customs require

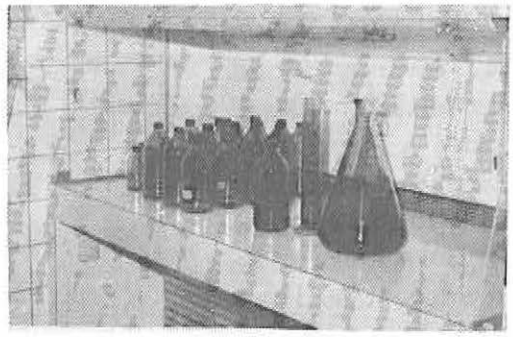


Fig. 13

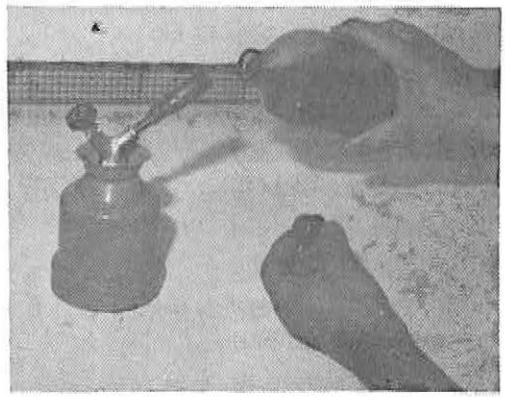


Fig. 14

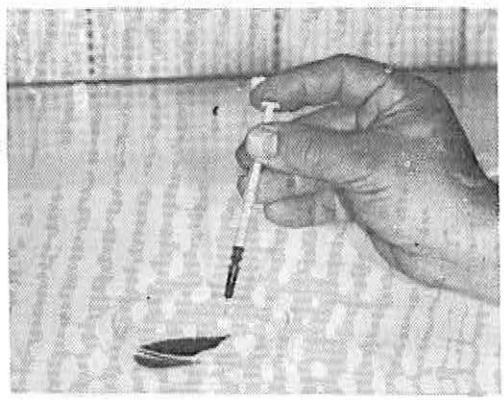


Fig. 15

the slaughtering of animals on the floor and the composition of the blood collection equipment needs to be adjusted accordingly.

- The routine blood collection set consists of several 25 litre polyethylene containers with wide screw caps (Fig. 19). A long paddle that can be rotated by a mechanical or an electrical drill is attached to two of the containers. In addition there is provision for the attachment of a wide funnel. A sufficient number (20) of polyethylene buckets (4–5 litre size) with tight closing lid complete the set. All materials are thoroughly cleaned and dried. It is important to ensure that the materials are sterilised or at least exposed to 80°C dry heat overnight. Preferably the decontamination treatments should be done in large closed autoclavable bags (Fig. 20) that are not opened until use at the abattoir to minimise the risk of microbial contamination.

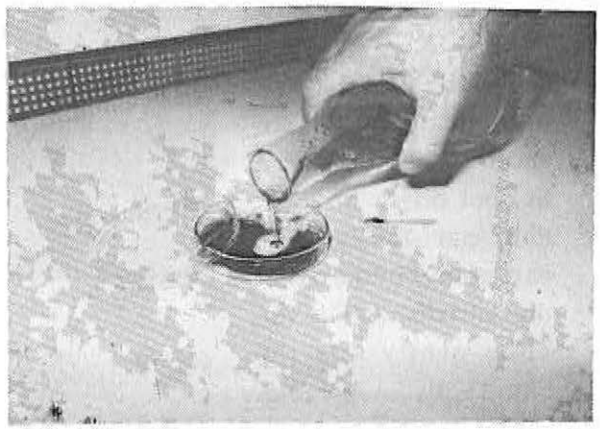


Fig. 16

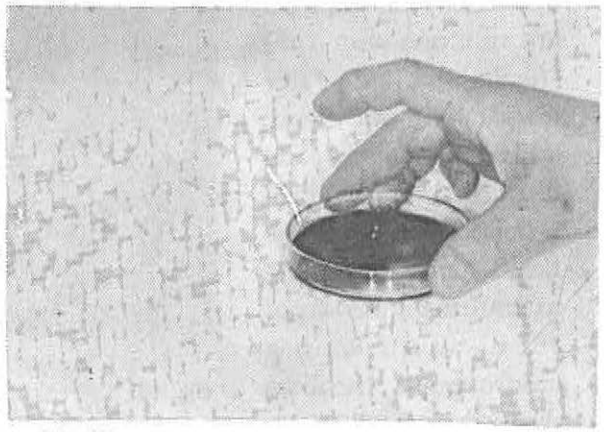


Fig. 17

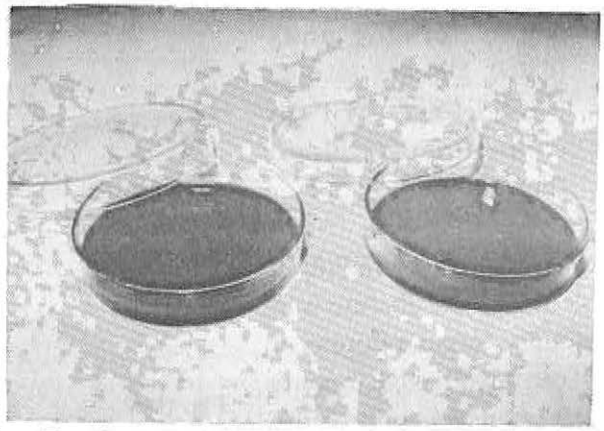


Fig. 18

- In the case of cattle slaughtered on the

ground, the lid is removed from the buckets just before use and blood is collected with these buckets from the cut throat (Fig. 21). The lids are replaced, the buckets are quickly taken to the 25 litre containers and the blood is poured in through the funnel (Fig. 22). The blood is either defibrinated by gently stirring the paddle with the drill or is heparinised. For the latter 800–1,200 i.u. Na+Heparin per l blood is added to the container before the first bucket of blood is poured in.

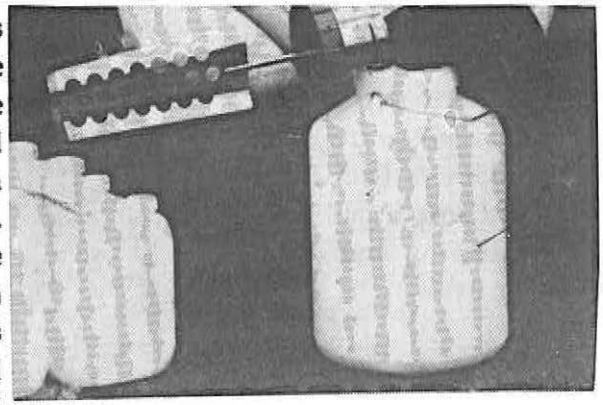


Fig. 19

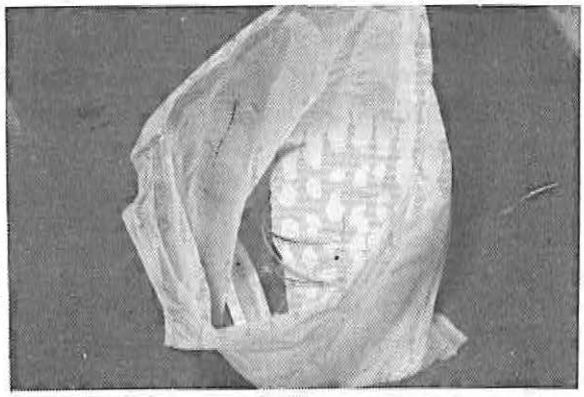


Fig. 20

- If a γ-radiation source is available at one's own laboratory, the blood is poured under sterile or aseptic conditions into clean heat-treated PE-containers that fit into the chamber of the radiation source and is microbially decontaminated using 0.5 kGy γ-rays (Fig. 23). Thereafter the blood is kept frozen until further processing and testing.

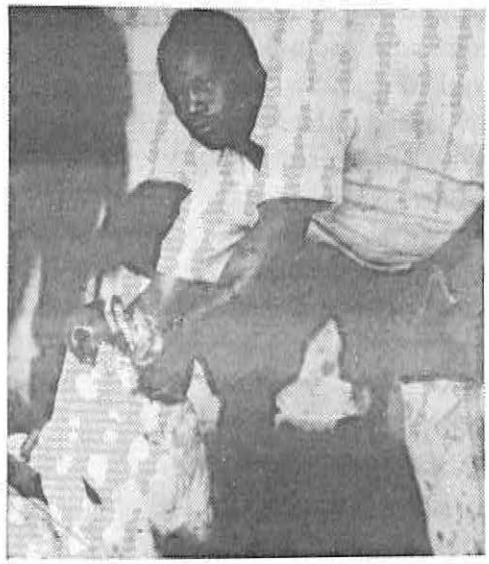


Fig. 21

- If the radiation source is not close to the laboratory, the blood is transferred into clean heat-treated PE-containers (4–5 l) and stored frozen until further processing and testing (Fig. 24).

In many abattoirs the animals are killed using a bolt shooting apparatus. Next they are hung and then the throat is cut. These conditions often permit the convenient collection of large quantities of blood for mass-reared tsetse fly colonies.

Fig. 22

- For blood collection the above 25 litre PE containers with the stirring device (paddle and drill) are additionally equipped with a 3 m silicone hose and a large funnel (Fig. 25). The funnel is held at the throat of the hanging animal. The blood is collected with this funnel. It flows through the tube into the container, where it is defibrinated by stirring (Fig. 26).

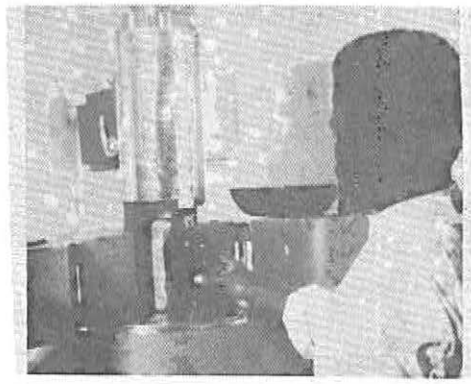


Fig. 23

- Freshly collected blood from several 25 litre containers can be poured through a funnel with a

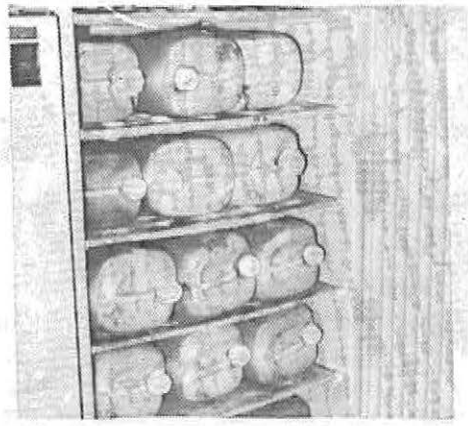


Fig. 24

sieve into a large 80–200 litre PE barrel and regarded as one collection batch. After collection the blood is transferred into clean heat-treated 4–5 l PE - containers (Fig. 27) and stored frozen until further processing and testing.

For routine tsetse fly colony feeding using the *in vitro* feeding regimen, quality assurance of the blood is essential. In addition to the nutritional quality of the blood or the risk of microbial contamination of the blood, other factors in the blood may affect the fly colony performance. It is therefore advised to screen each batch of collected blood by conducting a standard feeding test with a small group of flies (Van der Vloedt, unpublished).

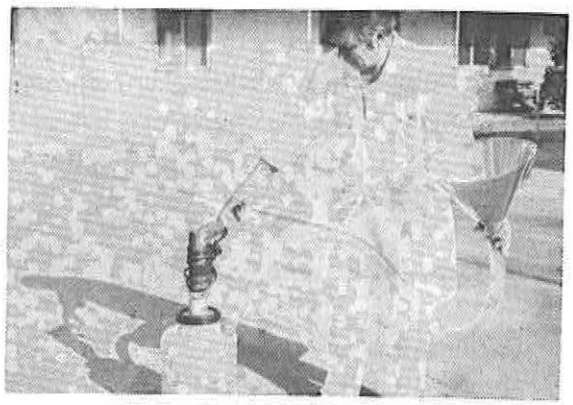


Fig. 25

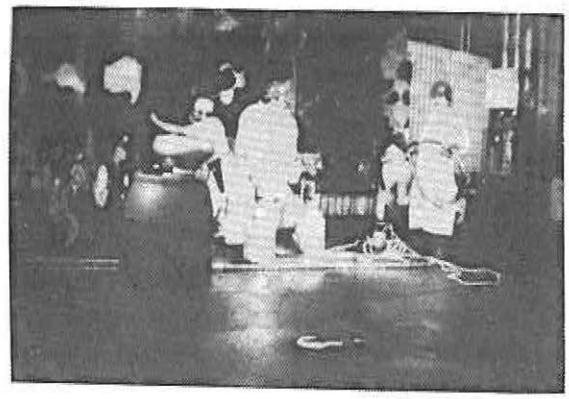


Fig. 26

- All materials used for blood processing must be sterilised or, in case of PE flasks, be treated overnight at 80°C.

- For a feeding test, at least 500 ml of the blood to be tested is thawed. A bacteriological sample is taken from the storage container (see above).

- The same blood diet is prepared for testing that will later be fed to the tsetse fly colony. Some

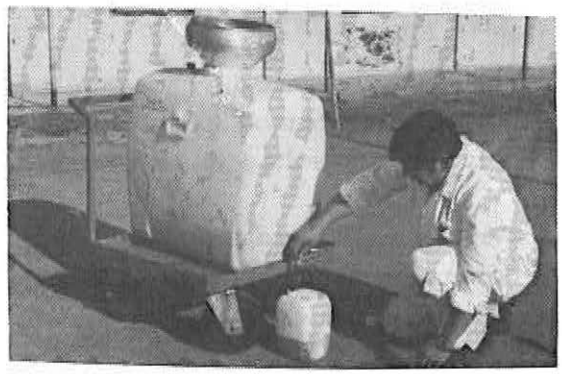


Fig. 27

Glossina spp., such as *G. austeni*, can be fed on pure cattle blood, others, e.g. *G. tachinoides* perform better if a mixture of 75% to 25% fresh-frozen cattle and pigs' blood is offered.

- The diet is mixed and portioned to 20 ml polyethylene scintillation vials (Fig. 28). Thereafter a 1–1.5 kGy γ-radiation treatment is applied. Bacteriological samples are taken before and after irradiation. The γ-radiation treatment eliminates on average 96% of the microbial contamination.

Fig. 28

- Contaminated diet is discarded. Retained diet is stored frozen until use.

- For a feeding test some 30 emerged teneral females are required. Fifteen females each are held in small PVC cages (4.5– 5 cm high, 10–11 cm diameter). The cages have a hole with a rubber stopper on the side and are covered on top and bottom with terylene bobbinet, small enough to keep the flies in but large enough to permit larvae to crawl through. For species that deposit big larvae a larger mesh size of netting may be selected for the bottom of the cage. The check intervals for abortions suggest Tuesday as the preferred day to start the 25 day feeding tests. The females are offered their first blood meal on the day of emergence and are fed the diet for 25 days, except on Sundays. The use of a special record sheet (Fig. 29) is recommended.

- The females are mated with mature males at their highest receptivity to mating, which for most species is on day two of the females' adult life. One or two days later the sexes are separated.

- The survival of females is recorded. Dead females are dissected and the occurrence of mating scars (*palpalis* -group) and inseminations are noted.

Entomology Unit, Joint FAO/IAEA Programme

IAEA Agriculture Laboratory Seibersdorf
Record Sheet for Tsetse Blood Diet Quality Control

BLOOD CODE: 1/91 (pig) 25% date collected 19. Apr. 91 storage - 20 °C
P/90(cattle) 75% date collected 23. Okt. 90 storage - 20 °C

Test-No.	15
year	1991

date processed 3 Jun 91 pH 7.96
additives 10^{-4} M ATP osm. pr. 275 mOsm

MICROBIAL SCREENING (CFU/ml)

cont. A 1/91	cont. B P/90	diet pre y	diet after y
2	39	15	0

SURVIVAL & FECUNDITY

G. tachinoides
test species

day	DATE	dead af	dead bf	fem. surv.	No. of. pupae prod.	No. of. abortions in dish
1	4.Jun.91			30		
2	5					
3	6					
4	7			30		
5	8					
6	9					
7	10					
8	11			30		
9	12			30		
10	13		1	29		
11	14			29		
12	15					
13	16					
14	17			29		
15	18			29		36
16	19			29		
17	20			29		
18	21			29	3	
19	22					
20	23					
21	24			29	18	2E
22	25			29		
23	26			29		
24	27			29	2	
25	28			29	1	2E,1I
Σ					24	7E,1I

females: dates 4.Jun. EM, 5.Jun MAT, 7.Jun 91 SEP
males: 25. May EM

other remarks:

weight class distribution

	A	B	C	D	E
Σ	2	14	7	1	-

DISSECTIONS

N	MS	Sp	UT
1	+	+	-
2	+	+	III
3	+	+	-
4	+	+	E
5	+	+	II
6	+	+	III
7	+	+	II
8	+	⊖	-
9	+	+	III
10	+	+	II
11	+	+	III
12	+	⊖	E
13	+	+	III
14	+	+	II
15	+	+	III
16	+	+	-
17	+	+	III
18	+	+	II
19	+	+	II
20	+	+	III
21	+	+	E
22	+	+	II
23	+	+	III
24	+	+	E
25	+	+	II
26	+	+	-
27	+	+	III
28	+	+	II
29	+	+	E
30	+	+	II

FS 16	29	PT	24	PB	14	PD	1	E+I	4	AB	2
FS 25	29	PA	2	PC	7	PE	0	II+III	20	BL	0

QF 1,25

Fig. 29

- No later than when a female's age is 8 days, a small dish is placed below each cage (Fig. 30). The dishes are searched for abortions under a binocular microscope on days 10, 15, 20 and 25 after female emergence. Larvae are allowed to pupate and their individual pupal weight on the day of larviposition is recorded. If a pupal sorting machine is available (used in larger *Glossina* spp. colonies of at least 40,000 females to sort the produced pupae to size classes), all pupae may be sorted at the termination of the test. The numbers of pupae in the various size or weight classes are recorded.

- All females surviving 25 days are dissected and the occurrence of mating scars (*palpalis*-group), insemination, uterine content, the follicle next in sequence to ovulate and reproductive abnormalities (oviduct blockages, abortions) are noted.

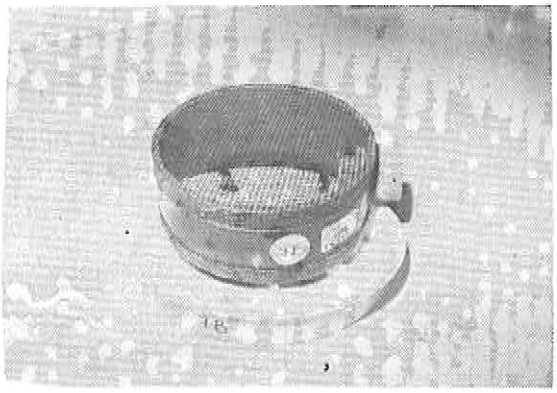


Fig. 30

- From the collected data a quality control factor (QF) may be calculated and be used as the criterion to decide whether or not the tested diet is suitable to be fed to the mass reared colony. Parameters that are included in the calculation are listed in Table 1. Table 2 gives for some *Glossina* spp. the mean pupal weight and figures of pupae which should be regarded as A-, B-, C-, D- and E-class pupae, respectively. The QF is determined as described in Table 3.

Table 1. Parameters used for calculating the blood-diet Quality Factor (QF)

Parameters	Explanation
First reproductive cycle:	
FS 18	number of females surviving on day 18
FS 25	number of females surviving on day 25
PT	total number of produced pupae
PA	number of A - class pupae
PB	number of B - class pupae
PC	number of C - class pupae
PD	number of D - class pupae
PE	number of E - class pupae
Second reproductive cycle:	
E+ I	No. of inseminated FS 25 with early pregnancy stages *in utero*
II + III	No. of inseminated FS 25 with late pregnancy stages *in utero*
BL	No. of inseminated FS 25 with oviduct blockage
AB	No. of inseminated FS 25 that aborted (empty uterus follicle next in sequence to ovulate is not mature)

Table 2. Mean weight of pupae and pupal weight ranges in the size classes for some tsetse fly species

Species	Mean pupal weight ± standard deviation (mg)	Weight range for pupae in the size classes (mg)				
		A	B	C	D	E
Glossina tachinoides	173 ± 1.9	8–14	14–17	17–19	19–21	21–27
G. palpalis palpalis	30.9 ± 3.8	13–21	21–27	27–31	31–35	35–43
G. fuscipes fuscipes	32.8 ± 3.7	15–23	23–29	29–33	33–37	37–45
Glossina austeni	23.1 ± 3.6	12–18	18–21	21–24	24–27	27–33
Glossina pallidipes	35.9 ± 3.9	20–29	29–33	33–37	37–41	41–50
Glossina brevipalpis	64.4 ± 5.7	36–56	56–61	61–66	66–71	71–91

Table 3.Calculation of the blood-diet Quality Factor (QF)

QF = [positive parameters from first reproductive cycle
+ positive parameters from second reproductive cycle
- negative parameters from first reproductive cycle
- negative parameters from second reproductive cycle]/ No. females

QF = [FS 25 + PT (PB x 0.3) + (PC x 0.4) + (PD x 0.5) + (PE x 0.6)
+ (E+I x 0.3) + (II + III x 0.6)
- (PA x 0.2)
- (AB x 0.5) - (BL x 1.0)] / [FS 18 + FS 25]

A. Van der Vloedt, modified

- Before a limit for an acceptable diet quality factor can be established (the suitability of diets may vary for different species), some replicates of diet quality control feeding tests should be conducted. It is suggested to start with a QF of 1.00, as the limit for acceptable blood. Once a sufficient number of feeding tests (at least 50) has been conducted, the parameters from the first reproductive cycle (see Table 1) can be correlated with the respective QF results. In order to reduce the dissection workload a trend analysis then can be used to calculate a QF_c from the first cycle parameters prior to dissections. Thus only groups of females with a QF_c score below the established limit are dissected to calculate the QF as listed in Table 3. Fig. 31 shows as example the system of a routine large scale blood collection, diet processing and quality control, as conducted at the Entomology Unit, IAEA Laboratory Seibersdorf (Joint FAO/IAEA Programme).

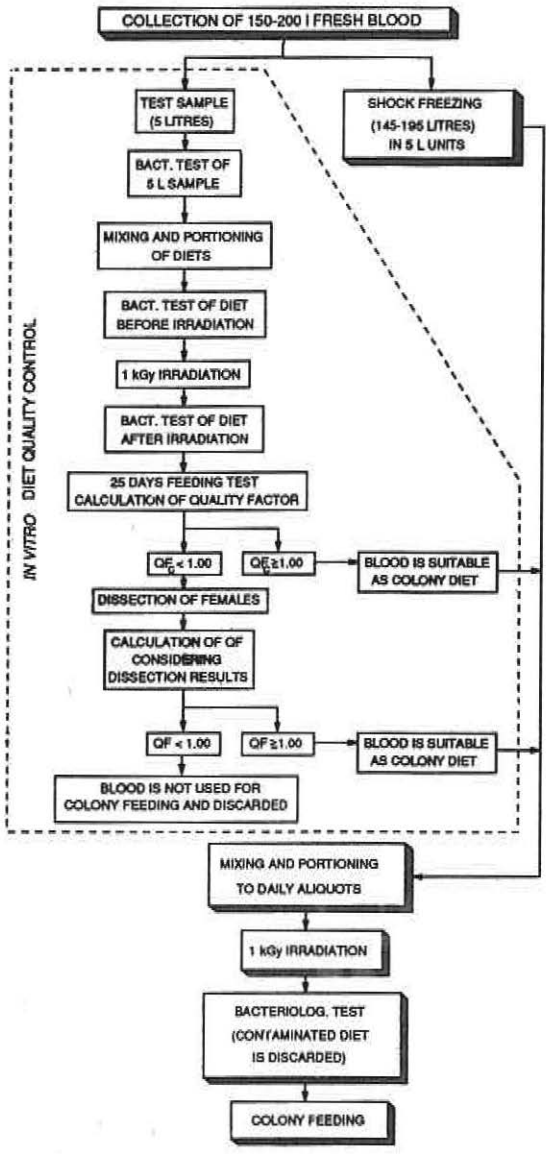


Fig. 31. Production of colony blood and diets

- Once a diet has been declared to be of acceptable quality for colony feeding, it is transferred to a separate freezer. Here only blood is stored that has passed the quality control screening. For the membrane feeding of tsetse fly colonies the 4–5 litre PE containers with the

quality screened blood are removed from the freezer the day before blood processing and are thawed in air at room temperature. If a diet consisting of cattle and pigs' blood is required, blood is mixed and stirred in conical flasks under sterile conditions (Fig. 32). Sterile glass bottles, holding 200–1,000 ml, are filled half-full with the diet to enable the freezing of the diet bottles.

Alternatively 100–300 ml PE bottles, heat treated with 80°C during the previous night, may be filled. The diet bottles are firmly closed.

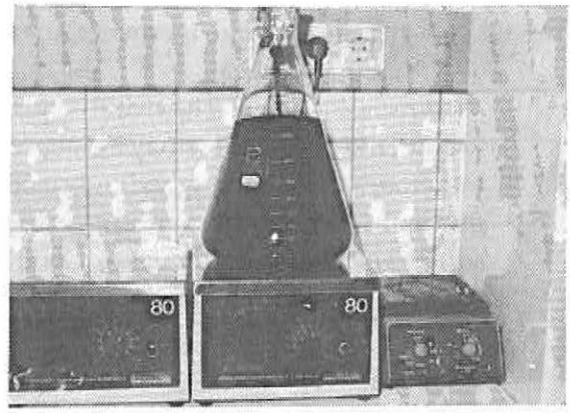


Fig. 32

- If blood from several containers is processed on the same day, it is important that the conical flasks and the glass bottles are labelled according to the respective storage containers (Fig. 33). Then the bottles are treated with 1–1.5 kGy γ - radiation.

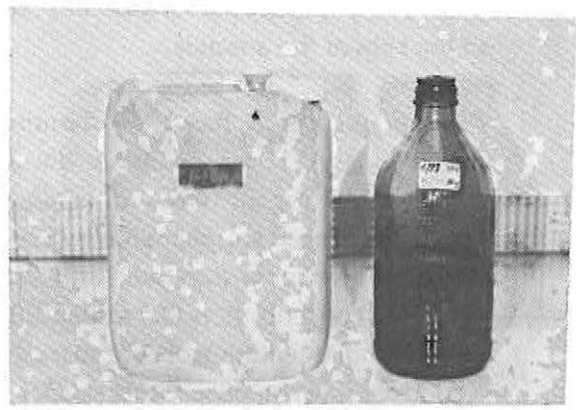


Fig. 33

- After the γ-treatment, bacteriological samples (see above) are taken from two or three diet bottles of each container. The diet is stored frozen and is only used if it passes this last bacteriological screening. If contaminated diet is found, the blood in all bottles bearing the same label as the contaminated diet above, i.e. blood from the same storage container, should be discarded. Only blood that passes this screening system is used for fly colony feeding.

If there is sufficient freezing capacity, it is advised to have enough tested blood in frozen storage to last for at least three months of *in vitro* feeding.

4. The Membrane Feeding of Tsetse Flies

If possible, the feeding of tsetse flies should be done in a separate room that can be kept under aseptic conditions overnight (e.g. with UV light). Also the room should not be used for other daily colony routine work before feeding. Good blood uptake by tsetse flies through the membrane may be influenced by several factors:

- *Disturbances and light:* The vicinity and the movement of staff during feeding (odour, moving shades) may influence the feeding response. Light sources therefore should be designed and positioned so that indirect illumination of the room is achieved and movement of staff does not occur between the light source and the feeding benches. Good feeding response has been achieved with dimmed illumination of the feeding area to 12 –14 lx.

- *Humidity:* Some tsetse fly species, such as *G. fuscipes fuscipes* or *G. palpalis palpalis*, require increased air humidity not only in the holding room, but also in the feeding area. This is usually not necessary for the *in vivo* feeding of the same species, possibly because there is a suitable micro climate close to the skin of the animals.

- *Phagostimulants:* It has been demonstrated that phosphonucleotides, particularly ATP, may enhance the feeding of haematophagous insects (Galun and Margalit 1969). For *in vitro* feeding of tsetse flies in past years the diet has been supplemented with 1 mM ATP. However, as has been demonstrated for *G. p. palpalis* and *G. tachinoides*, which recognise ATP at very low concentration (0.5 µm and 0.01 µm, respectively) (Galun 1988), lower supplementation of ATP to fresh-frozen blood is sufficient to induce a good feeding response. Other species, such as *G. austeni*, were successfully fed fresh-frozen bovine blood without ATP for several generations.

The daily feeding of tsetse flies using the membrane feeding technique is conducted as follows:

- Bottles with quality screened blood-diet are removed from the freezer in the morning and thawed under cold running water. The bottles are then dried and transferred to the feeding room.

- The sets with feeding trays and membranes that were heat sterilised at 120°C overnight are removed from the heat sterilising oven and placed onto the heating units (Fig. 34).

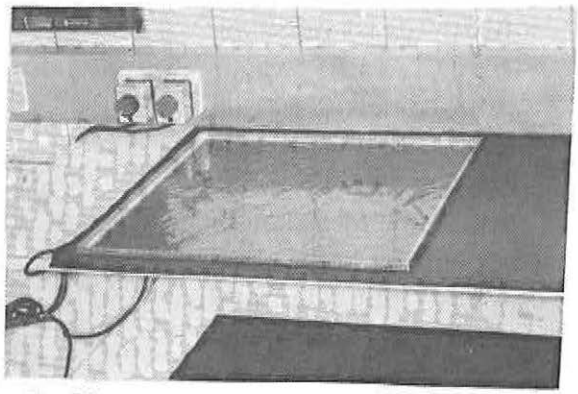


Fig. 34

- If necessary, 0.1 mM or 1 mM ATP is added with a 100 or 200 µl pipette to the diet from a sterile stock solution (Fig. 35). Gently swirl the bottle to ensure even distribution of the phago-stimulant in the blood (do not shake the bottle!). The ATP stock solution should be prepared under sterile conditions with autoclaved distilled water for not longer than a week in advance and is stored refrigerated. Example: The daily feeding of a 5,000 female tsetse fly colony requires up to 3 litres of blood per week.

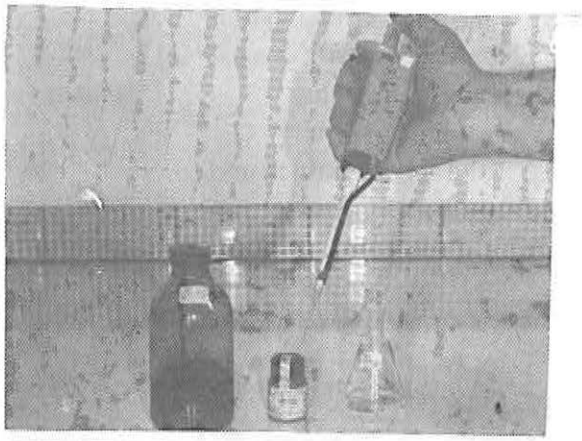


Fig. 35

Example: 100 ml diet each is supplemented with 0.1 mM ATP using a 200 µl pipette. A one week's supply of stock solution is prepared by dissolving 22 mg ATP in 8 ml sterile distilled water.

- To reduce the risk of contamination of the feeding unit, before lifting up the membranes from the feeding trays, make sure that doors are closed and all equipment that could cause air turbulence is switched off (fans, air-conditioners, humidifiers, etc.).

- Lift up one membrane by two thirds from a feeding tray and pour approximately 100 ml blood diet onto the feeding tray (Fig. 36). Avoid the formation of air bubbles in the blood while pouring and during the subsequent replacement of the membrane onto the feeding tray. Then continue with the next feeding unit, etc.

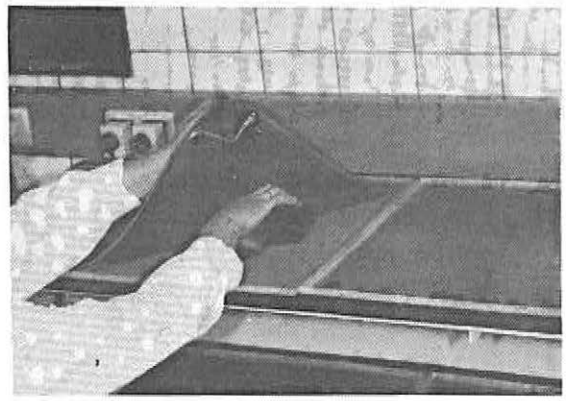


Fig. 36

- Take a 4–5 cm thick and 20–25 cm long plastic rod or a roll of tissue paper and distribute the diet on the feeding tray evenly by rolling the rod over the membrane (Fig. 37).

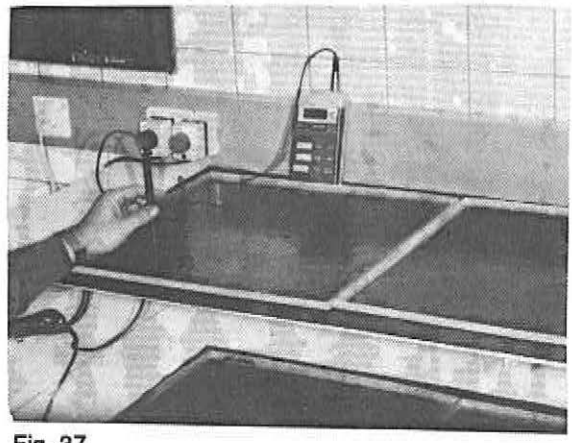


Fig. 37

- Air-conditioners and humidifiers can now be switched on again. It should be noted that the climate in the feeding room should not be adjusted for the comfort of the staff but maintained at optimal handling conditions for the flies.

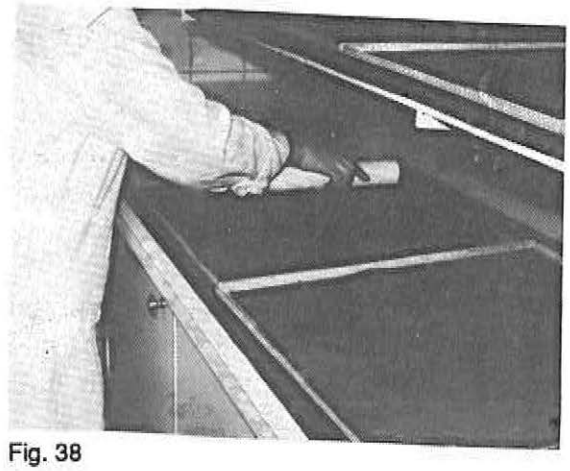


Fig. 38

- By use of a thermocouple ensure that the

membrane surface temperature is approximately 35°C (Fig. 38).

- For *in vitro* feeding of tsetse flies, the fly cages are turned and are placed upside down onto the membrane (Fig. 39). Thus the same side of the cage netting will always be down while non-feeding (holding position) and during membrane feeding the other, cleaner side will be down (feeding position).

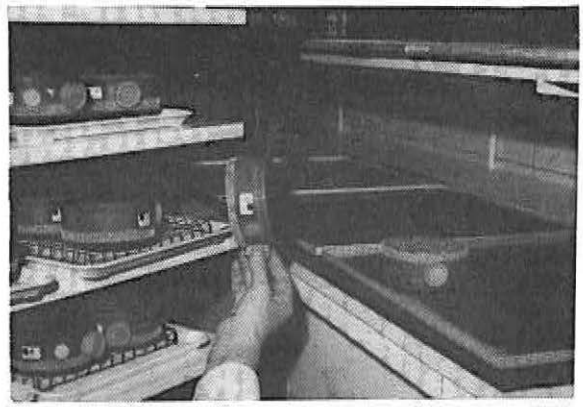


Fig. 39

- The feeding now starts with the **young flies first**, followed by the next older ones, etc. The cages remain on the membrane for 10 minutes and are then replaced by the next set of older flies. One membrane feeding unit as described above with 100 ml of blood each can be used to feed up to 1,500 tsetse flies in less than one hour.

- After use, the feeding trays and membranes are thoroughly washed in cold water and rinsed with distilled water. The membranes are then replaced on the feeding trays and are heat sterilised overnight at 120°C for use next day (Fig. 10).

- Some handling of young flies, i.e. the sexing upon emergence and after mating, is usually conducted in a special fly chiller (modified chest freezer, adjusted to +4°C). It is important to ensure that the flies recover for at least 2.5–3 hours from the chill-immobilisation prior to feeding. The membrane feeding of flies chilled in the morning should be conducted with a new (sterile) *in vitro* feeding set in the afternoon or, preferably, even next day, if the flies have sufficient energy reserves.

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The mention of names of specific companies or products should not be construed as a recommendation or endorsement on the part of the IAEA.

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Standard *in vivo* Rearing Procedures for Various Tsetse Species

J. U. Wanyonje

Insect and Animal Breeding Unit (IABU),
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Abstract

***Glossina** species of tsetse flies are important vectors of human and animal trypanosomiasis. ICIPE has selected these vectors as target arthropods that need sound IPM and IVM control strategies. In this regard, core programme, the Tsetse Research Programme and research units, the Chemistry and Biochemistry Research Unit, the Cell Biology Research Unit and the Sensory Physiology Research Unit are carrying out research on various aspects of arthropods ecology, biology and control. For research work to succeed, reliable sustained artificial production of quality arthropods are required for experimental purposes.*

The paper outlines the methods used in the colonisation and rearing of the **Glossina** *species of tsetse flies which are directly under the Insect and Animal Breeding Unit (IABU), namely the* **Glossina morsitans morsitans, G.m. centralis, G. f. fuscipes, G. austeni,** *and* **G. pallidipes.**

Introduction

Tsetse flies belong to the genus *Glossina*, the sole genus of the family Glossinidae within the order Diptera. Their natural distribution is confined to the African continent where they are the vectors of human and animal trypanosomiases. Twenty-two separate species exist, but together with sub-species and races

approximately 30 different types of tsetse flies are recognised. Only 6 species of tsetse flies are implicated as vectors of trypanosomiasis. However, it is likely that all species are potential vectors.

Rearing Methods

Steps	Key points
Materials and methods	
1. Starting an initial colony	• Rearing of *Glossina* spp. began in ICIPE in 1970 with pupae supply from Bristol. • Feeding of tsetse flies has been done *in vivo* on rabbits.
2. Environment	• The flies are kept in a room with all natural light excluded. • Light intensity is controlled by a tungsten switch for keeping the light dim or bright as required. • A photoperiod of 12 hour light 12 hour dark is maintained by a rotational timer switch (Switchgear Control Ltd.—Nairobi). • Temperature and humidity controlled at 25 ± 1°C and 70 ±10% respectively are considered optimum holding conditions for most *Glossina* spp. • The air is changed and circulated within the breeding room by means of air conditioner. • The humidity is propagated by humidifiers and the heating of the insectaries is done by wall heaters. • Insectocutors are installed at strategic points in tsetse insectaries and handling areas to attract and exterminate any loose tsetse flies trying to escape into the outside environment.

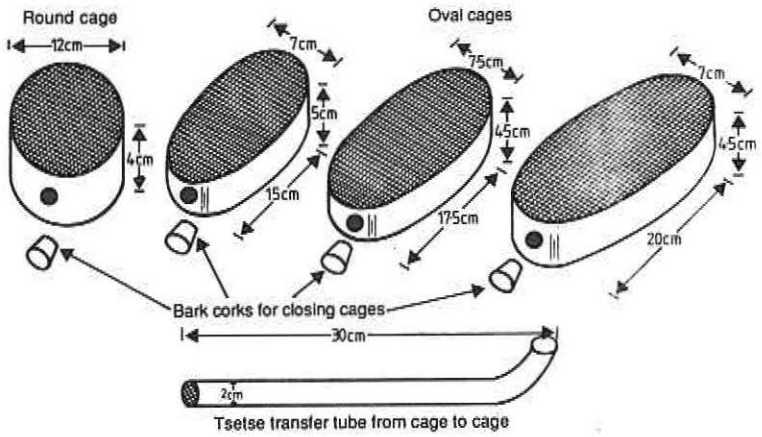

Fig. 1. PVC cages of various sizes

Steps	Key points
3. Tsetse cages	• Tsetse flies are housed in cages made from various sizes of polyvinyl chloride tubing obtained locally (Fig. 1). • The tubing is cut into suitable lengths and closed with terylene netting stuck on both sides with PVC glue. • The flies are added to or removed from the cage via a hole cut in the tube.
4. Lariposition	• The cages rest on plastic coated 2.5 cm mesh wire netting held 137 x 44 cm above painted metal shelves 137 x 44 cm which act as false floor for falling larvae. • Thirty rabbits feeding tsetse cages are placed on each shelf.

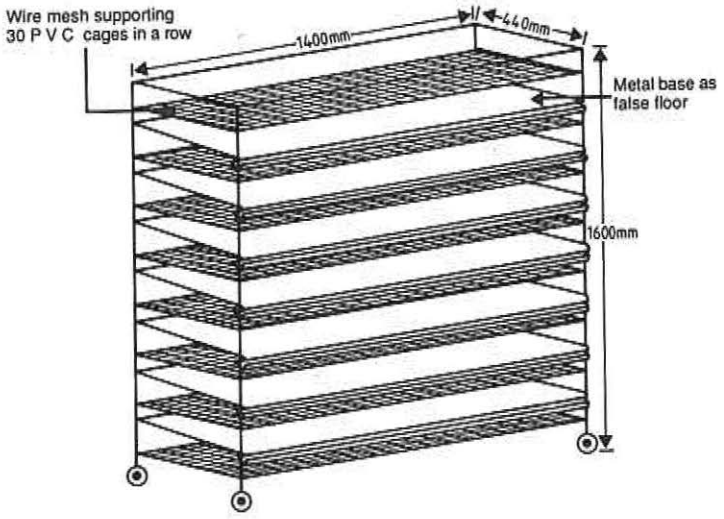

Fig. 2. Mobile holding rack for mated females of the colony

Steps	Key points
	• One whole rack has 7 complete shelves which can store upto 210 tsetse cages.
	• The larvae crawl through the large netting, fall on the metal false floor, then roll onto collecting trough (137 cm length x 5 cm width x 2 cm depth) for pupation.
	• The racks are handy and provided with wheels for ease of movement between the breeding and feeding areas (Fig. 2).

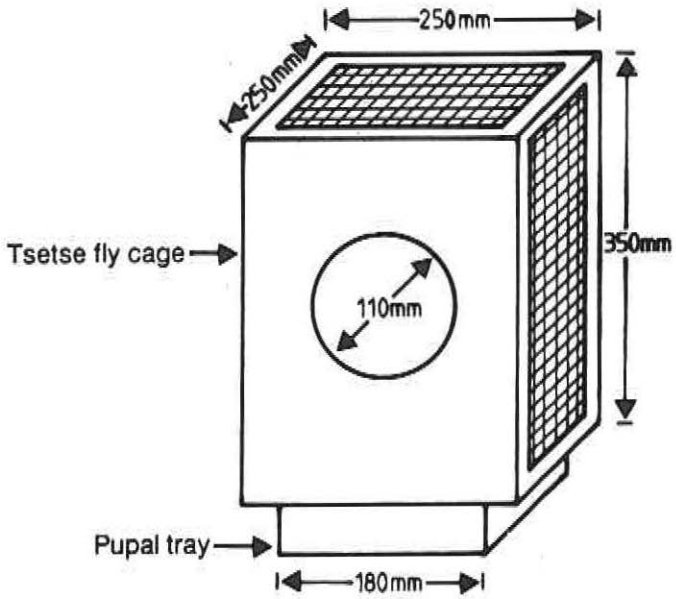

Fig. 3. Tsetse emergence cage

Steps	Key points
5. Tsetse emergence	• The pupae are placed about a week before they are due to emerge in a wooden framed cage covered with galvanised mosquito wire netting 31 cm high x 25 cm broad x 38 cm deep with a removable aluminium tray to hold the pupae (Fig. 3). • The newly emerged flies crawl up the sides of the cage to expand their wings and harden. • To remove the flies, the pupal tray is replaced by an aluminium plate, the upper cage is also replaced by an aluminium plate then placed in the cooled box. • When immobilised the flies are removed and the pupal tray replaced.

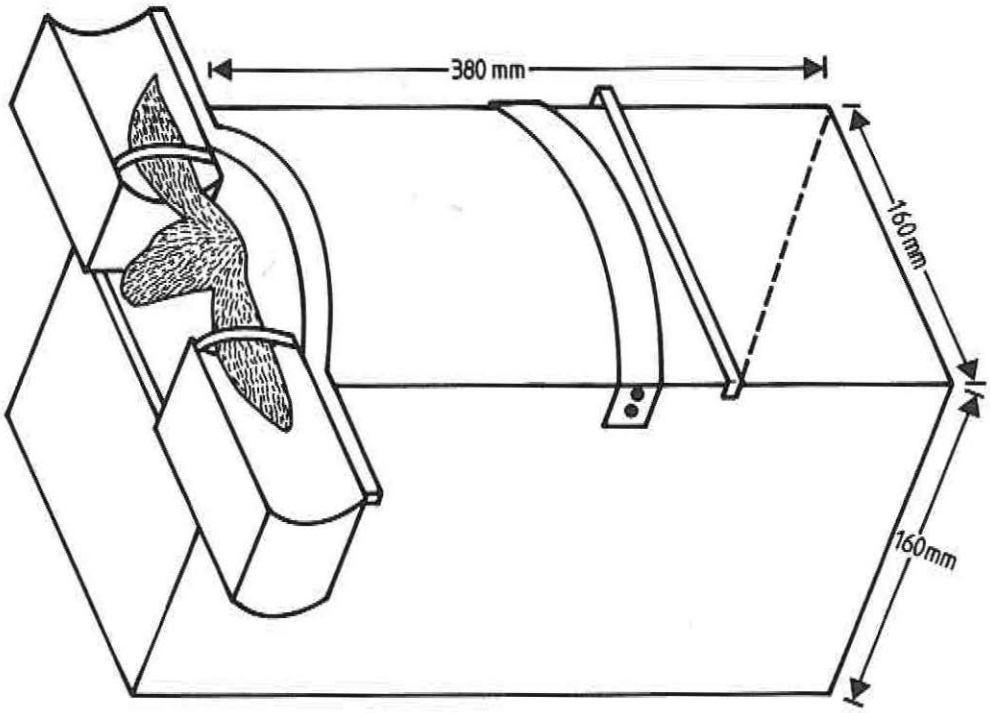

Fig. 4. Rabbit holding box

Steps	Key points
6. Handling of flies	• Tsetse flies are handled during sexing, mating and separation, by cooling them at 4 to 1°C in a cool box modified from a deep freezer (Fig. 4). • The air is cooled by contact with the cold sides of the cabinet and the temperature of the air is regulated by means of a thermostat mounted in the return air louvre. • The flies are immobilised by the cold air within a few minutes and are then tipped from the cage onto a mirror where they are sorted and brushed into PVC cages. • The flies recover within 1–3 minutes.

Steps	Key points
7. Mating	• Two-day old females that have recently had two blood meals are mated with males over 5 days of age that have not been fed within the previous 4–24 hours. • The males are picked using a glass tubing (30 cm long x 2 cm diameter) and introduced into female cage via a funnel. • Twenty-five males are put into each cage of 20 females and corked. • Two days later all males are removed from female cages to leave females alone.
8. Feeding	• The flies are offered food for about five minutes daily except on Sundays by strapping the cages with elastic onto rabbits' ears. • A maximum of 300 flies should be fed on each rabbit every third day, but due to acute shortage of suitable rabbits this figure has been considerably exceeded for short periods.
9. Recording	• Details of numbers, age, emergence, mating and separation dates are recorded on a special label stuck onto each cage. • Age group number (AGN) is also recorded on a label stuck on each shelf of the rack. • A record sheet is kept for each age group of 20–30 cages kept in a hard cover file. • Details of fecundity, longevity, mortality and the general performance of the colony are all recorded in the record sheet.

Steps	Key points
Results and discussion	
10. Advantages of rearing method	• The use of PVC cages has both advantages and disadvantages over the "Geigy" type wire framed cages. • The PVC cage is cheap, unbreakable and easily made. • Furthermore, suitable PVC tubing is manufactured locally and is readily available. • The netting remains taut throughout the life of the cage, but easy to repair in case of damage. • A record label may easily be stuck to the side of the cage. • The tubing is available in a number of diameters to suit all types of hosts.
11. Cleanliness of insectaries and rearing equipment	• At the end of each working day, clean working benches and swab them with 70% alcohol soaked in cotton wool. • Clean floors using Teepol detergent. • Wash rearing equipment using Teepol detergent three times a week, rinsing them thoroughly in hot water.

Conclusion

Standard *in vivo* rearing of tsetse flies started at ICIPE in 1970 using rabbits as hosts. This methodology has been used successfully for over 20 years now. It has worked successfully in other tsetse research institutions all over the world.

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Rearing of Phlebotomine Sandflies

M. J. Mutinga and C. C. Kamau

Medical Vectors Research Programme (MVRP),
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Abstract

Sandflies belong to the family, Psychodidae and sub-family Phlebotominae. They are small hairy dipterans measuring about 3 mm long with large eyes, long legs and pointed wings held at an angle of 45°. In both tropical and sub-tropical areas phlebotomine sandflies have a wide distribution in various ecological zones that range from arid zones to wet tropical rain forests. Though sandflies seemingly appear to be harmless due to their small size, they cause great harm to man and animals by both causing nuisance and transmitting diseases. Sandflies transmit protozoan and viral diseases, the worst of which are the various forms of leishmaniases which cause about 1,000 human deaths annually with over 12 million people infected worldwide.

Laboratory colonies of vector species of sandflies facilitate investigations into their biology, bionomics, behaviour, taxonomy and observations on parasite vector and vector-host interaction. This knowledge is essential in planning control strategies of sandflies that may lead to the reduction of the leishmaniasis scourge.

Introduction

Phlebotomine sandflies are of great economic importance in both the tropical and subtropical countries. They are vectors of the various forms of leishmaniases of both man and animals. Presently the disease is found in approximately 80 countries with 350 million people at risk and causing over 400,000 new cases

annually. In Africa about one million people are infected with leishmaniases. African countries in which leishmaniases is a serious public health problem include Algeria, Chad, Ethiopia, Kenya, Libya, Morocco, Senegal, Sudan and Tunisia.

The sub-family Phlebotominae contains about 600 species and subspecies of sandflies. Among these, 71 are proven or suspected vectors of leishmaniases. The species of sandfly that are vectors belong to either the genera *Phlebotomus* and *Sergentomyia* in the Old World or to the genus *Lutzomyia* in the New World. In Kenya, for example, there are 38 documented species of sandflies and of these the proven or suspected vectors are *Phlebotomus martini, P. vansomerinae, P. ciliae, P. duboscqi, P. pedifer, P. guggisbergi, Sergentomyia garnhami* and *S. ingrami.*

Laboratory Colonies

The establishment of sandfly colonies is important for understanding the taxonomy, biology, physiology, and behaviour of vector species. The reared sandflies can also be used for many other experiments in the laboratory for example in the understanding of host-parasite interactions.

Sandflies are highly adapted to their natural breeding and resting habitats and are therefore very difficult to rear in the laboratory. There are three major problems that workers who want to initiate a sandfly colony encounter. Firstly, the determination of favourable conditions for inducing oviposition and development of first instar larvae. Secondly, the death of females shortly after first oviposition. Finally, the problem of larval stages and gravid females being susceptible to dryness, and at the same time being prone to drowning in even slight excess film of water (Mutinga *et al.* 1989a).

The first successful colonisation of phlebotomine sandflies was that of *P. mascitti* by an Italian, Grassi in 1907. Bayma (1923, 1936) reared *Lutzomyia intermedia* and *P. papatasi*. Larval development in colony was later studied by Whittingham and Rook (1923). In Eastern Africa it is only in the last decade that colonies of the vector species of sandflies have been established in laboratories. The first study was on *P. pedifer*, the vector of *Leishmania aethiopica* (Mutinga 1971, 1972). In 1981 the biology of *P. martini*, the vector of *L. donovani* was studied by Kapur and Mutinga. *P. martini* was subsequently successfully colonised by joint efforts of ICIPE and the Kenya Medical Research Institute (KEMRI) (Beach *et al.*

1983). Since then 10 species of sandflies have been colonized. These are *P. duboscqi, P. guggisbergi, P. martini, S. adleri, S. africanus, S. antennatus, S. bedfordi, S. garnhami, S. ingrami* and *S. schwetzi* (Mutinga *et al.* 1989a).

Rearing Methods

Field Collection of Adult Sandflies

Sandflies in different physiological conditions are collected from their natural breeding and resting sites. Fed and gravid female sandflies are sorted out, from males and unfed females, and put in vials to oviposit and hence initiating a colony. The unfed females collected should be allowed to mate and feed in the laboratory on either laboratory animals or wild favoured hosts (see Figures 1–8).

Steps	Key points
1. Location of breeding and resting habitats	• Differ according to geographical location. These include animal burrows, termite hills, tree holes, caves, homes, rock crevices and cracks in the soil (Mutinga *et al.* 1989b)
2. Methods of collection	• CDC light trap: Left overnight in suitable breeding/resting habitats. Attracted by light in the traps, the flies are fanned into the trap and collected in the morning. • Updraft trap (Mutero *et al.* 1991). Flies are also attracted by light at night. Used in animal burrow. • Manual aspirators. Used in the morning between 0600–0700 hr for most habitats located in open areas or any time in the morning or evening for enclosed cool habitats like caves. • Human bait catches. Done at night by having somebody sit by a source of sandflies and capture all those coming to probe on him using a vial. • Emergence and funnel traps left in known breeding and resting sites (Bettini and Meli 1988; Comer and Corn 1991).

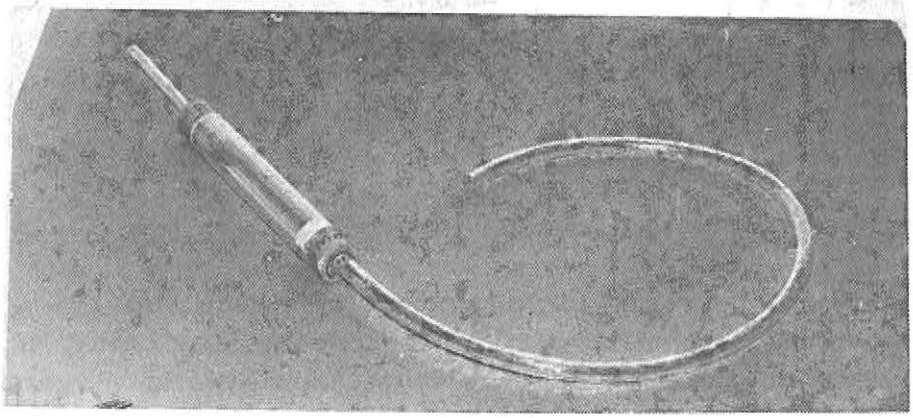

Fig. 1. Manual aspirator used for collecting sandflies from their natural breeding/resting habitats. Other traps used are the CDC light trap and the updraft trap

Steps	Key points
	• Incubation of soil collected from breeding sites (Mutinga and Kamau 1986).
3. Transportation to laboratory	• Collected fed female sandflies are kept in oviposition vials (described later) ready for oviposition and transportation.
	• Unfed females and males are transported in holding cages.
	• Vials with gravid females are kept moist by transporting in cooler boxes. Ensure minimal shaking.
Equipment and facilities	
1. Rearing facilities	• Spacious air-conditioned room to accommodate working benches, rearing chambers, a refrigerator, a sink and shelves for keeping caged adults.
2. Equipment	• Environmental chambers or ovens for maintaining immature stages (eggs–pupae) and gravid females in conditions of: (a) darkness (no direct light), (b) high relative humidity. Optimal 70–90% (most species), range 60–95% for other

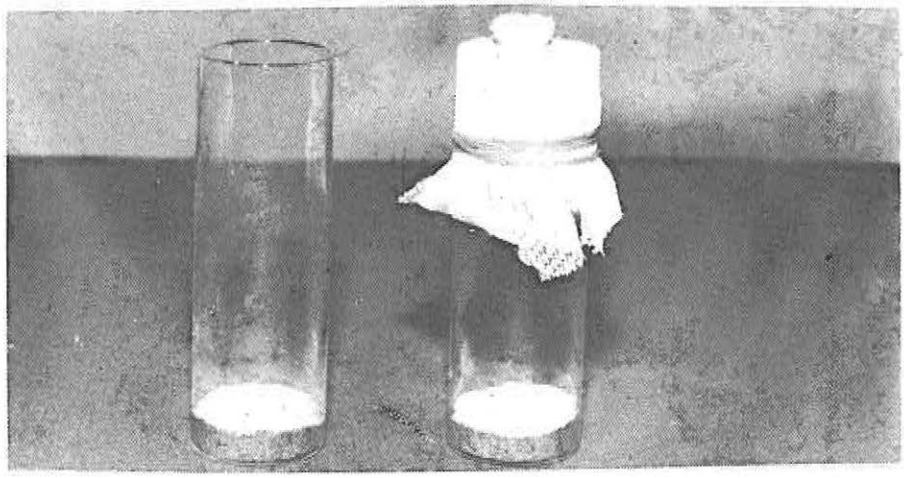

Fig. 2. (a) Glass vials used for keeping gravid female sandflies ready for oviposition

Steps	Key points
	species depending on their geographical origin. The immature stages and the gravid adults are very sensitive to desiccation. (c) Controlled temperature: Optimal 27–29°C. Temperatures as low as 17°C can be used depending on the species' geographical origin whether highland or lowland.
	• Oviposition vials made of polyethylene (plastic) or glass measuring 2.9 x 2.7 cm. The vials are lined at the bottom with wet toilet papers or plaster of Paris, filled a quarter or half-way from the bottom. A filter paper is then placed on top of the wet material to act as a laying surface and prevent entanglement of sandfly legs especially when the toilet paper is used.
	• Polyethylene rearing petri dishes 9.0 cm in diameter and 4.0 cm long with a hole at the bottom and 2.0 cm base of plaster of Paris. In the oven the dishes are kept in a large tray that is lined at the bottom with wet cotton wool.

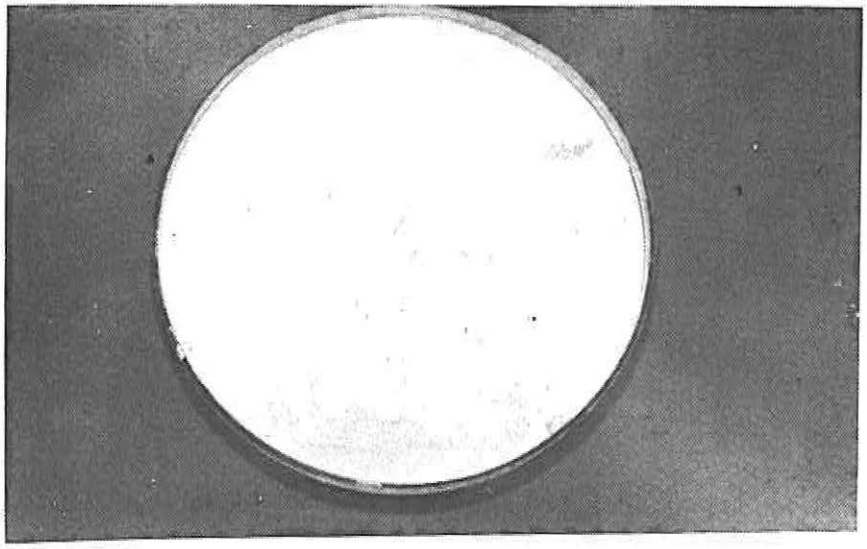

Fig. 2. (b) Plastic dish used for rearing immature stages of sandflies

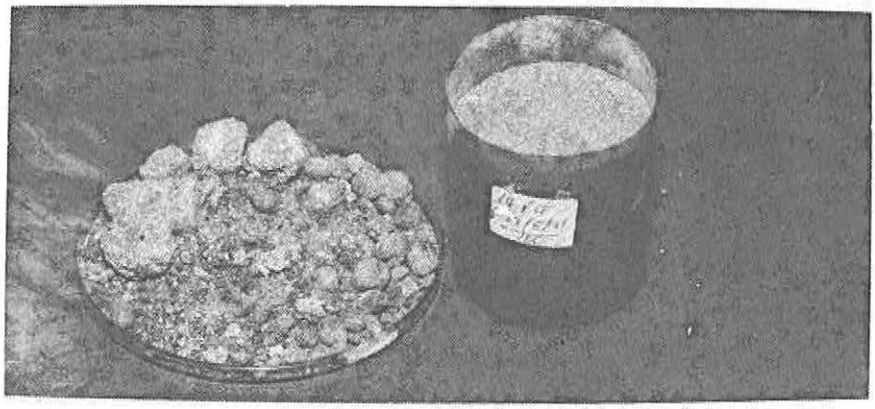

Fig. 3. Laboratory-prepared sandfly larvae food

0.2 mm

Fig. 4. (a) Sandfly developmental stages

Steps	Key points
Larval diets	
1. Commercial food	• Derived from vegetables or mixed animal plus vegetables. This food is normally used for fish and is easily available, inexpensive and sterile.
2. Laboratory-prepared food	• Rabbit dropping plus Purena Rabbit chow (pellets) or bread crumbs (with or without beef or liver powder extract) mixed and dried. Wet the mixture and let it age to prevent development of fungus or sterilise.
Description of sandfly developmental stages	
1. Egg	• Elongate, oval-shaped about 0.4 mm in length with rounded ends. It turns from pale to dark-brown with age. Under the electron microscope the egg has species or even subspecies specific sculptures (Irungu *et al.* 1986).
2. First instar larvae	• Tiny, clear and shiny. Easily missed in the rearing dish due to its resemblance with the white plaster of Paris. Head is slightly visible since it is blackish. This instar has one pair of caudal bristles.

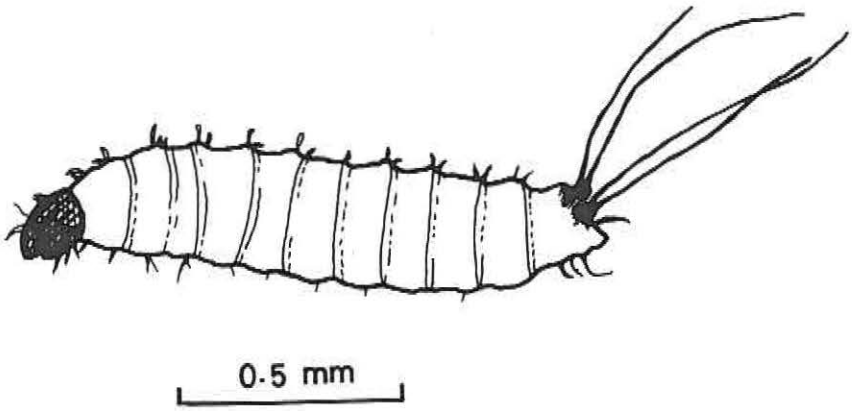

Fig. 4. (b) First instar larvae. Notice the one pair caudal bristle at the distal end

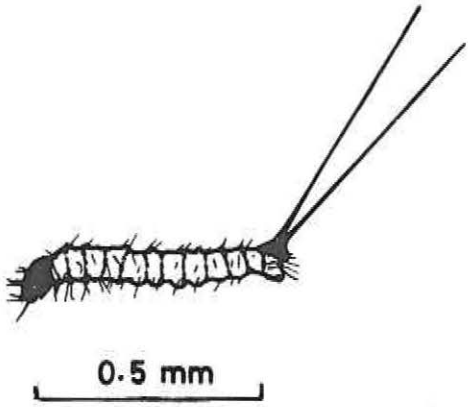

Fig. 4. (c) Second instar larvae

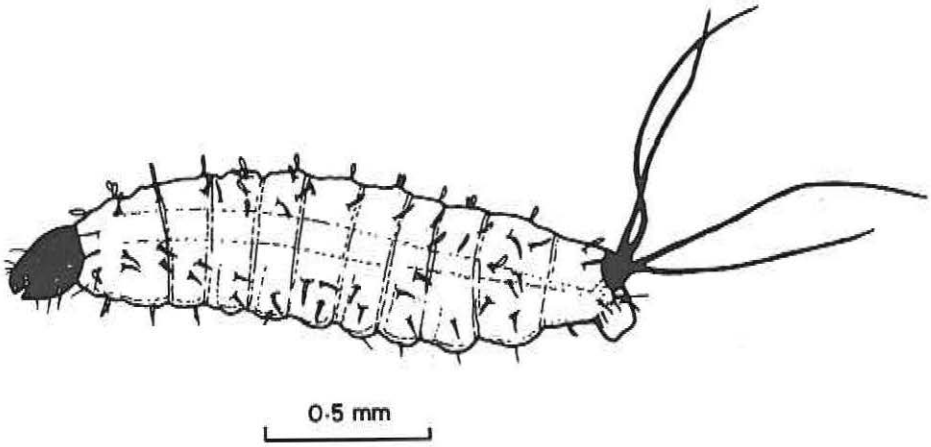

Fig. 4. (d) Third instar larvae

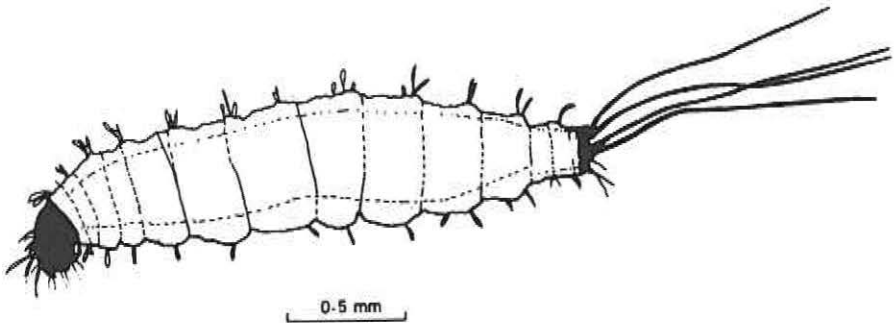

Fig. 4. (e) Fourth instar larvae

Steps	Key points
3. Second instar larvae	• Two caudal bristles like in all other later instars, the outer being shorter than the inner. The last abdominal segment which bears the caudal bristles is chitinised and brown in colour. The head is darker than in first instar. The size of the instars increases progressively with age.
4. Third instar larvae	• Yellowish brown body hairs. Longer caudal bristles than in second instar.

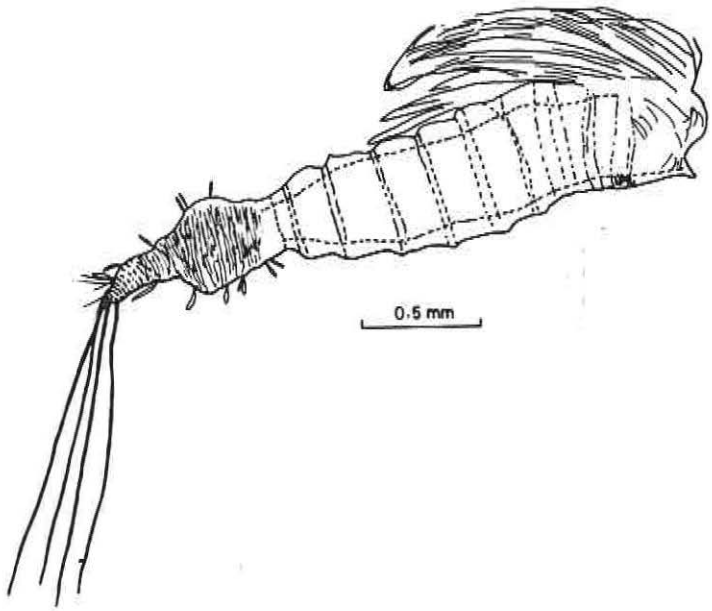

Fig. 4. (f) Pupal stage

Steps	Key points
5. Fourth instar larvae	• Long and large. Chitinised tergites of last two abdominal segments (eight and nine). Body hairs longer than in third instar larvae. Posterior clasper on the ventral side of the ninth abdominal segment are very prominent in this instar. • When ready to pupate the larvae attach the posterior segment to the side of the rearing chamber and then hang downwards. Others pupate on food and fecal debris on the plaster of Paris.
6. Pupal stage	• Sandfly pupa is characterised by the attachment to its distal end of the remains of the 4th instar larvae ecdysial skin with its 4 bristles.

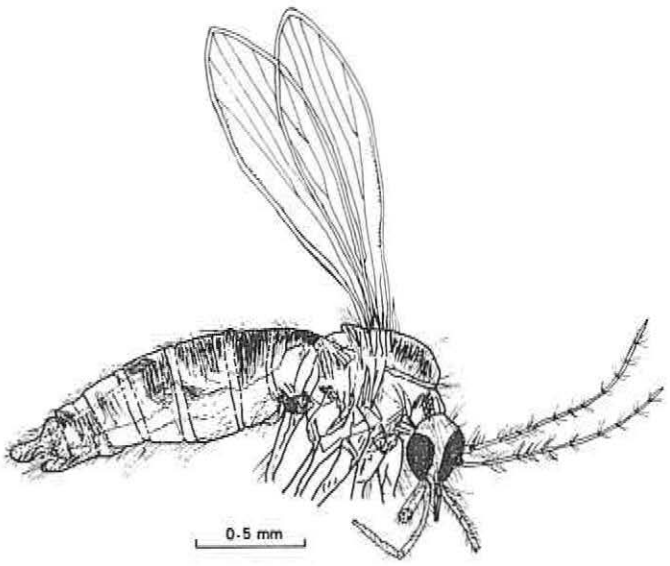

Fig. 4. (g) (i) Male adult

Steps	Key points
	• Newly formed pupae are whitish in appearance. Older pupae look yellow, but wing sheath is extremely white.
7. Adult stage	• Small hairy insect on average 3 mm in length with large eyes, long legs and pointed wings held at a 45° angle. Males are easy to distinguish by the conspicuous terminalia at the end of their abdomen.
	• Most sandflies are light yellow with a few being dark or dark grey in colour.

Management of various stages

1. Egg laying and maintenance	• Females that have oviposited and died are removed from the vials and washed in detergent saline (1%), rinsed and dissected in Locks solution or sterile saline, mounted using gum chloral and after clearing and drying they are identified (Abonnenc and Minter 1965; Lewis 1982).

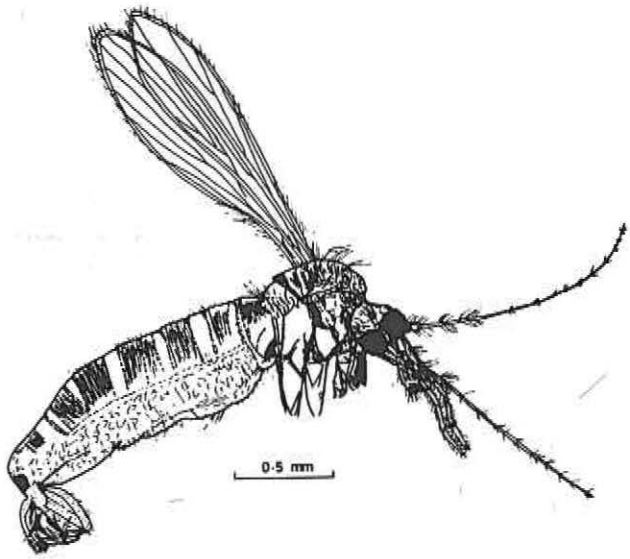

Fig. 4. (g) (ii) Female adult

Steps	Key points
	• The laid eggs are removed from the oviposition vials by flooding them out. After drying, the eggs are then placed in the rearing dishes.
	• The number of eggs laid is influenced by diet (both bloodmeal and larval diet) and environmental conditions (temperature and relative humidity). At temperature greater than 30°C though development is faster, eggs may not be laid.
	• Oviposition occurs in about day 6 and 9 for *Sergentomyia* and *Phlebotomus* species respectively, after females have taken a bloodmeal but can be delayed by a day or two by keeping the vials slightly dry. This improves egg laying.
	• Number of eggs laid vary greatly with species. On average about 40–50 eggs are laid by most species.

Steps	Key points
	• Of the laid eggs between 10–50% have been reared successfully.
	• Most females die after first oviposition. Of the eleven species of sandflies colonised in our laboratory only *P. duboscqi* survive oviposition. For this species females can undergo up to eight gonotrophic cycles (Mutinga *et al.* 1987).
	• *S. gamhami* in Kenya (and other species in other countries) appears to have egg diapause. Eggs of such species should be maintained carefully until they hatch. In *S. gamhami* such eggs can take as long as 6 months before they hatch.
2. Larval feeding and maintenance	• Food is sprinkled sparingly into rearing dishes as soon as the eggs turn pale-brown (about two days from hatching) to avoid starving the larvae when they emerge.
	• Regular check to ensure proper amount of moisture and replenishment of food and removal of excess fungal and predaceous mites is essential.
	• High temperature and relative humidity result in faster development. Food quality also affects rate of larval development and pupation.
	• Water is necessary and is absorbed through food and integuments. Water of about neutral pH (6.8–8.0) is best for homeostasis. Larvae cannot tolerate desiccation at all but can tolerate flooding for a short duration.
	• Proper aeration in the incubator and in the rearing dish minimises moisture condensation on the lid of the rearing dishes that could drown the larvae.

Steps	Key points
	• Larvae are affected adversely by direct light and heat. • Larval development of *Phlebotomus* species takes significantly longer (mean 55.3 days) than of *Sergentomyia* species (39.9). • In all stages *Phlebotomus* species are larger than the *Sergentomyia* species.
3. Mass rearing	• When a colony is established individual oviposition vials are replaced with large jars where batches of gravid females are introduced for oviposition. Larvae hatch in these jars and feeding is handled the same way as described above. This reduces too much handling and labour.
4. Adult sandflies bloodmeal feeding	• Females of all haematophagous insects require a bloodmeal in order to complete their gonotrophic cycle. • Sandflies can feed on a wide range of hosts including man, hamster, chicken, guineapig, dog and lizard. In general *Phlebotomus* species prefer homeiotherms while *Sergentomyia* species prefer poikilotherms. • *Phlebotomus* species feed more readily on hamsters and produce more eggs than when fed on lizard. In contrast, *Sergentomyia* species feed more readily on lizards and produce more eggs than when fed on hamsters. • To enable sandflies to feed and also to prevent the animals from eating them, hamsters are anaesthetised using Pentabarbitone (Sagatal®) while the lizards are restrained using wire mesh. • Most sandfly species take between $^1/_2$– 4 hrs for most members in a batch to feed.

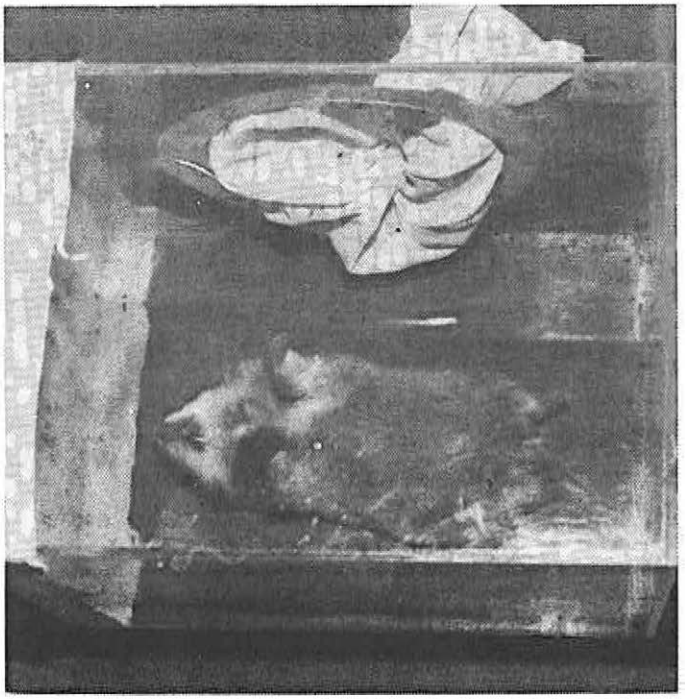

Fig. 5. (a) An anaesthetised hamster in a sandfly holding cage

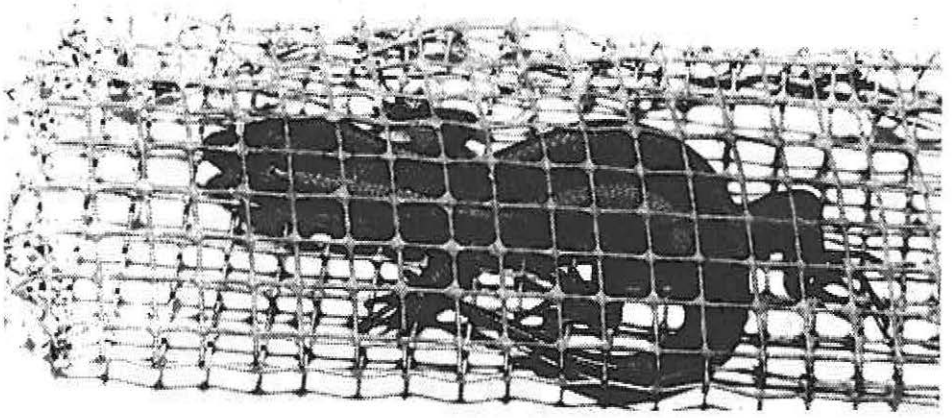

Fig. 5. (b) A restrained lizard. These are favoured bloodmeal sources in a sandflies colony

Steps	Key points
	• Unless conditions are optimal females refuse to feed on either bloodmeal or sugar and hence die.
5. Sugar feeding in adult sandflies	• Sugar does provide both male and female sandflies energy for general metabolic needs and also prolongs their longevity.
	• Sandflies prefer a wide range of sugar solutions including: glucose, sucrose, fructose, maltose, and raffunose.
	• Plants leaves or fruits which the flies probe on directly can also be used for supply of sugar. We have found it economi-

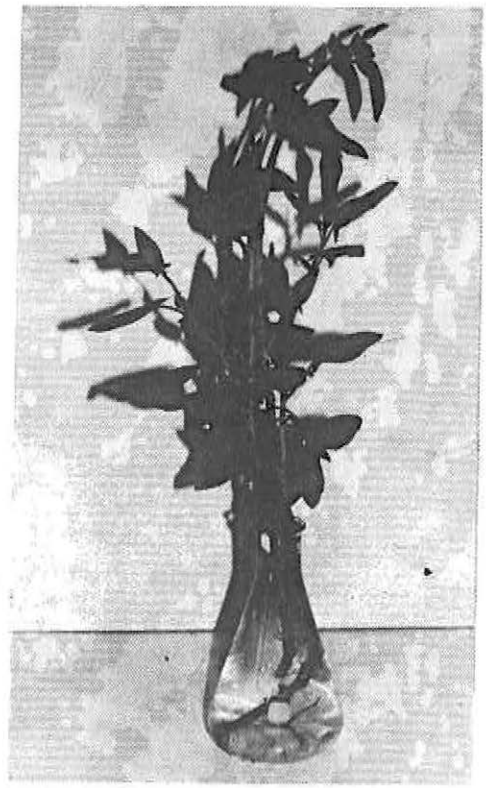

Fig. 6. *Rumex usambarensis*, a plant that is a favoured source of sugar in a sandfly colony

Steps	Key points
	cal to feed them on a juicy shrub *Rumex usambarensis.* • Rate of sugar acceptance is not affected by concentration, pH, sodium chloride content, colour or temperature. Optimal sugar solution is a highly concentrated sucrose or fructose solution in distilled water with or without colour. • Sugar concentration had no appreciable effect on fly longevity. • Larger holding cages results in higher feeding rates.
6. Adult maintenance	• Adults are kept in holding (Fig. 5) cages at room condition. • In comparison species of the *Phlebotomus* genus require warmer conditions when feeding. • Copulation takes place any time from after emergence to oviposition. Thus keeping the males and females together all the time enhances chances of fertilisation. • When in oviposition vials or jars, gravid females should be supplied with sugar. • Females live longer than males. *P. duboscqi* female for example was recorded to live for 45 days while males hardly lived for 20 days (Mutinga *et al.* 1987).

Problems encountered in rearing sandflies

1. Low fertility	• Most gravid females do not lay all the developed eggs, resulting in low colony productivity. In our colony this phenomenon of egg retention has been recorded in *P. duboscqi, S. ingrami* and *S. schwetzi.*

Steps	Key points
	• Almost all the species have a higher proportion of males than females. • Males also emerge before females and this is a problem in a colony of species with low productivity.
2. Contamination	• Fungal growth in rearing dishes presents a big problem especially if the dishes are infested during oviposition or at the early larval stages. Fungus that grow in rearing dishes include *Rhizolus stolonifer* and *Aspergillus terreus.* Since *A. terreus* is a soil fungus, it would seem that the fungus are introduced into the colony through field collected sandflies. Control of fungus is crucial to the success of a sandfly colony. This problem can be minimised by (a) removal of fungus daily from those dishes that are heavily colonised using an entomological pin, (b) sterilising working equipments regularly, (c) fungicides may be used judiciously so as not to affect the larvae.

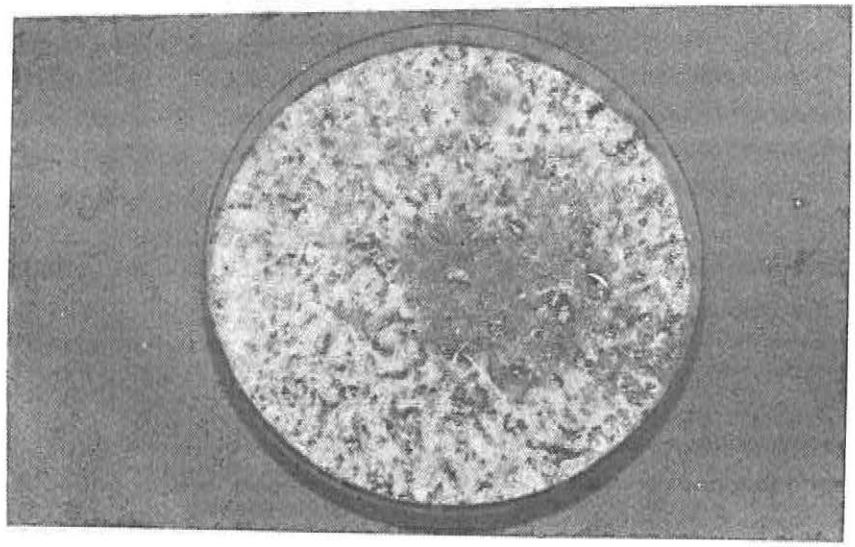

Fig. 7. Fungal contamination in a sandfly rearing dish

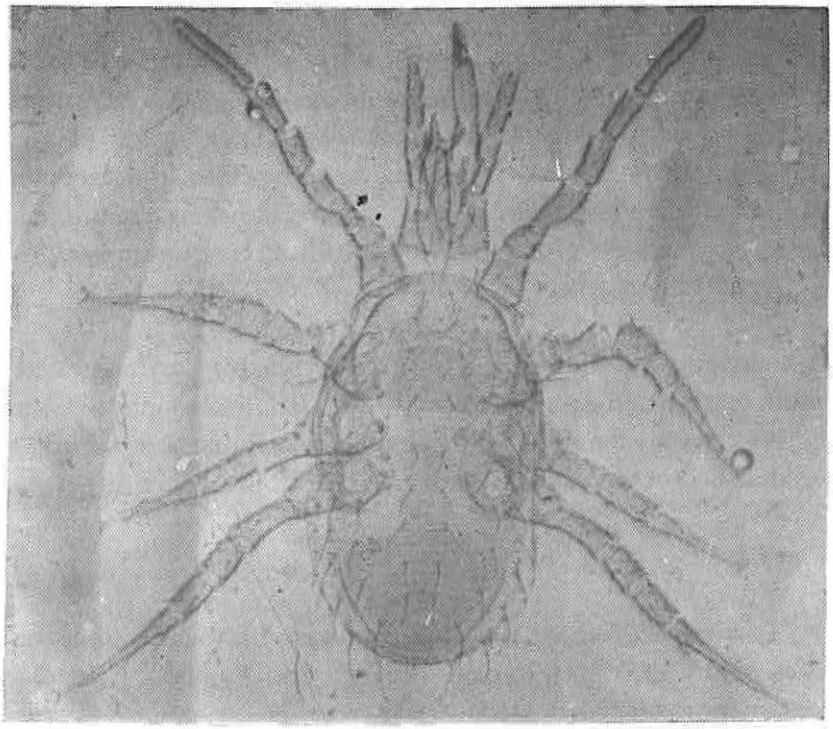

Fig. 8. A predatory mite, a serious pest in a sandfly colony

Steps	Key points
	• Predacious mites are a menace in a sandfly colony. Rather than feed on fungus as they normally do, in a sandfly colony these mites prefer the eggs and the early larval instar causing great losses. These ubiquitous mites find their way into the colony even under conditions of scrupulous cleanliness and rapid, skilled work. Management of the mites is by direct killing with a pin in the infested dishes and by freezing the rearing dishes after emergence of adults.
3. Labour	• Due to contamination and sensitivity to desiccation the rearing of sandflies is very labour intensive.
4. Rearing conditions	• Species-specific requirements of temperature and humidity are necessary especially for species from different geo-ecological zones.

Conclusion

Ten species of sandflies have been reared to several generations in the ICIPE's Medical Vectors Research Programme laboratory. From these colonies investigations into developmental biology, survival, fecundity, longevity, vector-plant interaction, vector-host and host-parasite interactions, and the potential of biological control have been carried out. Investigations are continuing in the laboratory as vector species of sandfly are tapped from their natural environment and reared in the laboratories.

The knowledge thus acquired will be useful in planning an integrated vector management system for the control of sandflies and the leishmaniasis disease they transmit. This can only be achieved if colonies of the important sandfly species become established in the laboratory for easy availability to the scientists.

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Rearing of Ticks Under Laboratory Conditions

D. K. Punyua

Livestock Ticks Research Programme (LTRP),
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Introduction

Ticks comprise of only three families and about 800 described species. Most species require at least three hosts to complete the lengthy, physiologically slow life cycle. While some species may require as many as 12 hosts, a few feed only on one or two hosts. Most tick species of economic importance parasitise domestic stock, i.e. cattle, sheep, goats, dogs, cats, pigs, camels, yaks, buffalo, chickens etc. Some common species also parasitise wild animals. These ticks are strictly or moderately host specific. The three families comprising the superfamily Ixodoidea are:

(1) Argasidae or "soft ticks"—with 150 species in five genera: *Argas, Ornithodoros, Otobius, Antricola* and *Nothoaspis.*

(2) Ixodidae or "hard ticks"—with over 650 species in four subfamilies and 13 genera: *Amblyomma, Aponomma, Boophilus, Cosmiomma, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Margaropus, Rhipicenter, Rhipicephalus, Nosomma,* and *Anomalohimalaya.*

(3) Nuttalliellidae — intermediate between "hard" and " soft" tick— with only one described species.

Most argasid species remain sheltered in burrows on niches close to colonies, nests, or roosts frequently or seasonally revisited by birds or mammals. Thus, protected by limited exposure time during feeding and by microhabitat, and assured periodically of ample food from resting immature or adult birds or

mammals, argasid species inhabiting sheltered microhabitats, notably, have always fed on the same kind of host, the one generally available under these situations. Hungry ones may feed on the exceptional host venturing into their habitat (some survive but others die from the effects or develop poorly following such bloodmeals).

The Ixodidae as a group have been more adaptable biologically and ecologically than Argasidae.

Ticks Life-Cycles

All ticks have complex developmental cycles. In argasids, the life cycle consists of the egg, larva, 2–7 nymphal stages and an adult.

In the ixodid family the nymphal stage is reduced to a single instar. They usually feed once in each instar (except the larvae of the sub-genus *Ornithodoros* which do not feed at all, but remain in the egg until they are ready to make the larval-nymphal moult).

The main differences between the three instars are both morphological and physiological. The larvae are relatively smaller in size than the nymphs or adults of the corresponding genus. They have three pairs of legs and physiologically they are sexually immature. Nymphs are also relatively smaller than the adults but bigger than the larvae. They possess four pairs of legs, resemble morphologically the adult females, but lack the "porose" areas which are found on the dorsal side of the basis capitulium. Physiologically they are also immature. The adults are bigger than the first two stages with four pairs of legs. The males and females have distinct morphological and physiological features. Sexual maturity, in some genera, is attained after a blood meal while in others (e.g. *Ixodes* species) sexual maturity is attained before taking a blood meal. For this reason some species of this genus have non-feeding males, since they fertilise females soon after moulting from the nymphs.

Blood feeding in larval, nymphal and adult stages result in regular alternations of free-living and parasitic existence together with changing hosts.

Ticks life cycles are divided into four types on the basis of the number of hosts changes and moults. These are multi-hosts, three-hosts, two-hosts and one host.

Multi-hosts — mostly characteristic of most argasids. This is associated with irregular but numerous feeding nymphal instars and several adult gonotrophic cycles.

Three hosts — characteristic of most hard ticks. Ticks with this life cycle remain on the host only while feeding. The larva hatches from the egg (which is usually laid on the ground) and climbs up the nearest vegetation and waits for a passing host which, if suitable, attaches and feeds. After engorgement it drops to the ground to moult. The emerging nymph also climbs the vegetation and awaits a host. After feeding, it also drops to the ground to moult. The adult (male or female) also waits for a passing host on the vegetation. After feeding the two sexes meet and copulate on the host. After engorgement the fertilised female drops to the ground and lays a batch of eggs which later hatch into larvae. The female soon dies after laying eggs. The male is capable of fertilising several females and can thus remain on the host for longer periods.

Two-hosts— In this life cycle the larva attaches to the initial host and even after engorgement moults to the nymph while still attached to the host and detaches only as an engorged nymph. It moults to an adult which also seeks for a new host.

One-host —In this cycle all the moults occur on the first host and only fertilised females detach to lay eggs on the ground.

In the Ixodidae the eggs laid by one female in one batch may number one or several thousands. Generally all hard ticks are slow feeders and each feeding period may be of several days' duration.

Development in the non-parasitic phases of ticks takes place on the ground and is very much dependent on environmental factors.

After the engorged female drops to the ground to lay eggs, it would take a few days, depending on temperature, for the first egg to appear. They are laid over a period of several weeks. The period of egg development ranges from a few weeks during the warm months to a few months during the cool seasons of the year. Larvae take between 3–10 days on the host depending on the resistance status of the host, tick species and the environmental temperatures. Nymphs feed for 5 – 7 days while the adults take between 6–15 days to engorge on a susceptible host.

The method described by Bailey (1960) and modified by Branagan (1969) and again Irvin and Brocklesby (1970) is currently used for rearing ixodid ticks in East Africa and some other parts of the world.

Laboratory Facilities

It is essential that certain facilities and items of equipment are available. This includes a small room, which is set aside and used solely for the purpose of tick rearing and storage. It should be protected from direct sun rays, from extreme temperatures, away from harmful chemicals. Modern automated incubators set and maintained at various controlled temperatures and humidities are currently available in the market. In the absence of such sophisticated incubators, conversion of a large discarded refrigerator cabinet or an insulated or lagged cupboard would be satisfactory. In this system heavy wire-mesh shelves should be installed and a low wattage electric light bulb should be mounted on the bottom shelf. At the bottom of the cupboard or incubator a large tray should be placed containing either water or clean damp sand. The water together with the heat generated by the light bulb will maintain a relatively high humidity inside the incubator. This could be substituted by placing large aluminium cans (15 cm diameter x 20 cm high containing clean damp sand) or air tight desiccators containing saturated salt solutions which give specific relative humidities.

While the method of tick application differs slightly depending on the animal host to be used for feeding, the tick species and even the tick stage, the basic set up is essentially the same.

Rabbit Hosts

When using rabbits for tick feeding the following should be observed:

(a) The rabbits should be tick naive (susceptible).
(b) They should be healthy and mature.
(c) They should be clean and kept in clean cages.
(d) One week before tick application, both ears of the rabbit should be treated by applying a few drops of liquid paraffin in order to rid it of wax and ear mites.
(e) On the day of tick application shave the base of the ears with a clean pair of scissors or shaving machine avoiding possible wounds as much as possible.

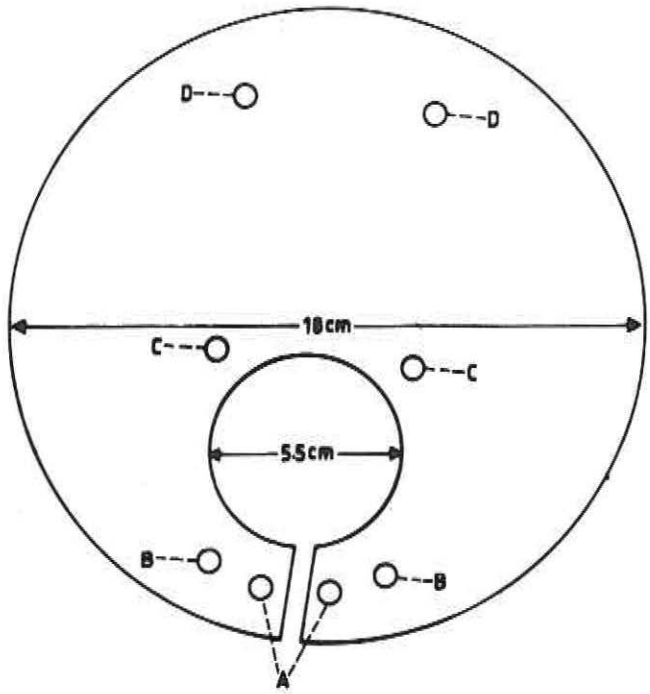

Fig. 1. The rabbit collar for tick feeding

(f) Wipe the ear with 70% alcohol soaked in cotton-wool.
(g) Rabbits should be kept in individual cages which are provided with water and food in small containers.

Collars made from heavy 5 mm thick leather are needed for rabbits to prevent them scratching off the ear-bags which contain the feeding ticks. They are designed as shown in Fig. 1. The whole collar is approximately 18 cm diameter. The cut hole (5.5 cm diameter) is located at the lower half of the collar.

Four pairs of 5 mm holes are positioned as shown and designated A, B, C, D, in the figure. Once the cut hole is placed round the rabbit's neck, a long cord is put through the holes marked "A" and the ends taken back under the forelimbs of the rabbit and tied together across its back. Another set of cords are passed through holes marked "C" to those marked "B" and their ends taken back under the forelimbs also and again tied together across its back. This will ensure that the

collar remains upright and prevent flopping sideways. This way, the rabbit would still have freedom of movement in its cage.

Animal ears are more commonly used as tick feeding sites than other parts of the body (Fig. 5). Ear-bags are made of white calico cloth material, approximately 16 cm long and 8 cm wide with both ends opened. A long (15–20 cm) cord is sewn to one end of the bag. To secure the bag onto the ear, a thin film 3 cm wide of non-toxic adhesive material is applied to the clean shaven base of the ear. A similar thin layer of the same material is also applied onto the inner side of the base of the ear-bag. The rabbit ear is then slotted into the ear-bag and fixed to the adhesive material. The bag is pressed to the base of the ear such that there is no gap which would allow any tick to escape. This is reinforced by an application of a 5 cm wide Elastoplast tape wound firmly and evenly round the neck of the bag in order to gather together the folds of cloth. Ensure that the Elastoplast tape is not very tight which may result in the swelling of the ear and become oedematous to the detriment of the ticks. Leave the ear aerated for a few minutes to release the harmful petroleum base vapour of the adhesive material.

Hungry ticks collected from the ground, from a laboratory colony or (depending on the degree of engorgement, partially fed ticks from other hosts may now be applied into the ear through the open top end of the ear-bag. It is then folded and a piece of adhesive tape is tightly wound round it, leaving the 15–20 cm cord hanging. The cord for the left ear-bag is threaded through the right "D" hole while that for the right ear is threaded through the left "D" hole and the two ends are tied behind the leather collar.

As indicated earlier, slight differences of application are noted for various tick stages and species. Adults and nymphs of most tick species (especially those which prefer feeding on the ears) are better emptied into the bag, but larvae, which are normally held in collection glass tubes, are better when the whole tube is left overnight in a closed ear-bag with the cotton-wool stopper removed. The ticks will move from the tube to the ear at their own time and attach. While adults of *Amblyomma variegatum* are known to attach better if males are applied 4– 5 days earlier than females, this can also be achieved by pushing the whole ear of the rabbit into the 2.5 cm x 7.5 cm tick holding tube in a closed bag and left overnight.

Ear-bags for cattle, sheep and goats are made slightly larger and since they do not require collars they do not require cords at the top end of the bags.

Ticks have been fed on other parts of the host's body especially on the neck and back. The most common material used is a soft stockinet which is secured to a patch of skin by the same method as that of the rabbit ears described above.

The most commonly used method involves putting a caged rabbit into a muslin sack with ticks. After the ticks attach, it is transferred from the sack to one of the cages whose false floor is made from wire mesh over a turntable. The ticks, when engorged would drop onto a receptor tray under the cage (Camin *et al.* 1971; George 1971). The second technique is the use of plastic cylinders (zipper vials cut in half) glued directly to the freshly clipped back of a rabbit (George 1971; Gregson 1971) (Fig. 2) A snap-on lid is put on the open end of the cylinder which would be removed as soon as the ticks attach to allow engorged ticks to drop from the host.

Mice have been encased in cylinders made from multi-perforated light metal sheets (Eichenberger 1970) (Fig. 3). Others have caged mice in wire-mesh cages

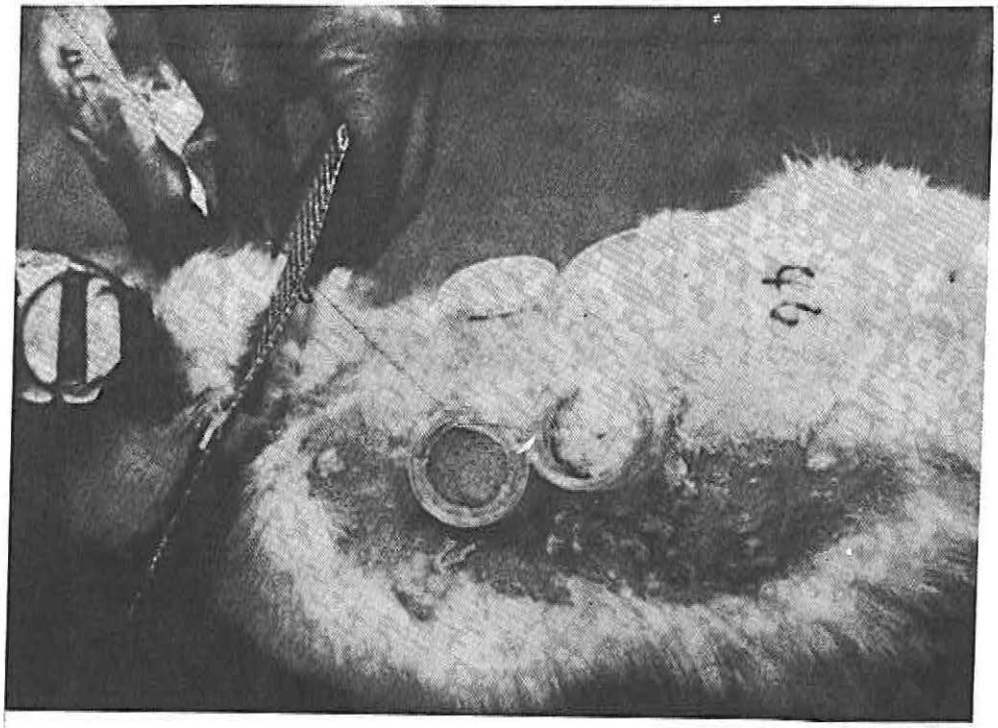

Fig. 2. Plastic feeding chambers glued to the back of a rabbit. This rabbit has a hardware cloth collar (From John E. George 1971)

and the engorged ticks would drop onto a collector tray which is also placed on water so that no ticks can escape. Large animals like cattle have also been infested with ticks over the whole body and the animals made to stand on specially designed pens with collection trays placed below the pens (Bennett 1974) (Fig. 4).

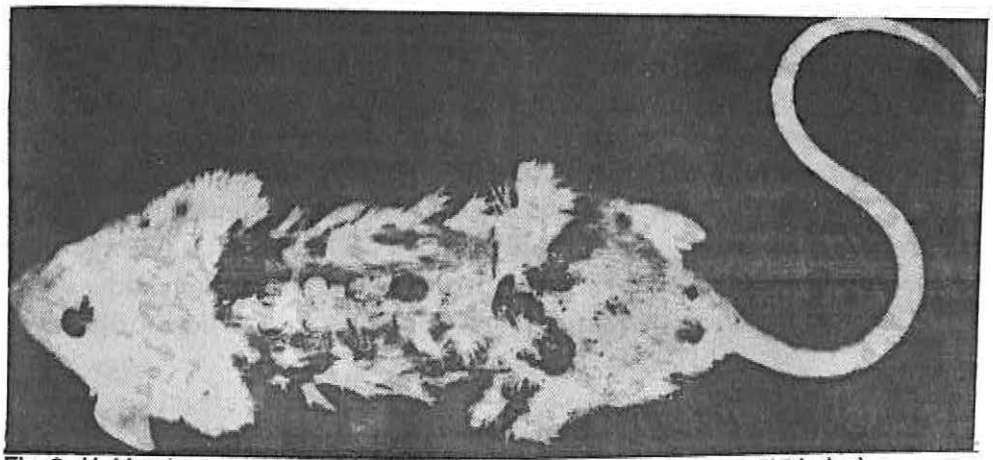

Fig. 3. *H. Marginatum rufipes* nymphae feeding on a corsetted mouse (15th day)

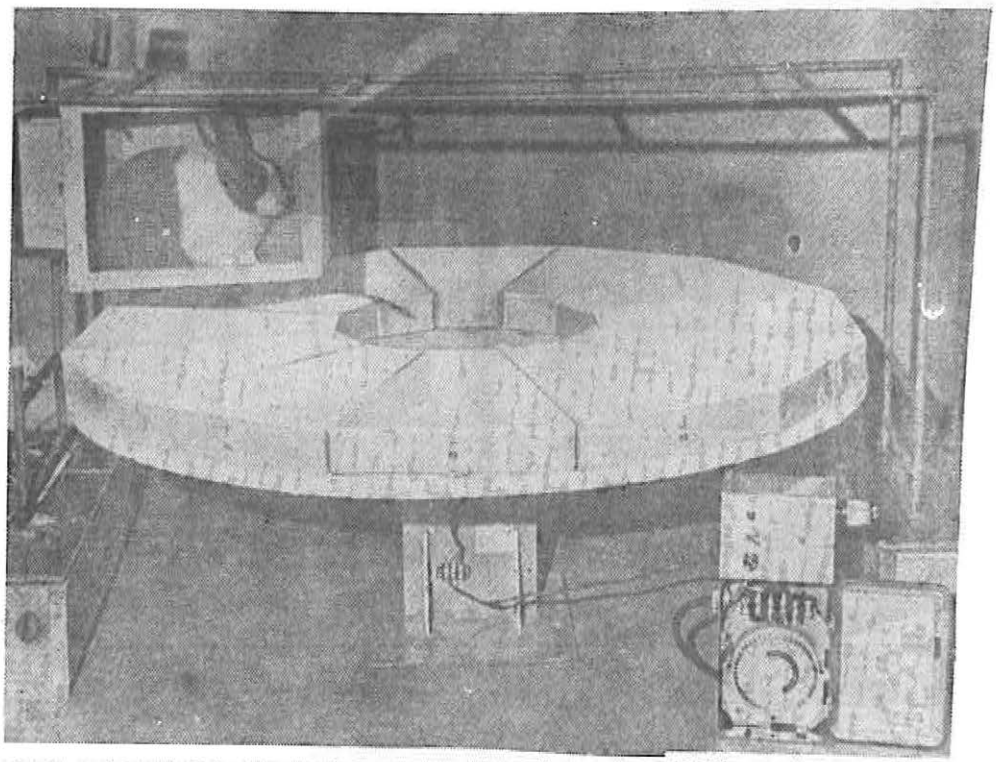

Fig. 4. Automatic tick collector in operation (From Camin *et al.* 1971)

After tick application, from the next day onwards, the ear-bags or any tick feeding chambers should be opened and checked for tick attachment (Fig. 5). This is done by removing the adhesive tape at the end of the bag. Access is therefore gained to the ear and any unattached ticks should be discarded. From the 3rd day onwards larvae of the three host species should be expected to drop. Nymphs start dropping from the fourth day onwards, while adults start dropping from the 6th day. These periods, however, differ from species to species. The two host tick species may not drop until after several days and for the one-host species adults would drop after 21 days. Detached engorged ticks should be collected into a white enamel tray and sorted out. Engorged ticks, especially the larvae, have a very delicate integument and should be handled with great care. Using a hair brush the engorged larvae should be counted out and put into 12 mm x 50 mm flat bottomed glass tubes in groups of 100–200. A clean non-absorbent cotton wool should be used as a stopper. The cotton wool should be covered by a layer or two of muslin gauze. This is to prevent entanglement of the emerging ticks in the wool which would, in turn prevent ticks from feeding. A piece of blotting paper is put inside the tube to add the surface area and absorb excessive moisture.

Engorged nymphs are much easier to handle. They can be counted out using a pair of soft wide mouthed forceps. They are kept in groups of 100–200.

Engorged females should be counted out and kept individually in 2.5 cm x 7.5 cm flat bottomed glass tubes. The bottom half of the tube should be covered with a clean blotting paper. When eggs are laid, they should not be allowed to come into contact with the glass surface. After the female has finished laying the eggs and she dies, the eggs should be transferred into 12 mm x 50 mm smaller glass tubes for hatching. Due to their delicate state they should be handled carefully by using a soft wide mouthed pair of forceps.

The engorged ticks (larvae, nymphs, adults) after they have been sorted should be transferred to controlled temperature incubators for development in aluminium cans with moistened sand.

Although conditions differ from species to species the most suitable condition is a temperature of 25°C to 28°C and relative humidity of 85% –96% . This can be achieved by using saturated potassium chloride salt solution for moulting (85% r.h.) and saturated potassium sulphate (K_2SO_4) for egg laying and hatching at (96% r.h.) (Winston and Bates 1960). At lower temperatures development is

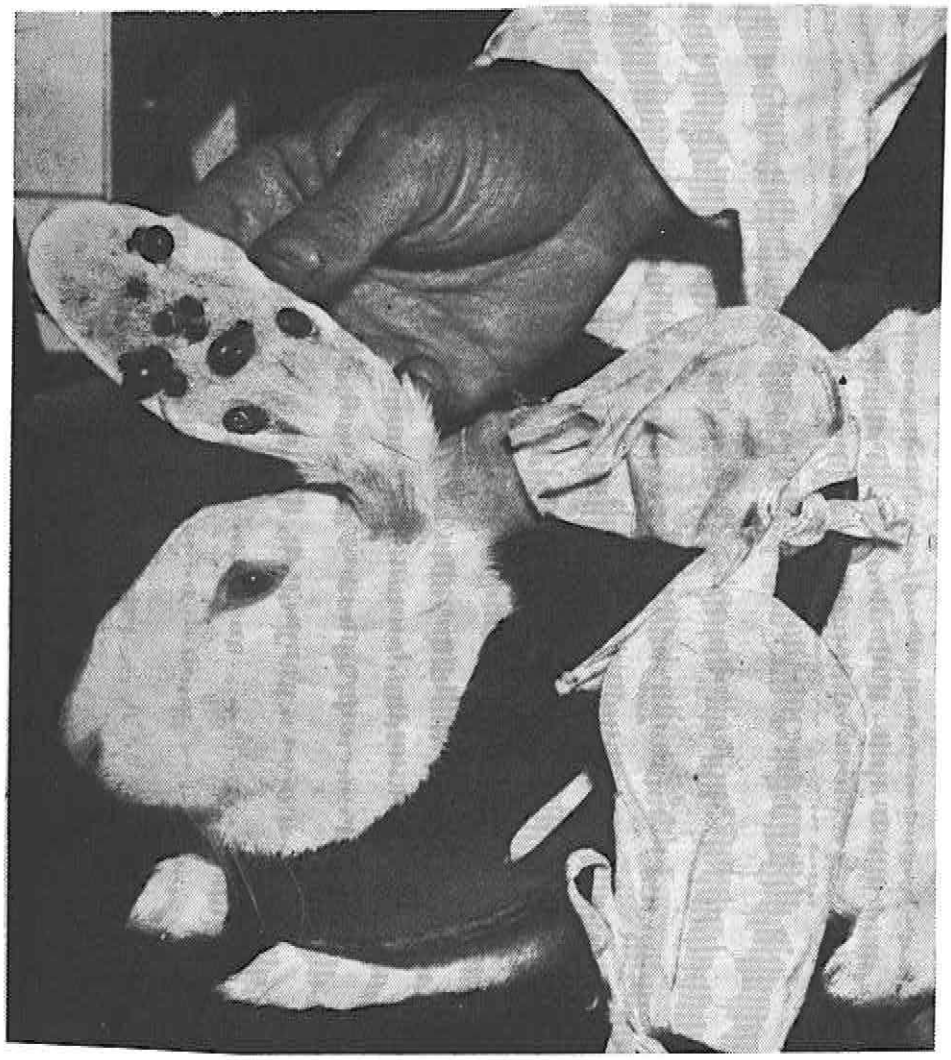

Fig. 5. Photograph showing collar and ear-bags. One ear-bag has been removed to show ticks feeding

slowed. For tropical tick species this process is negligible at 15°C and ceases altogether at 9°C (Punyua 1992).

After moulting or hatching the unfed stages are stored in a storage room which is maintained at 28°C and 85% r.h. Under these conditions the ticks may survive for fairly long periods ranging from 3–6 months for larvae; 9–12 months for nymphs and over two years for adults.

Rearing of Soft Ticks in the Laboratory

Soft ticks or argasids have all multi-host life cycles, while in some species a few stages are non-feeders altogether. This is because of the irregular but numerous feeding nymphal instars and several adult gonotrophic cycles. This implies that they are short time feeders. They also lay several small batches of eggs corresponding with the number of blood meals. For detailed description of the methods of rearing argasid ticks in the laboratory reference should be made to Kaiser (1966). This technique involves the use of screw-capped capsule attached to chicken or pigeon hosts by the use of plastic cement.

Tick Artificial Feeding Techniques

A number of tick artificial feeding techniques are currently available. These are being successfully used for mass rearing of soft ticks in the laboratory. The only known method for feeding hard ticks is still under development. Both of these techniques involve the use of membranes. For the soft ticks, a bat's wing membrane was stretched across one end and firmly attached to the sides of a 44 x 77 mm cylinder open at both ends by means of cellotape or masking tape. The sides of the cylinder was also completely covered with black polyethylene in order to enhance feeding of the ticks in the dark. Batches of 25 ticks were released into the cylinder which was then stood in petri dishes containing defibrinated blood. The petri dishes rested on a photographic dish warmer adjusted to give a constant temperature of 38°C. When fully engorged the females were placed with fully engorged males and kept in pairs in glass tubes. They were observed daily for egg laying and later the eggs were checked daily for hatching (Mango and Galun 1977).

Artificial feeding techniques for mass production of hard ticks are not yet available. Efforts are being made to develop these techniques (Waladde *et al.* 1991). Due to their long feeding periods and other factors, ixodid ticks are not easily induced to feed on artificial membranes. The device described by Waladde *et al.* 1991, involves a Baudruche membrane with one side made rough by the

addition of thin layers of cotton wool and animal hair, held in place with "Evo-Stik" impact adhesive cement diluted with xylene. The opposite side was relatively smooth and water proof. It was coated with two–three layers of the same adhesive alone. After treatment the membrane was dried at room temperature and divided into 6 cm^2 pieces. Each piece was fixed on the end of a hollow glass vessel (3.5 cm x 4 cm) thus forming dishes 4 cm deep, with the rough surface of the membrane facing inside the dish. Traces of xylene and any other toxic agents in the membrane were removed by rinsing the dishes with two–three changes of hot water (90°C) for one hour, and then drying them for 12 hours in an oven at 120°C. Each feeding device consisted of the dish described above which fitted into a second dish containing defibrinated blood. Care must be taken to prevent air bubbles being trapped between the membrane on first dish and the blood in the second dish. Blood should be treated with antibiotics (51 i.u./ml blood). Blood is replaced every 8–10 hours. The feeding device was kept in water bath maintained at 37°C.

The feeding surface to receive ticks formed the inside of the first dish. Cattle ear-wash concentrate and isotonic saline were applied to the membrane. Tick fecal pellets, collected from previous feeding trials, were scattered on two of the membrane preparations. After allowing the blood in the feeding devices to warm up to approximately 37°C, 30 pairs of unfed ticks are introduced into the first dish. Each dish had a confining lid, open at the top, but covered below by a cloth permitting air to circulate between the ticks and the experimental room.

Ticks have also been fed using capillary tubes (Purnell and Joyner 1967) and thin slices of cattle skin, (Kemp *et al.* 1975). These techniques are, however, not suitable for mass rearing of ticks in the laboratory.

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Breeding and Maintenance of Small Mammals for Feeding of Blood Sucking Arthropods

James M. Kagoiya

Insect and Animal Breeding Unit (IABU),
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Abstract

***Haematophagous arthropods are economically important vectors of human and animal diseases. For research scientists to work on the arthropods' ecology, biology and control, a reliable sustained artificial production of quality target haematophagous arthropods is required. These selected arthropods are fed either on mammals* (in vivo) *or on artificial blood* (in vitro).**

Mammals which have been used to feed haematophagous arthropods include goats, sheep, calves, rabbits, guinea pigs, rats, hamsters, mice, lizards and chicken.

This paper outlines methods which have been used to breed and maintain rabbits, rats, mice and hamsters for feeding blood sucking arthropods, i.e., tsetse flies, ticks, mosquitoes and sandflies at ICIPE.

Introduction

Laboratory mammals are living vertebrates (excluding man) that receive care and are kept more comfortable as compared to pets or to animals on the farm or in wild state. They are generally used as biological reagents or research tools for the investigation of problems pertaining to animals or human health and welfare. Laboratory mammals are bred in different conditions, management, housing and

feeding. The methods chosen for breeding are dependent on the quality and quantity required by the users. Breeding of laboratory mammals under commercial conditions may be different from those bred in the laboratory.

The methods discussed are the ones which have shown good results in breeding small laboratory mammals for feeding haematophagous arthropods.

1. Breeding facilities	The breeding facilities comprise routine breeding and experimental rooms for rabbits, rats, mice and hamsters. The walls of the rooms are finished with smooth impervious washable surface materials while the floors are made of reinforced concrete to overcome dentition by racks, trolleys and acids (urine). All windows provide maximum light and yet exclude direct sunlight. They are fitted with mosquito wire mesh to keep off vermin. The breeding and experimental rooms have forced air ventilation.
2. Cages	The mammals live in modern standard cages made of plastic materials, aluminium, metal or stainless steel.
3. Quality of mammals	The quality of the laboratory mammals has been improved over the years by the introduction of genetically proven breeders from reputable sources e.g. New Zealand white rabbits from Hylyne, England.
4. Feeds for mammals	Rabbits are fed on a specially manufactured balanced diet containing known ingredients that include extra vitamin E and K but no coccidiostat (see Table I). Rabbits fed on a diet containing coccidiostat have been implicated when laboratory reared tsetse flies maintained on those rabbits have died en masse. Rodents are also fed specially formulated diet in cube form.
5. Table 1:	Materials

Special rabbit pellets without coccidiostat but	(i) Pollard	–	30.5%
	(ii) Wheat bran	–	50.2%
	(iii) Soya bean meal	–	7.0%

with extra Vitamin E and K	(iv) Herring fish meal	–	2.5%
	(v) Bone meal	–	1.1%
	(vi) Breeder's premix	–	2.5%
	(vii) Lime	–	1.2%
	(viii) Vit. E mix.	–	2.5%
	(ix) Vit. K mix.	–	2.5%
	Moisture content	–	<10%
	Expected nutritive value of the diet		
	(i) Protein	–	18%
	(ii) Nitrogen free extract	–	52.0%
	(iii) Fat	–	2.5%
	(iv) Ash	–	7.3%
	(v) Fibre	–	12.0%
	(vi) Vitamins	–	142.72 ppm (Variation)

6. Cleanliness

In a system of intensive small laboratory mammals production, a high standard of hygiene and cleanliness is of primary importance and in this regard the motto is "clean it when dirty". Failure to observe this motto could result in high mortalities. Entry to breeding areas is restricted.

7. Quality control

(i) Regular assessment of solid parts in mammals blood.
- WBC, RBC and HB counts.
- Packed cell volume

(ii) Rabbit weaners should weigh 1.5 kg and above at 8 weeks. Rats should be 50 g, mice 10–15 g and hamsters 50 g at weaning.

8. Ethics on small mammals care

The small mammals require technician's total attention as they are caged. We love animals and inflict no pain or distress. Standard humane methods are used and

carcasses incinerated immediately. We occasionally invite experts on small mammals breeding in order to have their first-hand opinions.

Breeding Methods

The breeding stock is selected from parents with fully recorded history (heredity). This stock becomes the nucleus foundation colony and is isolated. Periodical replacements of this colony is done with only selected animals. The major desirable qualities are:

1. Fertility
2. Fecundity
3. Milk production
4. Maternal behaviour
5. Growth rate
6. Viability
7. General health, vigour and tameness.

Selection of males is considered more important than selection of females since there are few males as compared to number of females present in the colony.

The common breeding methods are:

1. Permanently mated groups:

(i) Monogamous — one male is allowed to run with one female.
(ii) Polygamous — one male lives with several females. Females permanently mated normally have postpartum estrus e.g. rats and mice.

2. Temporarily mated groups:

(i) Harem system — males and females are allowed to run together but are separated prior to parturition e.g. rats and mice.

(ii) Hand mating — the male and female are left together only for mating to occur, e.g. rabbits.

A. The Breeding and Maintenance of Rabbits *(Oryctolagus cuniculus)*

Steps	Key points
1. Procedure for introduction of breeders	• Buy 3–4 months old rabbits from a breeder with a record for producing rabbits of high quality. • Quarantine new breeders in clean standard holding cage (76 x 60 x 40 cm) in a quiet well-ventilated room free from draughts. • Provide plenty of palatable rabbit pellets and adequate fresh water. • Change dirty bedding twice a week and keep the environment clean. • Inspect the breeders daily for signs of ill-health. • Remove any ill breeder; kill humanely and instantly. Incinerate immediately. Disinfect all equipment. Incinerate droppings and beddings. Inform the supplier. • Introduce healthy breeders to production colony after 21 days of quarantine. • Tatoo each breeder's ear and assign it a breeding card attached to its own cage. Record appropriately.
2. Daily management	• Switch on lights each morning. Put on protective attire. Check room conditions. • Inspect the rabbits and isolate sick ones. Kill humanely. • Put dead rabbits into plastic paper bags. Label appropriately. • Take dead rabbits for postmortem and other pathological investigations. Incinerate remains immediately.

Steps	Key points
	• Feed does with clean rabbit pellets ad-libitum and offer plenty of fresh water.
3. Cleaning the animal cages	• Remove rabbits gently from cages. • Assemble all the dirty cages and their accessories to washing room. • Using hot, soapy water (Teepol) clean the equipment with a hand brush with nylon bristles. • Immerse the cages and their accessories into a suitable disinfectant. • Rinse with plenty of clean water under pressure (hosing). • Dry the cages and other accessories. • Put the cages back to their racks. • Return the animals gently.
4. Mating	• Take a mature doe (5–6 months) to a buck (6–7 months old) for mating. • Allow the doe to be mated twice. Return her to its cage and record date of mating, name and ear number of buck on a card attached to the front of her cage. • If mating does not occur, bring the female back after 6 hours. • Does can be assisted by hand mating:

Steps	Key points
	(i) Restrain the doe in the buck's cage. (ii) With right hand hold the ears and loose skin on the shoulders. (iii) Place the left hand under her body and between the hind legs. (iv) Lift the rear quarters to normal height for service. (v) After the buck mounts, the doe will normally respond and lift her weight from the hand. • Use the buck only for 2–3 times a week for short periods. • After 13–14 days, palpate the doe's abdomen gently to feel the presence of developing foeti which feel like 2 cm marbles. • A doe not successfully mated should be mated after 14 days.
5. Littering and management of offspring	• As average gestation period is 30 ± 1 days, put in one corner of the cage a nesting box (40 x 30 x 25 cm) containing clean, dry and soft wood shavings about 2–3 days before parturition which normally occurs in the mornings. • After birth do not disturb the female. • Check for any deformed or dead young ones which should be removed after 1–2 days. • Rub your hands in the bedding materials of nesting box before handling the young.

Steps	Key points
	• Leave the doe with 8 young ones and transfer the rest to another doe with a smaller litter before they are 3 days old.
	• Offer clean carrots daily to lactating doe to stimulate appetite.
	• Take a healthy doe for mating 4 weeks after littering.
6. Weaning	• Remove young ones from the mother after 8 weeks.
	• Sex the weaned rabbits and pair females to a single holding cage at 2,000 cm^2 floor area and 40 cm height.
	• Keep males individually after 12–14 weeks.
	• Keep rabbits for breeding separate. They should be from parents with desirable qualities (see breeding methods).

Fig. 1. Handling a rabbit during sexing

Steps	Key points
	• Mark ear of preserved young breeder (tattooing) and record the history appropriately on a card attached to the front of the cage. • Supply the rest of the animals to users as required.
7. Sexing	• Handle the weaned rabbits gently. With the right hand grasp the loose skin on the rabbit shoulders and place the animal on your lap (see Fig. 1). • With your left hand gently press open the sexual aperture with the thumb and forefinger. • Notice that in females a longitudinal slit is observed; in males the opening is round and the male organ can be made to protrude (see Fig. 2).
8. Handling	• To remove a large rabbit (>2 kg) from its cage, you need to control the head movement. Be confident and relaxed.

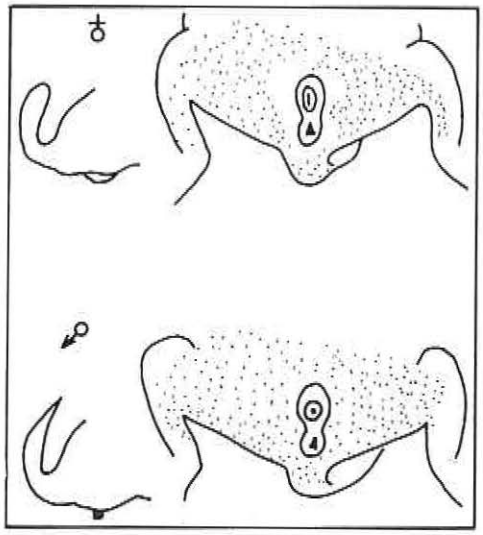

Fig. 2. Sexing of young rabbits

Steps	Key points

- Grasp the loose skin over the shoulders with the right hand and slide the left hand from the front of the rabbit under its body and between its hind legs.

- Notice that as you lift the animal, the legs will have nothing to kick against and that its body weight will be on the forearm. In order to move about with the animal, let the rabbit lie on your chest without releasing the scruff and place your other hand under its hindquarters to support the weight of the body (see Fig. 3). You may alternatively tuck the rabbit head under your left arm and support the hindquarters well. While in this latter position ensure that the eyes are covered and nostrils are free.

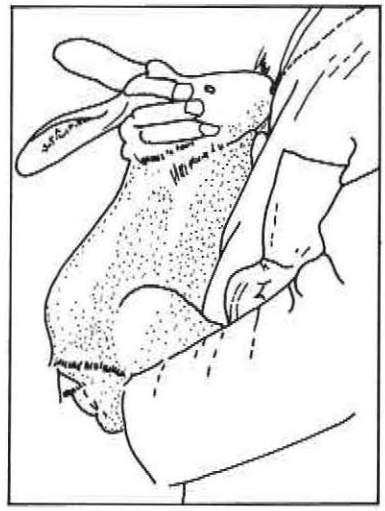

Fig. 3. Handling an adult rabbit

Steps	Key points

- Place the rabbit on non-slippery floor and make it feel secure and relaxed.

- To return the rabbit to its cage, prevent it kicking with its powerful hind legs by returning its rear end first.

Steps	Key points
	• While transferring a rabbit less than 2 kg from one cage to another, lift it by grasping the loin region firmly but not too tightly. The heel of the right hand should be towards the tail of the animal.

B. The breeding and maintenance of rats, mice and hamsters

Steps	Key points
1. Selection of breeders	• Obtain breeding stock from a reputable reliable breeder. • Obtain rodents that have desirable qualities (see breeding methods).
2. Supply and demands	• Check the levels of animals projections (anticipated demands) by users to determine the off-take levels of breeders so as to produce only what is required. • Quarantine new breeders for 21–30 days. • Assemble breeders in clean standard breeding cages in a quiet room free from draughts.
3. Mating	• Prepare appropriate clean cages for breeding and add clean dry soft wood shavings as beddings. • Select two sexually mature females and one male from the best lines and put them gently into each cage (outbred strains). • For inbred strains, select a breeding pair which should be brothers and sisters or close cousins. • Label the cages and enter history of animals into stock breeding records.
4. Daily management of rodent colonies	• On entering the rodent breeding room switch on the lights; put on protective overalls and check if air ventilation system is working.

Steps	Key points
	• Check the condition of animals and remove any dead or sickly looking ones. Kill the sick animals humanely and incinerate immediately. • Feed all rodents with special cubes ad-libitum. Carefully check in corners of the hamster cages for presence of hidden food before filling their food hoppers. • Give the rodents clean and fresh water using polythene bottles with ball tip nozzles.
5. Littering	• Note: The rodents have post-partum estrus. Gestation period for rats and mice is between 19–23 days while that of hamsters is 15–17 days. The average litter size for rats and mice is 8 while that of hamsters is 5. • Ensure the beddings are clean.
6. Weaning	• Prepare clean weaning cages (80 x 60 x 35 cm) and put clean and dry soft wood shavings as beddings. • Open the animal cages and transfer all the animals to be weaned into weaning cages after sexing them. Rodents are weaned after 3 weeks. • Put a maximum of 30–40 rats/hamsters and 60–80 mice into each weaning cage. Reduce stocking density level by 50% after 14 days. • Select animals for breeding from the best lines during weaning. • Record on card attached to the cages the number of animals weaned. Record the same in the stock breeding book.

Steps	Key points
	• Hold all weaned animals in a quiet, clean and well ventilated room for another 2–3 weeks before supplying to users.
	• Demate females when demand for animals is anticipated to be low.
	• Note: Economic breeding life for rats is 1 year, mice is 10 months and hamster is 8 months.

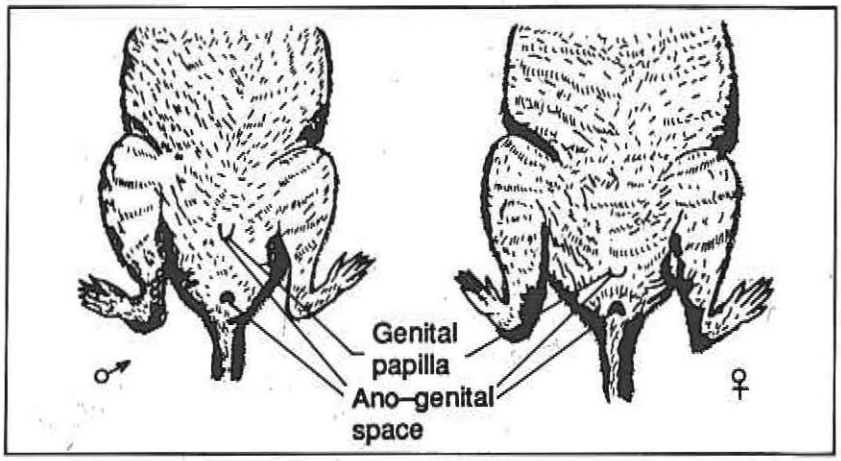

Fig. 4. External sex differences in young rats and mice

Steps	Key points
7. Handling and sexing rodents	**Mice (*Mus musculus*):** • Lift a mouse by holding the base of the tail between your thumb and forefinger. Support the weight of the body. A group of mice can be scooped up with both hands. • For sexing, allow the mouse to grip a firm base like the top of wire grids of cage. It will pull away. • Gently lift the hindquarters to reveal the presence of genital organs. The anal genital distance in male is greater than in female (See Fig. 4). The teats can be seen in females as this area has no hair. Descended testicles can be seen in males.

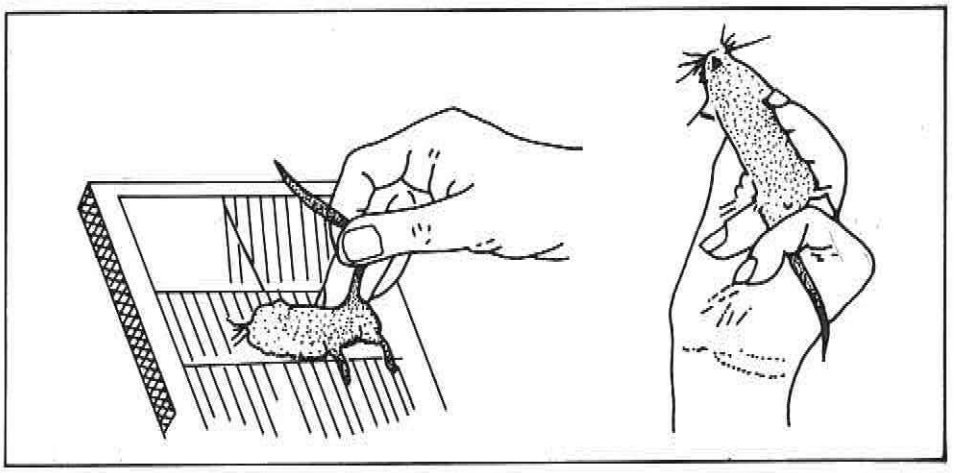

Fig. 5. Sexing and restraining a mouse

Steps	Key points
	• To restrain a mouse for injection gently grasp the skin of the neck between your thumb and finger. Turn the hand so that the mouse lies on its back. Restrain tail by the little finger (see Fig. 5).
	Rats *(Rattus norvegicus)*
	• NOTE: When approaching a rat, never hesitate, fumble or frighten the animal by waving the hands about. Be confident and relaxed.

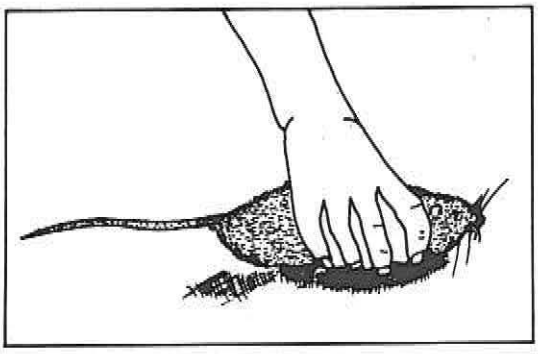

Fig. 6. Picking an adult rat

Steps	Key points

- Place the thumb and forefinger around the shoulder and chest and use the remaining fingers to lift the animal. Grasp the rat firmly but not too tightly (see Figs 6 & 7).

- Support larger rats with other hand while lifting (see Fig. 8 on restraining).

- Handle young rats during sexing as in mice.

- Note that in adult rats, the testicles of the male account for the distended area below the base of the tail.

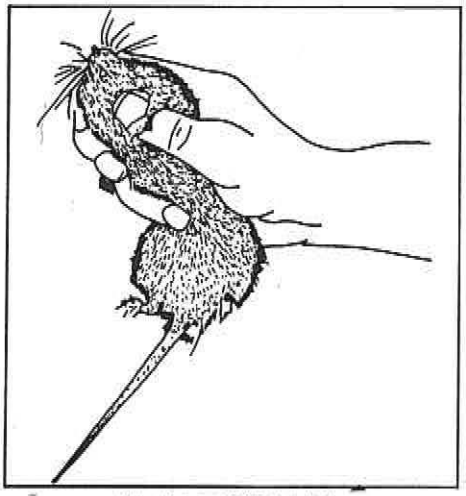

Fig. 7. Handling a rat

Steps	Key points

The Syrian hamster (*Mesocricetus auratus*)

- Using both hands scoop up a hamster (see Fig. 9). Alternatively pick it by the scruff of the neck.

- While cupped in the hands, roll the animal on its back to expose testes and scrotal sack in males.

Steps	Key points
8. Changing dirty beddings	• Remove cage from rack onto bench of collecting trolley. • Open the lid and place gently all the animals from the dirty cage into a clean cage with clean dry soft wood shavings. • Check the health of animals.

Fig. 8. Restraining an adult rat

Steps	Key points
	• Put the cage with animals back to the rack. • Scrape off dirty wood shavings from the floor of the cage, into a plastic paper bag in a waste collecting bin. • Assemble all dirty cages in washing room. • Using high pressure hosing clean the cages with plenty of water, liquid soap and handbrush.

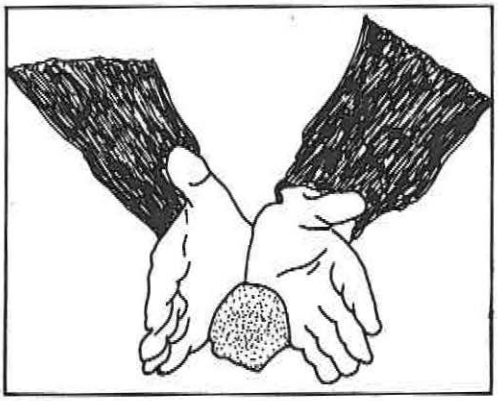

Fig. 9. Cupping a hamstar

Steps	Key points
	• Immerse the cages in a suitable disinfectant e.g. diluted Berol 25S or Tyrad (Jeyes). • Rinse the cages with adequate flow of clean water. • Put the clean cages on a drying rack. • Depending on the stacking level of each cage, change the dirty beddings 2 or 3 times a week.
9. Cleaning plastic water bottles	• Clean thoroughly all water bottles once a week with a bottle washing brush and a sterilising agent e.g. Milton. • Rinse the containers thoroughly with plenty of water to avoid any chance of toxic residues. *Note:* Remember cleaning is the foremost weapon in controlling diseases.

Conclusion

Laboratory mammals bought from outside may be diseased or under-nourished. We have managed to breed and maintain better laboratory mammals than average for the feeding of haematophagous insects. This has made it possible for the successful rearing in laboratories of various species of tsetse flies, ticks, mosquitoes and sandflies for research work.

The factors that have contributed to the successful breeding of mammals include the availability of selected breeding stock, proper housing, feeding, rigorous cages and rooms cleaning and disinfection, quality control and above all attention to details.

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