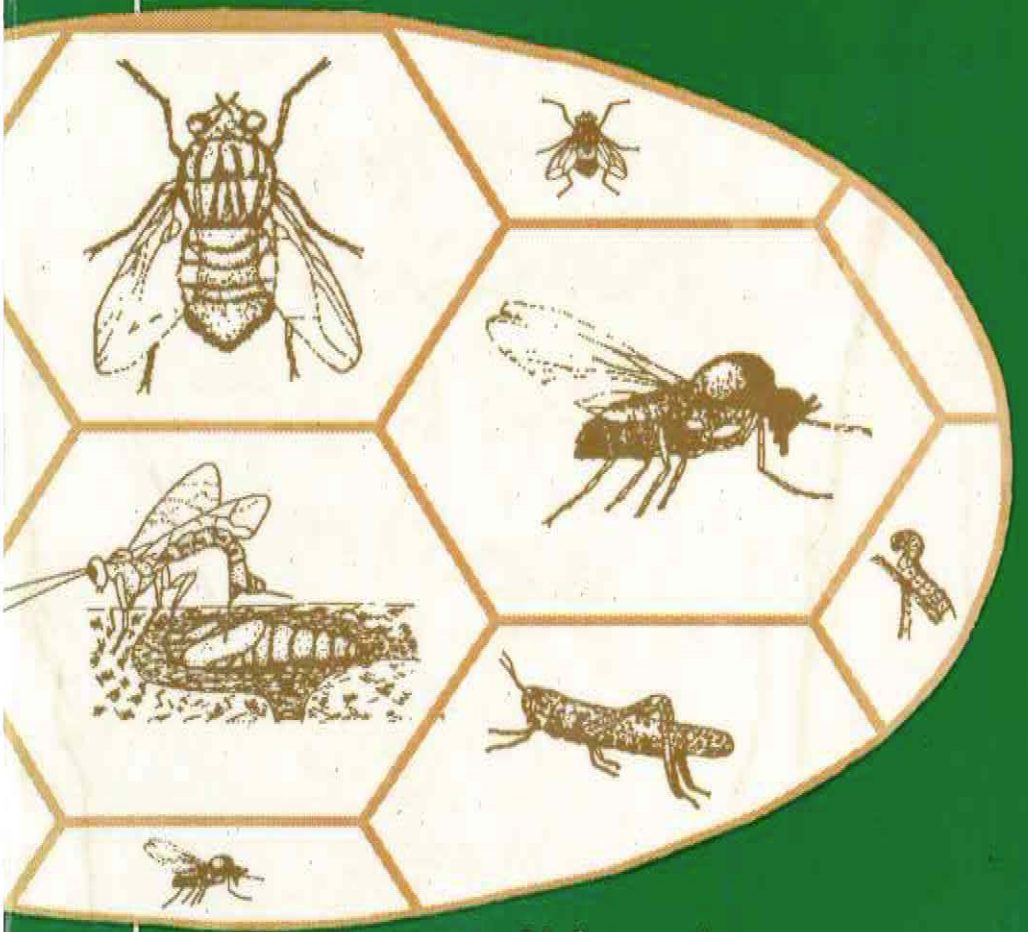
Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies

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



Volume 1

ICIPE
Science
Press

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

Volume 1

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

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
ICIPE, Nairobi

Table of Contents

Preface, *Thomas R. Odhiambo* v

VOLUME 1

(A) CURRENT STATUS AND FUTURE TRENDS IN INSECT REARING

Insect Rearing, the Sterile Insect Technique and the Management of Insect Vectors, *U. Feldmann* 1

Some Quality Control Parameters Used in the Rearing of Tsetse Flies, *U. Feldmann* 13

Contribution of Insect Rearing to the Management of Rice Pests, *R. C. Saxena* 31

Parasitoid *in vitro* Rearing: Successes and Challenges, *S.B. Vinson* 49

(B) METHODS OF LABORATORY COLONISATION OF INSECTS

Methods of Colonisation and Quarantine of Insects, *G. Hill* 107

Methods of Colonisation of Tsetse Flies, *H. K. Bandah* 119

(C) PRINCIPLES IN THE DEVELOPMENT OF INSECT REARING METHODOLOGY

Basic Requirements for Artificial Rearing of Insects, Mites and Spiders, *J.P.R. Ochieng'-Odero* 131

Principles for Insect Colony Management, *J.P.R. Ochieng'-Odero* 139

Basic Principles of Diet Development, Establishment and Feeding of Phytophagous Insects on Artificial Diet, *J.P.R. Ochieng'-Odero* 159

Contents

Ethological Aspects of Rearing Insects, *S.A. Lux* 173

(D) PRINCIPLES OF QUALITY ASSESSMENT AND CONTROL

Quality Control in Insect Rearing Systems, *F. Bigler* 189

Nutrition and Quality Control in Mass Rearing of Phytophagous Insects, *G.L. Bernon and N.C. Leppla* 211

Microbial Contamination in Insectaries: Contamination, Prevention and Diseases Identification, *P.P. Sikorowski and A. M. Lawrence* 221

Identification of Insect Pathogens Common in the Insectary, *M. O. Odindo* 247

Animal Health Implications in the Laboratory Breeding of Small Mammals (With Particular Emphasis on Rabbits), *I.G. Onyango* 273

Insect Rearing and Inhalant Allergies, *R. Kfir* 277

VOLUME 2

(E) THE REARING OF BENEFICIAL ORGANISMS

Rearing Parasites of Lepidopteran Stalk Borers of Tropical Gramineous Plants, *J.W. Smith Jr., and R.N. Wiedenmann* 285

Rearing of Predatory Mites using Artificial Food Source. A Case Study of the Phytoseiid Mite *Neoseiulus teke* Prichard and Baker, *R.S. Ochieng* 307

The Rearing of *Trichogramma*, *F. Bigler* 315

The Rearing of *Cotesia flavipes* for Classical Biological Control of *Chilo partellus*, *W.A. Overholt* 331

(F) REARING OF PHYTOPHAGOUS INSECTS

Rearing the Pink Stalk Borer, *Sesamia calamistis* and the Sugarcane Stalk Borer *Eldana saccharina* , *Z.T. Dabrowski* 349

Standard Rearing Procedures for the Spotted Sorghum Stemborer, *Chilo partellus*, *F.O. Onyango and M. D. O. Bungu* 367

Rearing of *Cicadulina* Leafhoppers, *Z.T. Dabrowski* 377

Standard Rearing Procedures for the Pod Borer, *Maruca testulalis* on Artificial Diet, *F. O. Onyango and P. O. Wagara* 393

Techniques for the Rearing of the Pod Borer *Maruca testulalis* (Geyer) on Host Plant, *R.S. Ochieng'* 401

Standard Rearing Procedures for the Maize Stem Borer *Busseola fusca*, *F.O. Onyango and A. G. Nyangwara* 407

Rearing the Cotton Bollworm, *Heliothis armigera*, *R. Kfir* 417

Methods for the Rearing of the Banana Weevil, *Cosmopolites sordidus* (Germar 1824), *A.M. Koppenhoefer* 421

Techniques for Rearing the Desert Locust, *Schistocerca gregaria* (Forsk.), *S.M. Ndugo* 433

(G) REARING HAEMATOPHAGOUS ARTHROPODS

Guidelines for the Rearing of Tsetse Flies Using the Membrane Feeding Technique, *U. Feldmann* 449

Standard *in vivo* Rearing Procedures for Various Tsetse Species, *J.U. Wanyonje* 473

Rearing of Phlebotomine Sandflies, *M.J. Mutinga and C.C. Kamau* 483

Rearing of Ticks Under Laboratory Conditions, *D.K. Punyua* 505

Breeding and Maintenance of Small Mammals for Feeding of Blood Sucking Arthropods, *J.M. Kagoiya* 519

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

VOLUME 1

Nutrition and Quality Control in Mass Rearing of Phytophagous Insects, *G.L. Bernon and N.C. Leppla* 211

Microbial Contamination in Insectaries: Contamination, Prevention and Diseases Identification, *P.P. Sikorowski and A. M. Lawrence* 221

Identification of Insect Pathogens Common in the Insectary, *M. O. Odindo* 247

Animal Health Implications in the Laboratory Breeding of Small Mammals (With Particular Emphasis on Rabbits), *I.G. Onyango* 273

Insect Rearing and Inhalant Allergies, *R. Kfir* 277

A. Current Status and Future Trends in Insect Rearing

Insect Rearing, the Sterile Insect Technique and the Management of Insect Vectors, *Udo Feldmann* 1

Some Quality Control Parameters Used in the Rearing of Tsetse Flies, *Udo Feldmann* 13

Contribution of Insect Rearing to the Management of Rice Pests, *R. C. Saxena* 31

Parasitoid *in vitro* Rearing: Successes and Challenges, *S.B. Vinson* 49

Insect Rearing, the Sterile Insect Technique and the Management of Insect Vectors

Udo Feldmann

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

Insects are reared for various reasons, such as for industrial purposes (e.g. *Bombyx mori*), for applied or basic research (e.g. *Drosophila melanogaster*) or for use in pest insect control programmes. The latter use involves the release of either laboratory reared beneficial insects that attack other species, i.e. entomophagous predators or parasitoids, or mass-reared genetically altered insects that specifically interfere with the reproduction of their own or very closely related species or subspecies.

The decision to use a genetic autocidal method of pest insect control may be made for various reasons:

- The prolonged use of other control methods may have led to the development of resistance, i.e. either resistance to the effects of an insecticide or behavioural resistance to attractants or other components used for control. A control programme that continues to rely on such methods is unlikely to meet its objectives.

- Many other methods of pest insect control have an insufficient target specificity which leads to undesirable side effects, such as when beneficial insects that normally keep potential pests under control are decimated. Damage to such beneficial predators and parasites may induce increase in insect numbers to reach pest status (secondary pest outbreaks). In addition public concerns are promoting legislative restrictions on the use of environmentally damaging methods of insect control.

- Some applications of genetic control methods have an attractive economic cost/benefit ratio, especially in integrated programmes when the population density is first suppressed by other methods.

The Sterile Insect Technique (SIT) is one of several methods of genetic control of pest insects. The principles of the SIT were first described by Knipling in 1955 and have since been applied for eradication or control of several economically important insect species. This method involves the following:

- reliable means to mass-rearing target insects,
- distributable excess of the insect that can be sexually sterilised by γ-radiation,
- sterile males that when released into the target area compete with native males of their species for mating, and
- females mated by sexually sterile males will not produce offspring.

Thus, the release of competitive sterile males that sufficiently outnumber the native fertile males results in a drastic reduction in numbers of offspring produced in the next insect generation. Provided (a) the released sterile males sufficiently interfere with the reproductive potential of the entire target population, (b) the releases are made over several generations and (c) either the invasion of mated wild females into the target area is prevented or the insect population is tackled within its entire distribution area (confined by climatic, geographic or genetic borders), the SIT can result in eradication of the target pest insect.

A pest control programme that utilises the SIT needs the coordination of many different activities. These activities are summarised under three main topics: 1. insect mass-production, 2. transport and release into the target area and 3. field related and other public project activities that can be summarised as pest management.

1. Mass-Rearing of Insects

Area-wide pest insect control programmes that use the SIT depend during a defined time period, on a consistent supply of large numbers of highly viable insects. Some of the factors that can have an impact on the quality and quantity of distributable sterile insect material are:

- Staff have to be technically qualified and sufficiently dedicated to work in daily shifts, possibly 7 days a week under insectary conditions that are unpleasant. A routine on-the-job training is beneficial for motivation of junior staff.

- Rearing procedures need to be developed that reflect the following keypoints:

  - Different developmental stages may require distinct maintenance conditions and handling methods that reflect the life cycle of the insects in nature.

  - Quality criteria for rearing the different insect stages need to be determined and implemented by a quality assurance system. This system must be continuously reviewed and updated.

  - During the planning and development stages of standardised rearing methods, it is important to consider and make provision for the automation of fly handling procedures.

  - If the rearing depends on the permanent availability of plants or animals or of their products, it is essential to assess the risk of varying availability, quality and price of such items on the production system. Alternatives must be explored such as an inexpensive high-quality artificial substitute.

  - If there is only seasonal demand for sterile insects, the possibility of using their diapause to stock-pile them until needed should be explored. Thus expenses for rearing can be minimised and the deployment of immature stages of sexually sterile insects can be organised in a way that ensures synchronised emergence with that of the native insects.

  - Special efforts should be undertaken not to release damaging stages of the insect. For example, development of genetic sexing strains of *Ceratitis capitata* permit, during rearing, the elimination of female flies that damage fruit by oviposition. This also results in diet savings and when males only are released, their mating potential is not diluted by the presence of sterile released females.

- The design of a mass-production facility and the development or selection of equipment takes into account various factors including the following:

  - External climatic conditions including diurnal and seasonal variations in temperature, humidity and direct exposure to sunlight will influence decisions on insulation of the building and the capacity of equipment for air conditioning, humidification, etc.

  - Provision of special construction and installation of back-up equipment is needed to ensure a constant supply of electricity and water.

  - Preventive measures such as air filter systems or positive air pressure should be incorporated in the planning of the rearing area to protect mass reared insects from disease, chemical contamination or parasitisation.

  - Appropriate countermeasures such as air filters and fly proof systems should be taken to protect employees from insect allergens, e.g. scales of Lepidoptera wings or saliva of haematophagous insects.

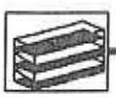
  - Means must be available to dispose of large quantities of waste materials from a mass-rearing facility.

  - Mass-rearing compartments should be arranged to facilitate the sequential steps of handling and incubation of the different insect stages.

  - Equipment should be used that standardises incubation conditions, facilitates or automates laborious handling procedures and permits the simple conduction of quality control.

  - All insect holding or handling procedures that have narrow and defined climatic or other quantifiable limits of tolerance need to be connected to an alarm system.

- The insect mass-production facility requires adequate maintenance and repair services.



- A constant and reliable supply of consumables and other materials is needed to insure the uninterrupted production of good quality insects.

The above points and prerequisites, while not complete, should give an impression of the complexity of the requirements needed to run an efficient insect mass-rearing facility.

Decisions on the most suitable type of a rearing facility to build (e.g. stationary or mobile) depends on the nature and location of the field programme.

- A stationary insect factory may be preferred for national campaigns, where the treatment area is relatively small and there are no plans to provide insects for programmes beyond the national borders.

- Large breeding centres capable of providing sterile insects to several neighbouring countries are advantageous for regional programmes. They should be built close to an airport with frequent and reliable flight connections to the consumers, i.e. pest insect control or eradication projects.

- An alternative would be mobile insect factories, such as on a large ship or a land based container system that can be shifted as the release area changes in accordance with the expansion of the eradication zone.

Advantages or disadvantages of above systems will not be discussed here.

2. Transport and Release of Sexually Sterile Insect Material

Methods for radiation sterilisation of insects are relatively simple and have been described elsewhere in considerable detail. Even so, it must be pointed out that the handling procedures during γ-radiation must be designed to ensure that only treated, i.e. sexually sterile insects, can be dispatched and that fertile flies cannot be accidentally released.

Methods for packing, transport and release should aim at the transfer of high quality sterile insects from the factory to the consumer with no or only negligible losses in survival and competitiveness. Sterile insects can be released at different developmental stages:

- For some diapausing insects such as the gypsy moth (*Lymantria dispar*), mass-reared progeny of irradiated adults (F-1 sterility) are stock-piled as diapausing egg masses. These egg masses are distributed in the habitat in spring time so that their hatch is synchronised with that of the fertile wild population.

- For most SIT programmes the late pupal or early adult stage is sexually sterilised by γ rays.

  - If late pupal stages are sterilised and shipped, chilling and/or anoxia can be used to reduce development of metabolic heat and thereby prevent emergence during shipment. Thus large numbers of pupae may be shipped in rather small volumes. The length of time insects can be held under these conditions is limited and must be determined.

  - If emergence of γ ray treated pupae is anticipated during transport, care has to be taken that the sterile adults have sufficient aeration, space to unfold their wings and possibly even food in their container. Packing systems providing these conditions are bulkier than ones with unemerged pupae.

  - When the programme requires relatively few sterile males for release, insects may be sterilised; transported and released as adults.

The transport conditions to which the insects are exposed need to be monitored. For large shipments special electronic equipment is available that record temperature, humidity, mechanical disturbances or the concentration of gases during transport. The recorded parameters should be evaluated immediately upon arrival and returned to the producers to enable them to take corrective action, if required.

The producer and the consumer may belong to the same organisation, but when not the consumer may have to pay for the sterile insects. Then it is essential that standard quality procedures for the product are agreed upon. These may include emergence rate, flight ability and, if the producer has to apply sexing methods that ensure the release of one sex only, the extent of contamination with the undesired sex.

Before starting shipments of (sterile) insects, the relevant national regulations on export and import of insects must be understood and terms agreed upon with the controlling agencies. Sterile insects are perishable and any delay in their transport can result in considerable loss of quality or even total destruction. Relevant agencies to contact include:

- Plant protection and veterinarian quarantine authorities,
- Customs authorities,
- Brokers and importation firms,
- Airlines and other transport businesses.

Sterile insects can be released for SIT programmes in several ways:

- Pupae can be held and flies released from racks positioned at release sites. The racks should provide maximal protection against predation and stress from climatic conditions and may be equipped with food for emergent adults.
- Releases can also be done directly in the habitat by staff opening cages containing sterile adults.
- Aerial releases are preferable in inaccessible terrain or when large areas have to be quickly treated. Aerial releases are usually done by packing and transporting the sterile insects in containers that are released from the aircraft and automatically open to liberate the insects.

3. Insect Pest Management

The relative merits of eradication versus control will not be discussed here; however, it should be pointed out that the SIT can be used to achieve both goals. Unlike most other control methods, the SIT does not bear the risk of selecting for resistant individuals in the target population, provided the established insect quality assurance system includes aspects of insect behaviour that are relevant to the mating success of released sterile males.

Status of Insect Rearing

Before useful insect pest management concepts can be planned and implemented a variety of information needs to be collected on the target area, on the pest and its interdependence with basic nonbiotic factors. Examples are mentioned below:

- The size and extent of infestation and the occurrence of seasonal population fluctuations needs to be determined. Seasonal variations are important for optimally timing operations.

- Socio-economic aspects of the pest and the availability of alternative control techniques need to be assessed.

- For planning field operations, data from intensive field surveys, maps, aerial photography and satellite imagery are very helpful. The latter are particularly useful for determining seasonal climatic changes and related fluctuations in the habitat of the insect pest.

- Information on possible prevailing tolerance or resistance against control methods will influence the planning.

- Preferably an entire pest insect population should be treated within its own geographic, climatic or genetic borders instead of tackling only the portion of a population that is found in a limited project area. If this cannot be done, suitable isolation methods need to be identified to prevent reinvasion by the pest insects.

Before any area-wide pest insect control programme is initiated in an area, information on other ongoing and planned activities in the area should be collected. Programmes or plans that influence the anticipated control methods or the agricultural development must be coordinated. It is essential that at this early stage all involved bodies agree on a detailed land use plan after the programme goals have been achieved.

When the SIT is part of an integrated control or eradication campaign, the following points deserve attention:

- The SIT can either be started *per se* or follow suppression of the population by other control methods. This decision is influenced by economic

considerations, natural pest population levels and fluctuations and by the number of sterile insects that can be supplied by the producer.

- "Conventional" methods for pest insect control are usually more efficient at the beginning of a treatment campaign than later, when the density of the target population is lower.

- The SIT has a converse efficiency pattern. It becomes more efficient with progressive reduction of the target insect population.

- If a pre-release suppression of the insect population needs to be conducted, methods should be selected that have maximal possible target specificity.

- Before initiation of sterile insect releases the number and location of release points or the flight routes of the release aircraft need to be determined, as well as the anticipated density of the released flies.

- The survival of sterile males and their dispersal in relation to the behaviour of like wild males requires frequent monitoring. For some insects, e.g. *Glossina* spp., dissections of females during advanced sterile male release operations provide information on the status of progress towards control or eradication (male induced sterility).

- An efficient pest insect control programme requires long term planning and coordination and flexibility in the day-to-day use of available control methods. Periodic ecological surveys on the target insect population provide information on required modifications of the releases in terms of the location of release points or the required density of sterile males.

- Any information on sterile male quality, their dispersal in the habitat, their behaviour and survival should be provided to the producer.

- Releases are often conducted at sites that are accessible to the public. Uninformed people may object to releases of sterile insects, particularly if a damaging stage is released (sterile female fruit fly stings) or if they cause any inconvenience (bites by blood sucking species). Frequent public infor-

mation campaigns should be held before and during the control operations against the pest insect.

Whenever eradication of a pest insect from the target area is the objective, methods need to be developed that confirm the elimination of the pest.

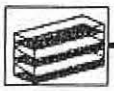
- Detection surveys can be conducted to confirm the eradication of the target insect.

- The elimination of insects that are vectors of animal diseases can be confirmed by the use of sentinel animals. Such animals have not been exposed to the disease or the vector before and are transferred to the eradication area. If sentinel animals show the vector borne disease, the vector control operations need to be continued.

- Some pest insects, such as the tsetse fly, have a very low reproductive potential and a low population density. During advanced control the population may not be detectable by means of conventional trapping. The release, recapture and examination of laboratory reared virgin sterile females for insemination may be a more sensitive indication for the prevalence of relic males.

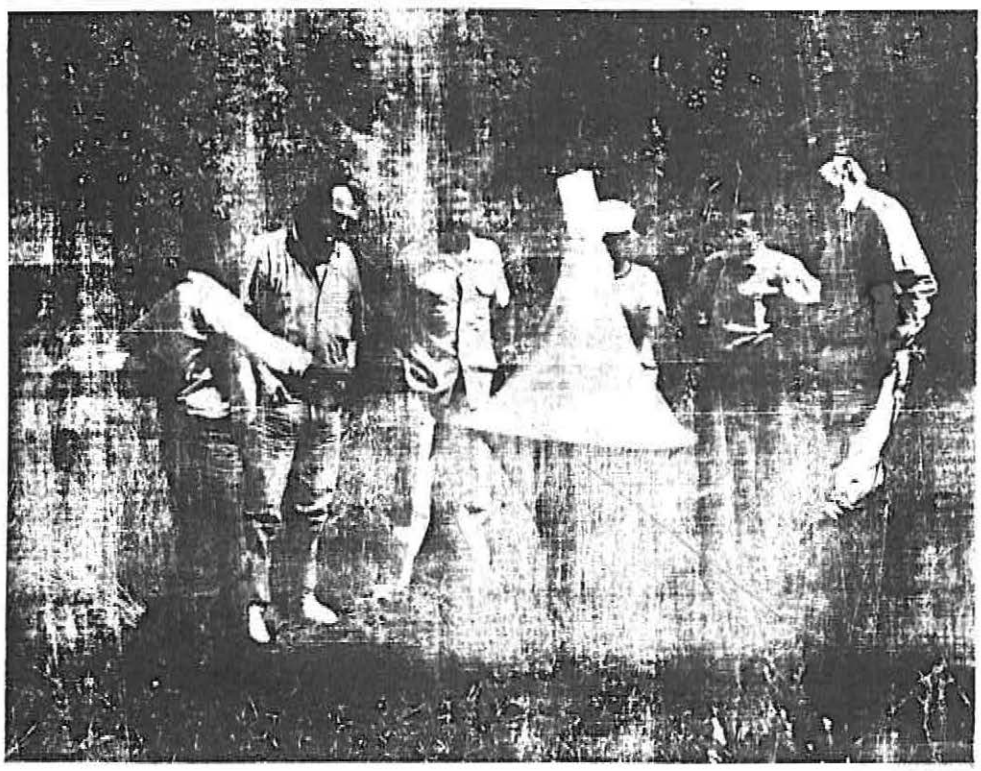
If the control or eradication area is not isolated, suitable barriers need to be erected and maintained. Routine infestation surveys need to be conducted and an action plan should be developed that is implemented in case of an outbreak.

The SIT has been used for the eradication or control of several pest insect species. Recent successes, i.e. eradication, has been reported against the melon fly (*Dacus cucurbitae*) in the Okinawa Islands and against the New World screwworm fly (*Cochliomyia hominivorax*) from Libya, where it had been accidentally introduced. Its effectiveness has been demonstrated against other pest insects, such as the tsetse fly (*Glossina* spp.) but the mass-production requires further refinement before major area-wide programmes can be implemented. Continued efforts should be invested into the improvement of genetic methods of pest insect control, such as the SIT. In addition to environmental advantages they are among the few control methods, against which the development of resistance is unlikely.



Dedication

This paper and two other contributions in the manual, namely "Guidelines for the rearing of tsetse flies using the membrane feeding technique" and "Some quality control parameters used in the rearing of tsetse flies" are dedicated to the late Dr. André Van der Vloedt of the Joint FAO/IAEA Division, who died on 31 December 1991 after returning from a tsetse field mission. Dr. Van der Vloedt was an enthusiastic proponent for the use of the SIT for tsetse control or eradication and we will gratefully remember his energy and commitment to our work for people in developing countries.



The late Dr. André Van der Vloedt, second from right, listening to a demonstration on the ICIPE tsetse trap in the Lambwe valley near Lake Victoria in Western Kenya. His colleagues were part of a review team assembled at the International Centre of Insect Physiology and Ecology (ICIPE) in 1989 to review the insect rearing programme and to evaluate its role in developing new pest suppression technologies

Some Quality Control Parameters Used in the Rearing of Tsetse Flies

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 tsetse fly is an insect with unique nutritional and environmental requirements and reproduction. Both sexes are obligatory haematophagous (blood sucking). Different tsetse fly species have distinct host animal and habitat preferences (Buxton 1955; Boyt *et al.* 1978; Staak *et al.* 1988). *Glossina* spp. that prefer to feed on domestic animals or occur in peridomestic habitats may be of economic importance if they cyclically transmit trypanosome parasites. Basic research and the development of appropriate methods for vector control or eradication require the supply of standard fly material. The use of sexually sterile tsetse flies for ecological monitoring target tsetse fly populations (sterile virgin female releases and recapture) or for target specific tsetse fly control (Sterile Insect Technique) usually require a regular supply of larger numbers of flies from a mass-production facility. The obligation to fulfill such demands for fly material requires very strict quality control procedures in many stages of the production line. Certain aspects of routine tsetse fly mass rearing operations that require special attention are mentioned below.

Optimal environmental requirements of tsetse flies vary with age and developmental stage, viz immature stages, young flies and adult female tsetse flies. Therefore quality control procedures include measures to maintain optimal climatic conditions at all times. All changes observed in quality control parameters (discussed below) should be analysed immediately, whether they are due to poor climatic conditions or other routine fly maintenance procedures. It is advisable to re-calibrate thermohygrographs routinely. A record book on handling procedures

and observations on other factors that may influence tsetse fly rearing should be kept.

Two important aspects of Quality Control that will not be covered in this chapter concern the assurance of optimal fly nutrition.

(i) The rearing of tsetse flies using *in vivo* feeding system requires that quality standards are established for the maintenance of a host animal colony and for the selection of animals used in tsetse fly colonies as "feeders". For guinea pigs, Oladunmade *et al.* (1990) suggested following quality control standards:

Maximum acceptable weekly mortality	1 %
Minimum required female fecundity every 6 weeks	0.6
Minimum offspring weight	75 g
Minimum "feeder" weight	550 g

(ii) Important quality control criteria for the rearing of tsetse flies using the membrane feeding technique, particularly the quality screening of the *in vitro* diet and the handling of special equipment and materials, are described in the chapter on "Guidelines for the Rearing of Tsetse Flies using the Membrane Feeding Technique".

1. Immature Stages of Tsetse Flies

Some *Glossina* spp. colonies, such as *G. tachinoides*, show a distinct diurnal larviposition pattern. Other colonised species lay their offspring more randomly. The duration of the free third instar larva stage differs considerably between different species (Feldmann *et al.* 1992). There is also a large variation within the same species and long crawling periods of third instar larvae correlate with higher weight loss from larviposition to pupation. Adverse conditions during crawling, e.g. low humidity or high temperature, may affect optimal development at this early immature stage. The larvae should therefore pupate in the breeding room. Special quality control measures appear not to be essential for this stage.

Optimal performance of the female tsetse fly, high fecundity and low mortality, is correlated with pupal size and weight. From very small and light pupae the emergence rate and number of strong emergent and viable flies is lower. The size

and/or the weight of pupae is therefore an important quality control parameter for offspring; this in turn is a reflection of the maintenance and feeding of the female fly and the transfer of nutrients from the mother insect to the developing larva *in utero* (Langley *et al.* 1978; Moloo and Pimley 1978). The procedures to follow in order to assess quality control parameters include:

- Collect larvae and pupae in the morning, transfer them to medium-sized dishes that are marked according to the experimental- or colony-unit and allow them to pupate and melanize in the breeding room until afternoon.

Depending on the size of rearing operations three different methods of quality control can be applied at this stage:

(a) • For a small group of flies producing up to 50 pupae daily or for experimental flies the pupae should be weighed individually and the weights are recorded.

- The mean pupal weight should be determined for each unit of females, i.e. a group of experimental flies or a female colony unit (see chapter 2. "Adult tsetse flies" below).

- For routine colonisation, small pupae should be kept apart from other pupae. Weak emergent flies, particularly those from small pupae, should be discarded.

(b) • For tsetse fly colonies producing up to 1,500 pupae per day, all pupae produced by each colony unit should be weighed together.

- The mean pupal weight should be determined for each female unit and small pupae treated as described above.

(c) • For larger tsetse fly colonies producing >40,000 pupae per month and maintained for longer periods, a special pupal size-sorting machine (Zelger and Russ 1976) (Fig. 1) and a pupae counting machine (Fig. 2) may be purchased or constructed. A pupal size sorting machine separates the daily pupae production into five or more distinct weight classes (Fig. 3). It is calibrated once and used thereafter to record the quality of daily pupae production. The counting machine also facilitates the quantification of pupae produced in each weight class.

- Mean pupal weight may be determined and/or the percent of pupae collected in the smallest size group (A-class) can be calculated.

- The small A-class pupae are either discarded or treated like the light pupae mentioned above.

- The average number of A-class pupae in a colony should be below 10%. Fig. 4 shows the age related distribution and the colony average of A-class pupae in a mass reared *G. tachinoides* colony.

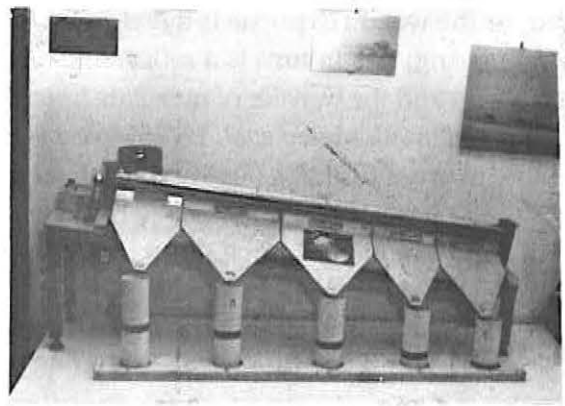


Fig. 1

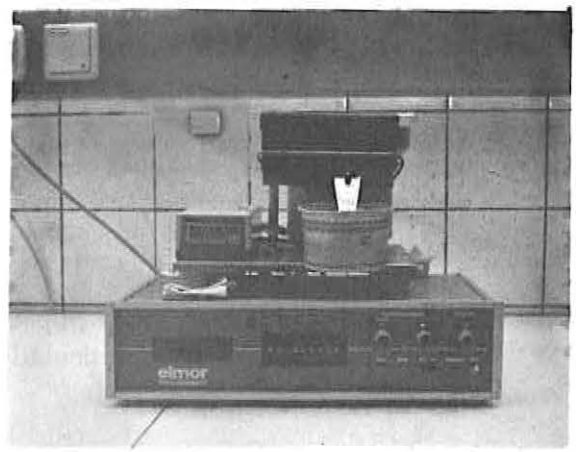


Fig. 2

Thereafter the pupae are handled as follows:

- After weighing or sorting, pupae are returned for incubation to a breeding room (Fig. 5).

- Once per week 100 (non A-class) pupae are removed from a single day's production, transferred to a separate dish and incubated together with other pupae deposited in the same week. These pupae are used as control for evaluation of fly emergence.

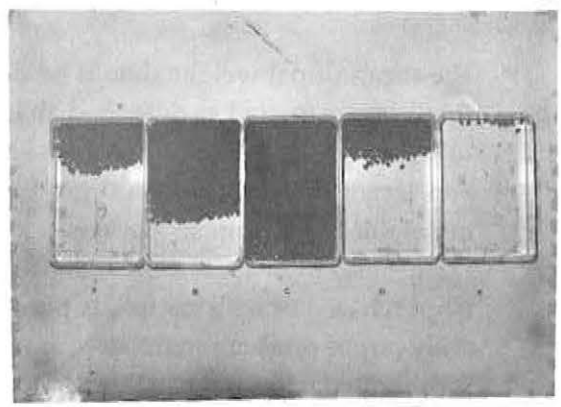


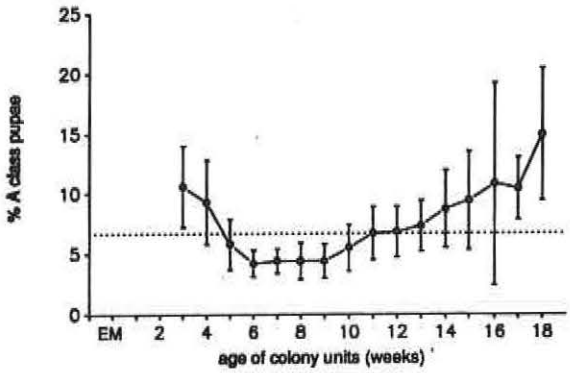
Fig. 3

- About 25 days after pupation, 250 – 300 pupae each are transferred to small dishes and covered with special emergence cages labelled according to the date of larviposition (Fig. 6). For small colonies producing 40, 80 or 120 pupae daily, pupae produced over one week, three and two days respectively, may be pooled into one emergence cage. Pupae for the weekly emergence control, however, are kept apart from other pupae.

- Emerged flies are collected daily. Males and females emerging from the weekly control are recorded separately.

- The percentage emergence rate and the male ratio are calculated. If the EM-rate is below 85%, all non-emergent pupae are dissected and recorded according to following categories (slightly modified after Bursell 1959):

Fig. 4. *Glossina tachinoides* under mass-rearing conditions: Percentage of produced small (=A-class) pupae in relation to the age of the female colony units



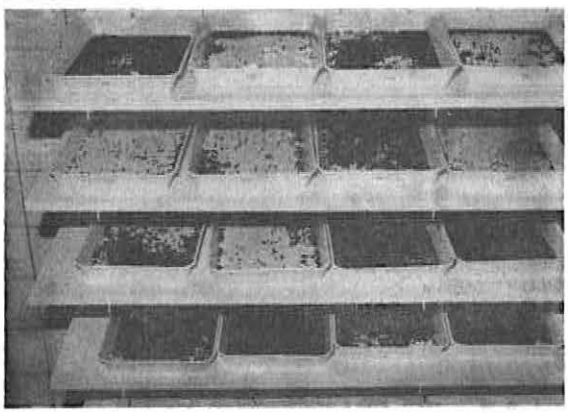


Fig. 5

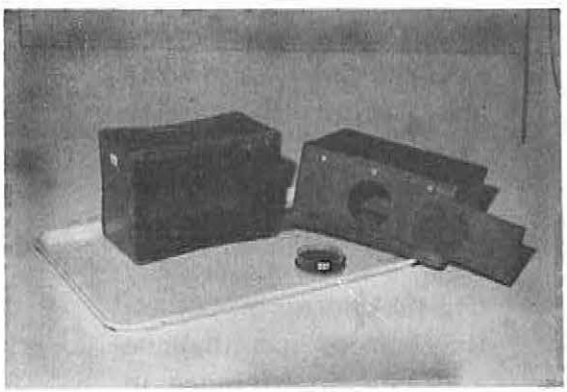


Fig. 6

- lysed (Fig. 7)
- early stage (no melanization, Fig. 8)
- red eye stage (only eyes are melanized, Fig. 9)
- fully pigmented males or females (cuticle still wet, Fig. 10)
- males or females, ready to emerge (cuticle dry, Fig. 11)

In attempt to trace the cause(s) of abnormal pupal development, the dissection results may provide useful indications as to when development was arrested.

- The pupal period is temperature dependent. The mean pupal period for emerged males and females is calculated. With many tsetse fly species an increased number of weak emerged flies is observed when the peak of female emergence occurs before day 32 post larviposition.

- Occasionally, i.e. every fortnight, the testes of some males from the weekly emergence control group should be dissected in order to examine their development (Fig. 12) and sperm mobility (Fig. 13). Underdeveloped testes or immobile sperm indicate a drastic interference in

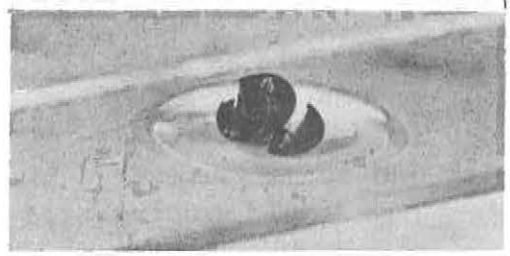


Fig. 7

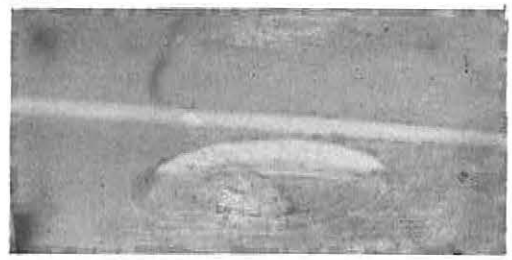


Fig. 8

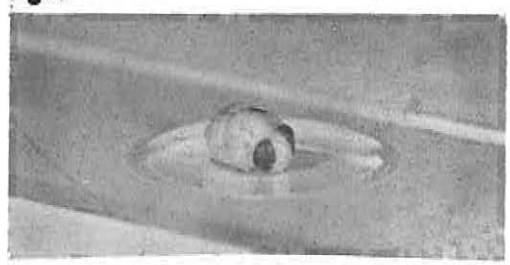


Fig. 9

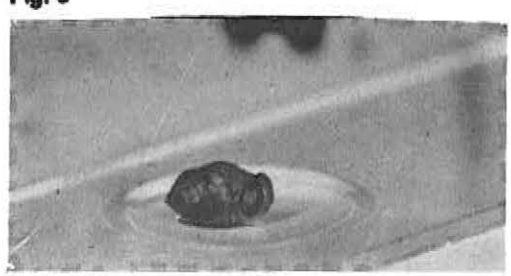


Fig. 10

Fig. 11

spermatogenesis or sperm maturation.

In most cases only incubation conditions require adjustment. Another example: A colony of *G. austeni* and its offspring are maintained at 23°C and 82% r.h. and emergence rates of 76.9 ± 5.2 % are recorded.

Dissections of non emerged pupae reveal that 58 –75 % of them are late stage males and females that only just failed to emerge. A test with different humidities during the last 7–10 days before emergence indicates that higher emergence rates are achieved if the pupae are maintained at increased humidity, i.e. 90.3 ± 2.7 %, during the last third of the pupal period. Without changing the climatic settings of the breeding room, increased humidity may be achieved in the vicinity of the pupae by placing moist sponges below slightly modified pupal dishes in the standard emergence cage (Fig.14).

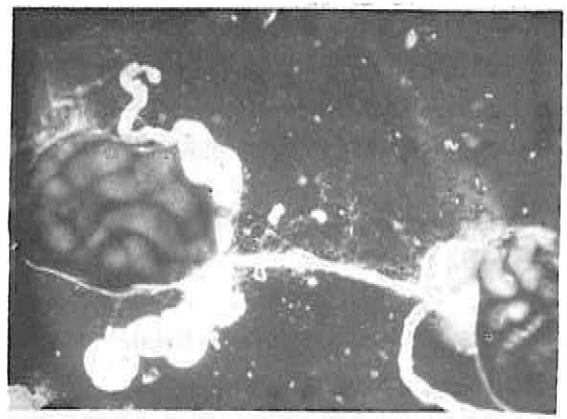


Fig. 12

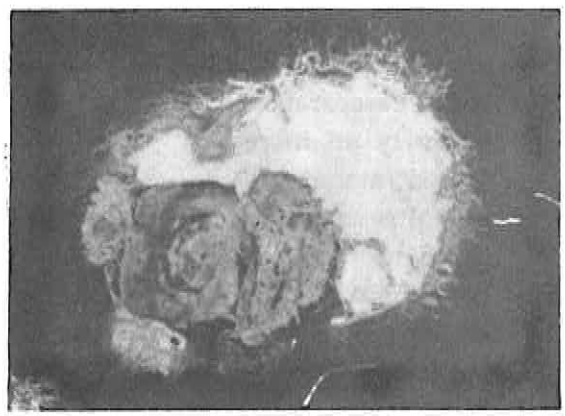


Fig. 13

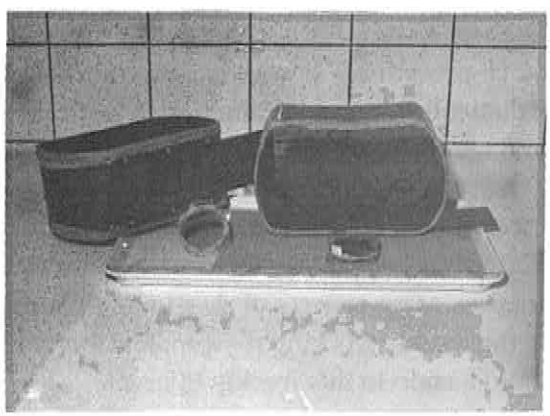


Fig. 14

2. Adult Tsetse Flies

Current methods of tsetse fly rearing involve considerable labour in handling young flies. Nevertheless it provides an opportunity to collect the first information on the performance of young adults. Special attention should be paid to:

- the appearance of flies, i.e. normality of body shape, crippled wings (Fig. 15);
- the feeding response in the first days and the rate of digestion;
- activity in the cage and immediate mating response;
- mortality before mating and after separation; particularly an increased "blood"-mortality (see below) after separation may indicate inadequate handling of young flies, e.g. too long or too cold a chilling period or insufficient time of recovery between chilling and feeding.

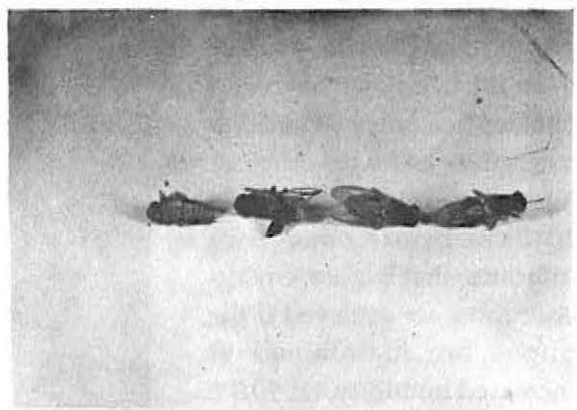


Fig. 15

In order to carry out quality assurance procedures in a tsetse fly colony it is essential to establish a strict system of data collection and evaluation. A laboratory colony of tsetse flies consists of several female units of different age. Fly performance is quantified by determining their survival or mortality, productivity or fecundity. This is influenced by environmental factors (incubation conditions), methods of fly manipulation and maintenance, nutritional factors and sources of intoxication and microbial contamination. Most aspects of day-to-day routine rearing procedures are influenced by the weekly working rhythm. It is therefore advisable to adapt the system of data collection to this weekly interval.

- Every week a colony female unit is formed with emergent females collected from Monday to Saturday. The unit bears the respective week number, i.e. 1 to 52 (in leap-years occasionally to 53) each year.

- For each colony unit a Unit Record Sheet should be prepared. Starting with the daily input of young females into the colony, this sheet should bear all relevant information on fly handling and unit formation, i.e. species, holding room, period of emergence, day of mating, mating regimen, day of separation of sexes, cage type, number of females per holding cage, diet, etc. All relevant data collected and any modification in the maintenance conditions for the unit must be entered into this sheet (Fig. 16).

- The performance of each unit should be evaluated on a weekly basis and be expressed in terms of daily mortality (%d.m.), fecundity (p/f/10d = No. of pupae produced per female per 10 day period) and the portion of A-class pupae (or mean pupal weight). In addition the unit's performance since its establishment should be presented for 4, 8, etc. week old units and upon removal of an old unit from the colony by calculating the percentage of surviving females and the number of produced pupae per initial female (ppfi).

- Weekly colony reports should be prepared. These should list the mean colony female mortality (possibly total mortality and % "blood" mortality separately for both producing and pre-producing units), the mean fecundity and the portion of A-class pupae. Furthermore, the report should include the following information: the current emergence rate (EM-control group), the number of males and females emerged, the size of the new colony unit formed, the total number of colony females, the total number of pupae collected during the week and the number of pupae or flies supplied by the colony for experiments or field projects.

2.1. Mortality

The survival of flies in any colony varies according to species. This is partially due to the different susceptibilities of species to the above influences and, to some extent, may reflect the degree of adaptation of the species to rearing conditions. Furthermore mortality shows an age dependent distribution (Fig. 17).

Entomology Unit, Joint FAO/IAEA Programme

IAEA Agriculture Laboratory Seibersdorf

Record Sheet for Weekly Tsetse Colony Units

45/91 (unit No. / year)

Species: *Glossina tachinoides*

MAT 5-11/11 (12-15.8.: 20 °C)

SEP 8-14/11 (at + 4 °C)

Cage, No. fem./cage a) until SEP. 20 ♀♀, 5 ♂ b) after SEP. 10 ♀♀, 10 ♂

Feeding, Treatment 75% FFBB : 25% FFP until 23. Nov. M5 in DIII, there after M4 in DI

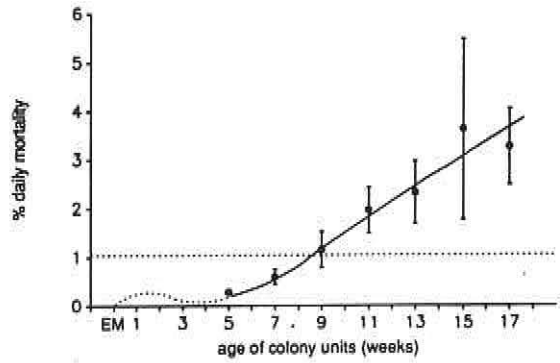
	DATE	No. of females dead	No. of females input	Σ	No. of pupae daily tot.	No. of pupae daily A	No. fem. died	No. fem. surv.
1	4/11		3600					
2	5		1920	5520				
3	6		1480	7000				
4	7		2280	9280				
5	8		2720	12000				
6	9							
7	10							
8	11							
9	12							
10	13							
11	14							
12	15							
13	16							
14	17							
15	18							
16	19							
17	20/11						—	
Σ	wk 2						12000	
18	21	85	4					
19	22	452	29					
20	23	1371	86					
21	24							
22	25	1877	140					
23	26	1423	356					
24	27/11	1692	413				—	
Σ	wk 3	6900	1028				12000	
25	28	1019	144					
26	29	846	248					
27	30	684	184					
28	1/12							
29	2	927	63					
30	3	851	114					
31	4/12	1712	53				—	
Σ	wk 4	6039	746				12000	
32	5	828	25					
33	6	1390	94					
34	7	1603	64					
35	8							
36	9	2205	148					
37	10	366	79				11/12 ex LA	
38	11/12	613	56				1113	
Σ	wk 5	7005	466				10 887	
39	12	521	48					
40	13	770	61					
41	14	1181	49					
42	15							

43	16	2254	105		
44	17	909	125		
45	18/12	1222	49	—	
Σ	wk 6	6857	437	10887	
46	19	785	23		
47	20	681	37		
48	21	923	27		
49	22				
50	23	1095	25		
51	24	547	33	25/12 ex 66	
52	25/12	1149	46	4945	
Σ	wk 7	5180	191	10090	
53	26				
54	27	2415	38		
55	28	1085	37		
56	29				
57	30	1950	60		
58	31/12	413	34		
59	1/1/92	500	20	—	
Σ	wk 8	6361	169	10 090	
60	2	586	25		
61	3	782	62		
62	4	897	35		
63	5				
64	6	2469	106		
65	7	679	79	8/1 ex 55	
66	8/1	1117	43	1109	
Σ	wk 9	6230	350	8981	
67	9	655	22		
68	10	539	37		
69	11	750	39		
70	12				
71	13	1225	75		
72	14	455	33		
73	15/1	1198	44	—	
Σ	wk 10	4822	250	8981	
74	16	757	46		
75	17	663	56		
76	18	1014	54		
77	19				
78	20	1165	43		
79	21	340	33	22/1 ex 44	
80	22/1	653	29	2191	
Σ	wk 11	4562	291	6790	
81	23	346	19		
82	24	431	41		
83	25	835	40		
84	26				

85	27	1172	55	
86	28	438	38	
87	29/1	663	34	—
Σ	wk 12	3885	227	6790
88	30	345	32	
89	31	319	40	
90	1/2	470	25	
91	2			
92	3	612	55	
93	4	430	37	5/2 Ex 70
94	5/2	464	19	2230
Σ	wk 13	2640	208	4560
95	6	244	8	
96	7	387	27	
97	8	501	29	
98	9			
99	10	573	46	
100	11	248	12	
101	12/2	243	10	—
Σ	wk 14	2196	132	4560
102	13	306	20	
103	14	209	12	
104	15	244	13	
105	16			
106	17	394	15	
107	18	150	13	18/2 Ex 38
108	19/2	257	11	2049
Σ	wk 15	1610	84	2511
109	20	136	8	
110	21	190	22	
111	22	160	20	
112	23			
113	24	272	22	
114	25	121	16	
115	26/2	147	10	—
Σ	wk 16	1026	98	2511
116	27	103	13	
117	28	135	17	
118	29	149	8	
119	3/3			
120	2	201	21	
121	3	64	12	4/3 Ex 23
122	4/3	66	13	1266
Σ	wk 17	718	84	1245
123	5	53	10	
124	6	89	16	Σ 123 - 127
125	7	96	13	ist 357
126-7	8-9	119	16	A 55

Fig. 16

Fig. 17. *Glossina tachinoides* under mass-rearing conditions: Percentage daily mortality in relation to the age of the female colony units



- Dead females should be removed from the cages about 10–14 days after unit formation and counted to reveal mortality resulting from early fly handling. Thereafter unit female mortality should be determined weekly or fortnightly.

To be able to trace potential problem sources it is essential to differentiate between types of female mortality as follows:

- Starvation (st): Dead females with extreme dorsoventrally flat abdomens and no ingested blood (Fig. 18, right). Some possible causes are: holding room climate is too warm or too dry, unprotected direct exposure to fan, feeding regimen inadequate or diet or host animal not attractive, heating unit of the *in vitro* feeding set not functioning or too low relative humidity of feeding room.

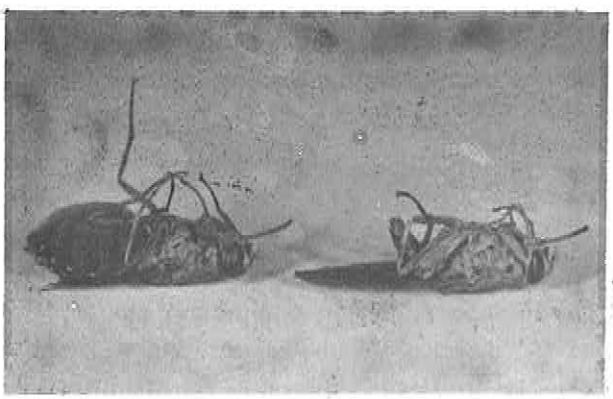


Fig. 18

- Blood-mortality (bl): Dead females with a large amount of undigested blood in the abdomen (Fig. 18, left). Some possible causes are: holding conditions too humid or insufficient aeration of breeding room, excessive diet ingestion (nutritional deficiency?), *in vitro* feeding set too hot, intoxication or microbial contamination.

- Pupa-mortality (p): Dead females with a larva that pupated *in utero*. Two possible causes are mentioned:

  (i) intoxication of the female in very late pregnancy, and
  (ii) too low humidity in the fly holding room. In the latter case the pupa-mortality is usually accompanied by an increased starvation rate.

- Other mortality (o): Dead females not falling in one of the above categories.

The percent daily mortality (%d.m.) is calculated for each unit as follows:

$$\%d.m. = \frac{\text{No. of dead females} \times 100}{\text{No. females surv. last mort. check} \times \text{No. of days elapsed since last check (usually 7 d)}}$$

If the overall mortality is consistently low, the intervals between checks in individual units may be increased (reduced workload). In case of high mortality shorter check intervals are advised. Data on mortality should, however, be collected each week from a representative portion of the colony. For example, units bearing odd week numbers are checked in one given week and alternating all units bearing even week numbers are checked the following week.

If the mean colony female mortality increases 1.2% per day or in case there is high mortality that exceeds the "normal" range for a certain age (see Fig. 17), immediate evaluation of the situation is required. In order to take appropriate steps it is advised to trace the source of mortality according to the following criteria:

- age dependent mortality pattern

- handling dependent pattern: increased mortality is recorded among all flies handled on the same day, in the same chiller, by one person, etc.

- holding position dependent mortality: irrespective of the age of the flies etc., increased mortality is recorded among flies kept in the same corner of the holding room.

Classification of mortality into the above types and a detailed evaluation of the mortality pattern should facilitate the tracing and elimination of the source of the problem.

2.2. Fecundity

Due to their adenotrophic viviparity tsetse flies have a very low reproductive capacity. A productivity of 5.2 pupae per initial female can be achieved with mass-reared *G. tachinoides*. In order to maintain a colony at constant size each female invested into the colony should produce at least 3 pupae, provided other QC parameters, such as the portion of A-class pupae or the emergence rate remain at acceptable levels. It is therefore important that the fecundity of colony female units is constantly monitored. The minimum average fecundity of a colony should be 0.60 pupae per female per 10 days. Fig. 19 shows the age dependent fecundity of a mass-reared *G. tachinoides* colony.

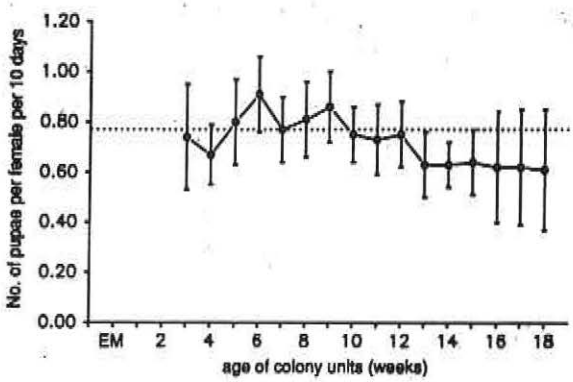


Fig. 19. *Glossina tachinoides* under mass-rearing conditions: Female fecundity in relation to the age of the female colony units

- The first larviposition of young colony females should be observed not later than at day 18 after emergence.

- Once per week (on the same weekday) the fecundity of each female unit is determined as follows:

$$p/f/10d = \frac{\text{No. of pupae produced during the previous week} \times 10}{\text{No. females recorded surviving previous week} \times 7}$$

The fecundity of the unit is thus expressed each week as the number of pupae per female per 10 days (the ovulation and larviposition cycles among tsetse fly females vary under normal conditions between 8 and 10 days). Example (ref. to Fig. 16): the *G. tachinoides* colony unit established in week 45/1991 (4–9 Nov. 91)

had 8,981 surviving females 9 weeks later (8 Jan. 92). During the following 7 days (9 – 15 Jan. 92) the unit produced 4,822 pupae of which 250 were A-class pupae. Thus, during the 9th age week of this colony unit, the calculated unit female fecundity was 0.77 p/f/10d and the portion of A-class pupae was 5.2%.

- If the onset of production is later than 18 days post emergence or in case low female fecundity has been determined, the adequate setting of environmental conditions in the holding rooms should be confirmed and a check for abortions on the trays or slopes below the fly cages should be made. Furthermore, a few females should be dissected to determine whether they have been mated and to detect reproductive abnormalities. Fig. 20 shows spermathecae that are 75% inseminated.

- Abortions, i.e. expulsion of eggs, first, second or early third instar larvae, may be caused by different factors. They are likely to be due to improper incubation/environmental conditions, stress (e.g. overcrowding of females in the holding cages), insufficient nutrition, intoxication or bacterial contamination.

- In the event that insufficient insemination of female spermathecae is detected, the applied mating regimen should be reviewed. This includes the ratio of males to females, the timing of mating and the age when females are most receptive to mating (species differences). Furthermore, it should be confirmed that the males that are used for mating are mature. This can be done by dissecting a few males and looking at the amount and mobility of sperm in the testes (Figs. 12 and 13) and assessing the developmental size of the male accessory glands (Fig. 21) (Pollock 1974). If dissections indicate that poor male quality may be the reason for insufficient female insemination, the male handling procedures and pupal incubation conditions require close attention and modification.

3. Quality Assurance for the Tsetse Fly Colony "Product"

Tsetse fly colonies are usually maintained for the purpose of supplying excess materials for experiments or for field projects. Most experiments require fly material of standard quality, particularly if successive series of tests or replicates are to be compared with each other.

- If the tsetse fly colony is large enough and a pupal size sorting machine is available, emerged flies from C-class pupae, the core group among five size classes, will represent fly material of equal quality, provided other external factors, such as incubation conditions, are not altered.

Fig. 20

- Methods for quality assurance of flies for field projects vary with the purpose for which the flies are to be used and with the following important criteria:

(i) Males released in vector control programmes (SIT) must be sexually sterile, mature and competitive. All these requirements can be confirmed by male dissections and simple laboratory tests (Fig. 22), i.e. mating with virgin fertile females, once or

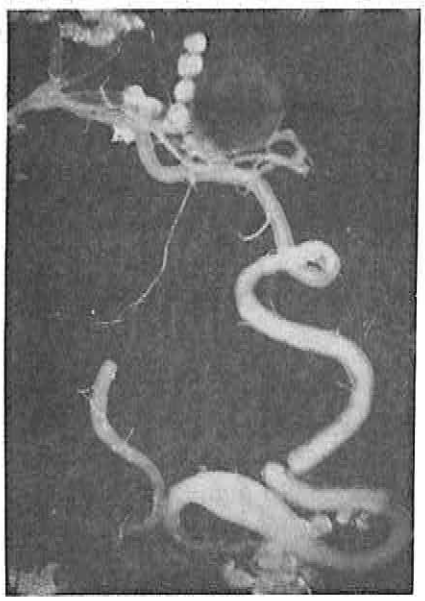


Fig. 21

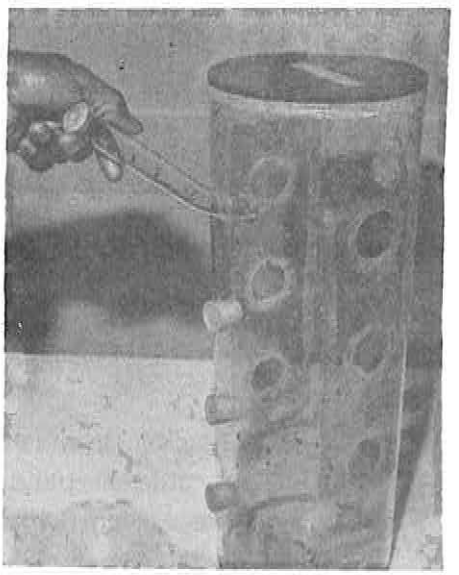


Fig. 22

repeatedly, in comparison or in direct competition with fertile males. Similarly, such methods can be used to evaluate the impact of transport procedures on the quality of the released flies.

(ii) Sexually sterile virgin females can be used for monitoring the existence of a relic fly population during or after vector control programmes. Their normal sexual attractiveness and receptivity to mating, their survival and dispersal are the main quality criteria.

The former can be confirmed by laboratory tests, e.g. by mating them with mature fertile males, in comparison or in direct competition with fertile females. The latter needs to be directly confirmed through trapping.

- The quality of pupae after shipment can be quantified under standard incubation and handling conditions using the quality assurance methods described earlier.

4. Acknowledgements and Remarks

The assistance of my colleagues Detlef Luger and Harry Baumgartner in preparation of the manuscript is highly appreciated and I want to thank Drs. W. Klassen, E. D. Offori, J. I. Richards and R. E. Gingrich for their support and useful comments on the manuscript.

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

References

Boyt, W.P., P.K.I. MacKenzie and the late R.D. Pilson. 1978. The relative attractiveness of donkeys, cattle, sheep and goats to *Glossina morsitans morsitans* Westwood and *G. pallidipes* Austen (Diptera : Glossinidae) in a middle-veld area of Rhodesia. *Bull. Ent. Res.* 68, 497–500.

Bursell, E. 1959. Determination of the age of tsetse puparia by dissection. *Proc. R. Ent. Soc. Lond.* 34, 23–24.

Buxton, P.A. 1955. The Natural History of Tsetse Flies. *Lond.Sch.Hyg.& Trop. Med., Mem.* No. 10, 816 pp.

Feldmann, U., D. Luger, H. Barnor , L. Dengwat, B. Ajagbonna, M. Vreysen and A. Van der Vloedt. 1992. Tsetse fly mass rearing: colony management, deployment of sterile flies and related research and development, pp. 167–180. In: *Proc. FAO/IAEA Seminar on Tsetse Control, Diagnosis and Chemotherapy Using Nuclear Techniques,* held at Muguga, Kenya, 11–15 November 1991. IAEA-TECDOC-634.

Langley, P.A., R.W. Pimley, A.R. Mew and M.E.T. Flood. 1978. Effect of diet composition on feeding, digestion and reproduction in *Glossina morsitans. J. Insect Physiol.* 24, 233–238.

Moloo, S.K. and R.W. Pimley. 1978. Nutritional studies in the development of *in vitro* feeding techniques for *Glossina morsitans. J. Insect Physiol.* 24, 491–497.

Oladunmade, M.A., U. Feldmann, W. Takken, S.O. Tenabe, H.-J. Hamann, J. Onah, L. Dengwat, A.M.V. Van der Vloedt and R.E. Gingrich. 1990. Eradication of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae) from agropastoral land in central Nigeria by means of the Sterile Insect Technique. pp. 5–23. *Proc. FAO/IAEA RCM Sterile Insect Technique for Tsetse Control and Eradication,* Vom, PL, Nigeria, 6–10 June 1988, IAEA-RC/319.3, STI/PUB/830.

Pollock, J.N. 1974. Male accessory secretions, their use and replenishment in *Glossina* (Diptera : Glossinidae). *Bull. Ent. Res.* 64, 533–539.

Staak, C., U. Kampe and G. Korowski. 1986. Species identification of blood-meals from tsetse flies (Glossinidae): Results 1979–1985. *Trop. Med. & Parasit.* 37, 59–60.

Zelger, R. and K. Russ. (1976). Puppentrennmaschine mit zwei Walzenpaaren. Z. *Angew. Zool.* 63, 257.

Contribution of Insect Rearing to the Management of Rice Pests

R. C. Saxena

ICIPE Field Station
P. O. Box 30, Mbita, Kenya

Abstract

Techniques are described to mass rear major insect pests affecting rice crop and stored rice. Efficient rearing of rice insects of good quality and vigour on suitable varieties, cultured medium, or artificial food substrates has contributed significantly to successful evaluation of rice germplasm and wild rices for identifying donors of insect resistance. Also, high volume screening of breeding lines for selecting potentially resistant varieties against insect pests and their biotypes is based on year-round supply of target pests. Mass-reared insects have also been used for insecticide evaluation, mechanisms of plant resistance, and other biological studies.

I. Introduction

Rice, *Oryza sativa*, feeds more than two billion people in Asia and several hundred million people in Africa and Latin America. It is planted over 145 million hectares — 11% of the world's cultivated land. Most of the world's rice is produced in irrigated and rainfed lowland rice fields where insect pests are a serious constraint. More than 100 species of insects attack rice; of these about 20 are major pests (Table 1) (Pathak and Saxena 1980). Together they infest all parts of the rice plant at all growth stages and a few transmit rice viral diseases. Also, a number of insect pests attack stored paddy and rice.

Yield losses due to rice insect pests have been estimated at about 30%. Losses of up to 50% were not uncommon in severely affected fields during brown planthopper outbreaks in Indonesia in 1974–76 (Oka 1979). Recently, green

Table 1. Major insect pests of rice (Pathak and Saxena 1980)

Common name	Scientific name
Crop Pests	
Striped stem borer	*Chilo suppressalis*
Yellow stem borer	*Scirpophaga incertulas* (= *Tryporyza incertulas*)
White stem borer	*Scirpophaga innotata* (= *Tryporyza innotata*)
Brown planthopper	*Nilaparvata lugens*[1,2]
Whitebacked planthopper	*Sogatella furcifera*
Small brown planthopper	*Laodelphax striatellus*[2]
Rice delphacid	*Sogatodes orizicola*[2]
Green leafhopper	*Nephotettix virescens*[2]
Zigzag leafhopper	*Recilia dorsalis*[2]
Rice bug	*Leptocorisa oratorius*
Black bug	*Scotinophora* spp.
Rice gall midge	*Orseolia oryzae*[1]
Rice whorl maggot	*Hydrellia philippina*
Rice stem maggot	*Atherigona oryzae*
Stalk-eyed fly	*Diopsis thoracica*
Rice leaffolder	*Cnaphalocrocis medinalis* and *Marasmia patnalis*
Rice caseworm	*Nymphula depunctalis*
Rice hispa	*Dicladispa armigera*
Rice water weevil	*Lissorhoptrus oryzophilus*
Rice thrips	*Stenchaetothrips biformis* (= *Thrips oryzae*)
Storage Pests	
Angoumois grain moth	*Sitotroga cerealella*
Rice weevil	*Sitophilus oryzae*
Maize weevil	*Sitophilus zeamais*
Granary weevil	*Sitophilus granarius*
Lesser grain borer	*Rhyzopertha dominica*

[1] Biotypes encountered.
[2] Also serve as virus vectors.

leafhoppers damaged approximately 500,000 hectares of rice amounting to a loss of about 0.5 million tons of rice (A. N. M. Rezaul-Karim and R. C. Saxena, unpublished information). Even greater damage is caused by rice viral diseases transmitted by these pests. Rice insect pest management deploying resistant varieties has therefore become a high research for sustainable rice production in most countries.

Mass rearing of insects is a major input for identifying donors of insect resistance in rice germplasm and evaluating breeding lines for selecting potentially resistant varieties. Also, insect rearing is a prerequisite for insecticide evaluation and other biological studies in laboratories or insectaries.

II. Insect Rearing and Progress in Breeding for Insect Resistance

The progress in breeding for insect resistance in rice has largely been dependent on the availability of germplasm collection and simple but efficient insect rearing and varietal screening techniques for identifying resistance donors and breeding lines (Fig. 1). Rice germplasm, comprising *O. sativa* and *Oryza glaberrima* cultigens, is large. Between 100,000 and 120,000 rice varieties and a few hundred wild rices are known to exist of which about 80% are conserved at the International Rice Research Institute (IRRI) in the Philippines (Chang 1989). Also, several hundred thousand breeding lines may be generated annually in a good rice breeding programme, such as at IRRI (Table 2). The high volume screening aimed at discarding the bulk of the susceptible germplasm and breeding lines in a greenhouse, screenhouse or field tests requires a correspondingly large and steady supply of test insects. With an efficient insect rearing programme, greenhouse screening can be conducted throughout the year.

A mass rearing programme also is valuable in field evaluation of varieties, particularly when "hot spots" of pest infestations are either lacking or located too far. Cultured insects can be used to artificially infest plants at the experiment station.

Efficient methods of rearing planthoppers and leafhoppers, stemborers, leaffolders, caseworms, and rice bugs have been developed at IRRI in the Philippines (Heinrichs *et al.* 1981, 1985; Saxena *et al.* 1990), while techniques have been developed in national programmes for rearing the gall midge (Prakasa Rao 1975; Arifin and Vreden 1977; Kalode 1980) and thrips (Nugaliyadde and Heinrichs 1984; Velusamy and Saxena 1990) and other insects. Methods of rearing the rice hispa have not yet been developed; most varietal evaluations are carried out in the field or using field-collected insects.

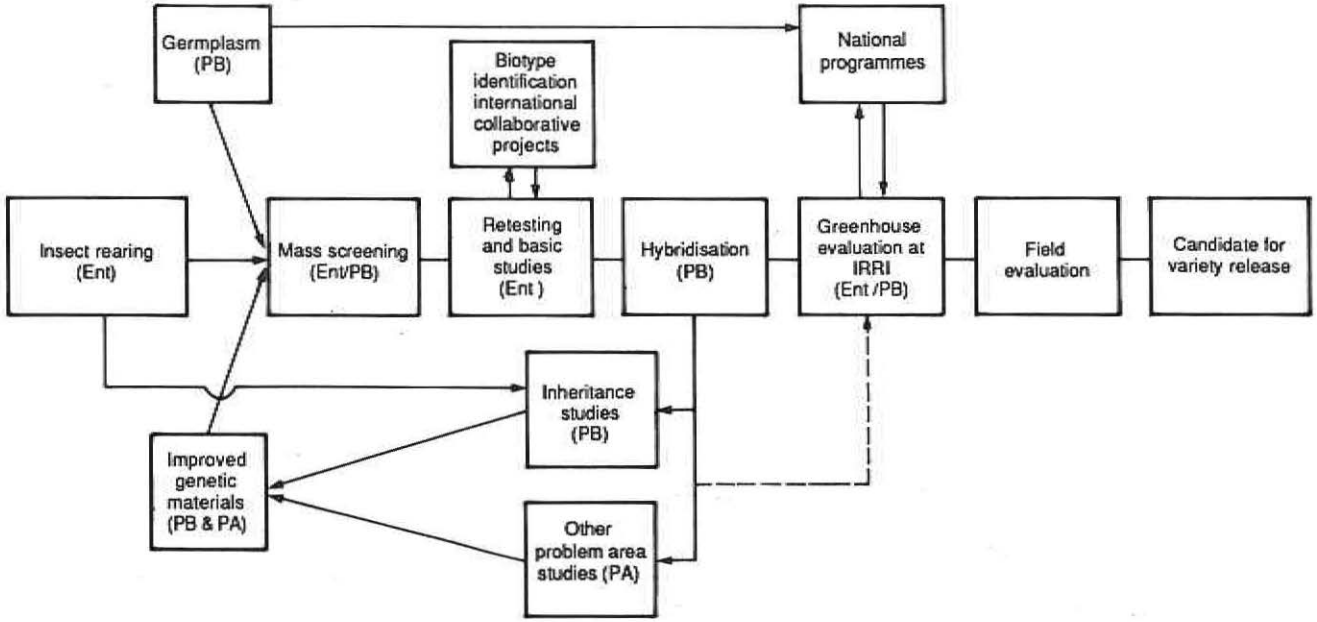


Fig. 1. The role of insect rearing in building of resistant varieties. Pest resistance and superior agronomic characteristics are bred into all varieties released through the Genetic Evaluation and Utilisation Programme. Entomologists (Ent), plant breeders (PB), and problem area scientists (PA) work together to obtain these objectives. Mass-reared insects are used in mass screening and retesting of germplasm and breeding lines, and for basic studies, inheritance studies, and mechanisms of resistance (Pathak and Saxena 1980)

Table 2. **Rice germplasm, breeding lines, and other materials screened for insect resistance. IRRI, 1988**

Insect	Germplasm			Breeding lines/other materials		
	Tested	Resistant		Tested	Resistant	
	no.	no.	%	no.	no.	%
Brown planthopper						
Biotype 1	1725	380	22	26253	21143	80
Biotype 2	190	8	4	35873	24895	69
Biotype 3	463	114	25	25902	19169	74
Whitebacked planthopper	470	3	1	1810	96	5
Green leafhopper	697	53	8	78362	43670	56
Yellow stem borer	876	16	2	2349	369	16
White stem borer	900	152	17	47	39	83
Rice leaffolder (*C. medinalis*)						
greenhouse	4410	0	0	276	0	0
field	0	0	0	1430	313	22
Rice leaffolder (*M. patnalis*)						
greenhouse	5047	0	0	276	0	0
Whorl maggot	2300	0	0	276	24	9
Caseworm	750	0	0	276	3	1
Black bug	16	0	0	536	154	29

III. Insect Rearing —General Considerations

Proper planning is a must to have an uninterrupted supply of insects of the right age and sex at the right time, reared either on natural or synthetic food sources. Because rearing conditions can influence the reaction of a test insect to a variety or a test chemical, it is of paramount importance that insects used in tests be of good quality and physiologically as uniform as possible. Here the term "quality"

Table 3. Brown planthopper biotypes and differential varietal reactions

Variety	Resistance gene(s)	Southeast Asia				South Asia		
		1	2	3	4	Bangla-desh	India	Sri Lanka
TN 1		none	S	S	S	S	S	S
Mudgo	*Bph* 1	R	S	R	S	S	S	S
ASD 7	*Bph* 2	R	R	S	S	S	S	S
Rathu Heenati	*Bph* 3	R	R	R	R	R	S	R
Babawee	*Bph* 4	R	R	R	R	R	R	R
ARC 10550	*Bph* 5	S	S	S	S	R	R	R
Ptb 33	2 (?)	R	R	R	R	R	R	V[1]
Sinna Sivappu	2 (?)	R	R	R	R	R	R	R

[1] Variable reaction.

refers to efficient performance of the function the test organisms are supposed to do.

The rearing techniques also should assure low insect mortality on susceptible cultivars or in the untreated control. Cultures should be examined regularly for any unwanted species and predators, and parasitised insects, if any, should be removed immediately. It is a good practice to infuse the culture periodically with wild insects to avoid possible inbreeding depression.

The occurrence of possible genetic variability in the pest species should be taken into consideration when raising pest colonies. If biotypes occur, then pure line populations on biotype-specific host varieties must be raised (Khan and Saxena 1990). For example, three sympatric brown planthopper biotypes have been identified in Southeast Asia and they differ from those found in South Asia (Table 3) (Saxena and Khan 1989). Biotypes also have been encountered in the rice gall midge.

IV. Mass-Rearing of Rice Insects

1. Planthoppers and Leafhoppers

In the tropics, planthoppers and leafhoppers are reared in cages placed in a well lighted and aerated greenhouse or screenhouse suitable for growing rice plants throughout the year. The greenhouse is divided into separate rooms with

screenwalls for rearing different hopper species or their biotypes. It is advisable to rear only local populations of planthoppers and leafhoppers, unless proper containment facilities are available.

A pushcart, a wheelbarrow, cages, and potted rice plants of a susceptible rice variety, such as Taichung Native 1 (TN 1), are needed for rearing hoppers. Bottomless cages placed in a galvanised iron water pan tray (Fig. 2a) are generally used for rearing planthoppers, while cages with four legs and closed bottom (Fig. 2b) are preferred for rearing green leafhoppers which are often attacked by the fungus *Metarrhizium anisopliae* (Heinrichs *et al.* 1985).

A transfer cage (Fig. 2c), which can hold water up to 30 cm deep is useful for transferring planthoppers from old to fresh potted plants. When water is drained into the transfer cage and as the water level rises, the planthoppers move from the base of the plants to leaf tips. They are then easily transferred to fresh plants in the rearing cages by tapping the leaves over fresh rice plants.

Initially, hopper nymphs and adults are collected from infested plants over a container with TN 1 rice seedlings. The collected insects are brought to the greenhouse. The target hopper or leafhopper species are selected for mass rearing.

To eliminate viruliferous hoppers, gravid females are caged on potted *Monochoria vaginalis*, a nonhost of rice viruses. A week later, virus-free potted rice seedlings are introduced into the cage containing infested *M. vaginalis* plants. Nymphs emerging on *M. vaginalis* soon move to rice seedlings and become the starters for a non-viruliferous colony. On the other hand, viruliferous insects can be produced by caging gravid females for 3 to 4 days on virus-infected 40 to 50-d-old TN 1 rice plants (Saxena *et al.* 1991).

Brown planthopper populations can be a mixture of several biotypes. It is therefore important to purify them by rearing the F_1 and F_2 progenies first on TN 1 and then testing the F_3 progeny for differential reactions on differential varieties: TN 1 susceptible to Biotype 1, Mudgo (*Bph*1 resistance gene) susceptible to Biotype 2, and ASD 7 (*bph*2 resistance gene) susceptible to Biotype 3. Also a brown planthopper population was found in the southern Philippines that thrived on varieties with either *Bph*1 or *bph*2 gene. Likewise, a brown planthopper biotype was discovered that thrived on *Leersia hexandra* grass but failed to establish on rice (Domingo *et al.* 1983).

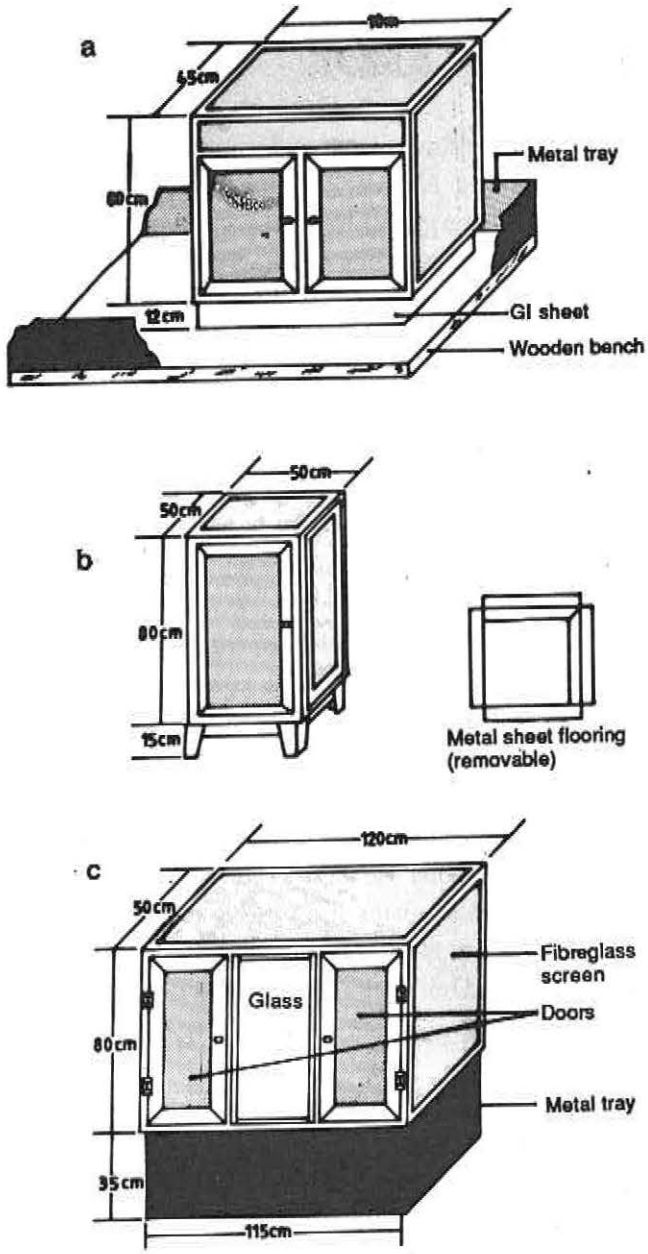


Fig. 2a. Bottomless planthopper rearing cage placed in a galvanised iron tray: potted plants sit in about 8 cm of water inside the cage; b. leafhopper rearing cage: leafhoppers are reared on plants in the cage; c. cage for facilitating the transfer of planthoppers: plants are placed in the metal tray and the water level is raised to cover the base of plants, forcing the hoppers to move upwards (Heinrichs *et al.* 1985)

A continuous supply of 30 to 40-d-old rice plants of a susceptible variety is needed for feeding and oviposition by the hoppers. Seedlings when 10-d-old are placed in clay pots (10 cm diam.) and fertilised with ammonium sulfate at 15 days after transplanting. Eight potted plants in each cage can maintain up to 1000 hoppers. Nymphs and adults of uniform ages can be generated easily if cages for oviposition and rearing are maintained separately. One should allow 4 days for oviposition before transferring plants to rearing cages.

2. Gall Midge

The gall midge occurs as a pest of rice in South and Southeast Asia and parts of West Africa. It thrives on rice, but also can survive on several grasses. A typical gall midge infestation is characterised by excessive tillering and the presence of onion leaf-like galls called "onion shoots" or "silver shoots".

The gall midge is a delicate insect and requires high humidity for survival. Although adults can be collected from infested fields using an aspirator, it is preferable to collect whole "silver shoots" (from which adults have not emerged) and place them in an emergence cage. When adults emerge, they are collected with an aspirator and transferred to an oviposition cage containing 16 pots of 15 to 20-d-old seedlings. Up to 30 pairs of gall midge can be released in the oviposition cage. Two cages should be infested daily for routine rearing. The gall midge lays eggs on the leaf surface. After 2 days, the potted plants with eggs are transferred to a mist chamber and replaced with fresh ones. In the mist chamber, gall midge eggs hatch on plants in 4 days and the newly emerged larvae crawl down the leaves to the growing points. After 4 days, infested plants are transferred to a water tray and the level of water is raised to cover 2 to 3 cm of the base of the plants. This assures high humidity and minimises parasitisation and predation. After 2 to 3 weeks, when "silver shoots" are formed, the plants are transferred to cages for adult emergence. Adults are collected early morning for various tests.

3. Whorl Maggot and Stem Maggot

The whorl maggot is widespread in South and Southeast Asia; the stem maggot is endemic in Japan. A satisfactory method for rearing these dipterous pests in the greenhouse has not been developed. The whorl maggot abounds in fields with standing water. The adult flies lay small, silvery eggs on rice seedlings soon after

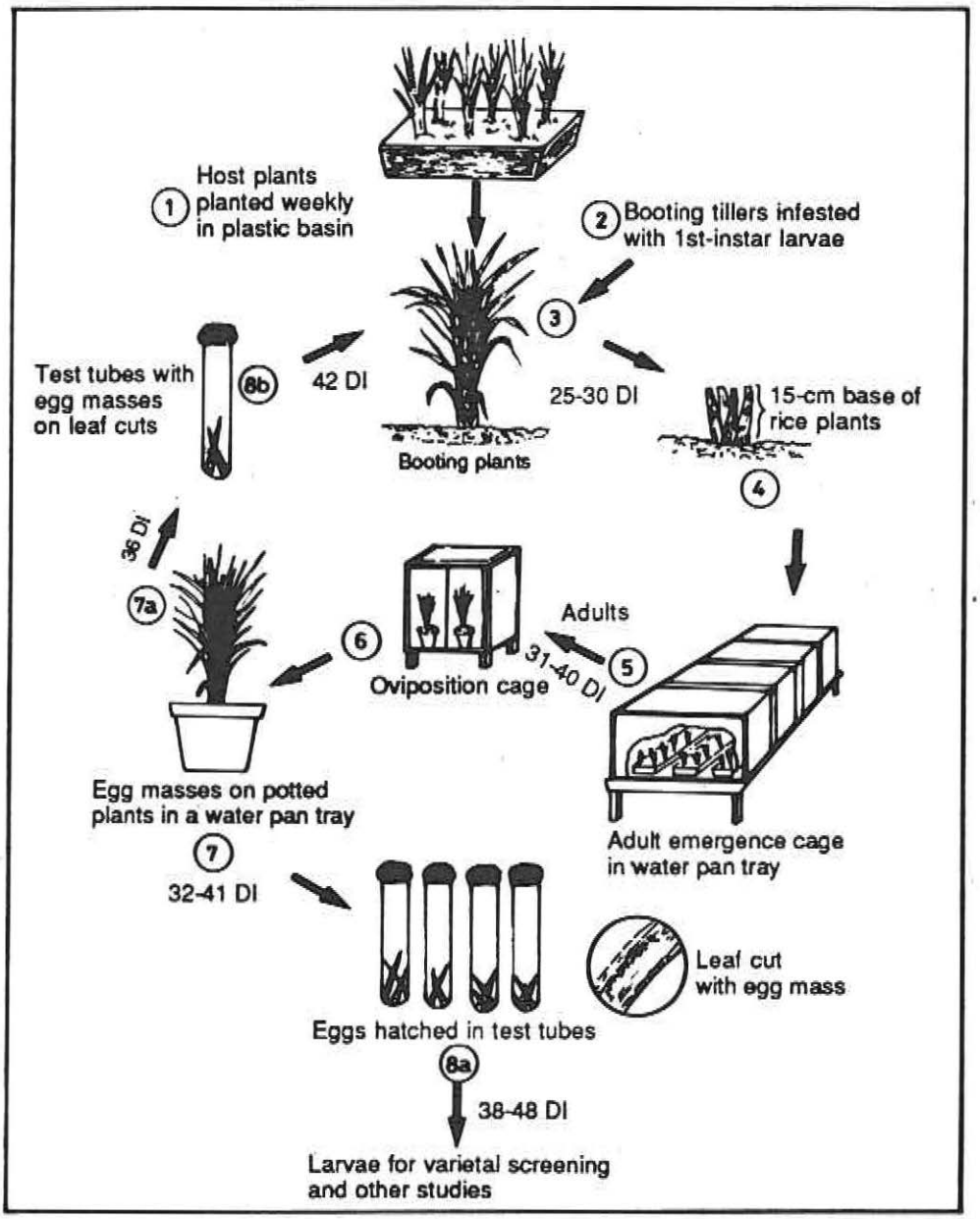


Fig. 3. Steps in rearing the rice yellow stemborer. DI = days after infestation (Saxena *et al.* 1990)

transplanting. The damage shows up at the tillering stage. Flies can be collected early morning by placing a cylindrical mylar-film cage (40 cm long, 5 cm diam.)

with a nylon mesh top over the rice seedlings in an infested field. Flies that fly upwards into the cage are trapped and carried to the greenhouse or laboratory for varietal evaluation or insecticide tests.

In the case of stem maggot, natural infestation in the field is used for varietal evaluation. Alternatively, adults collected in the field, can be brought to the greenhouse and allowed to oviposit on rice leaves in glass jars. Emerging first-instar maggots are then used to infest rice seedlings at 4 to 5 leaf stage, using a fine camel hair brush.

4. Stem Borers

The striped stem borer, the yellow stem borer, and the white stem borer are important pests of rice in Asia. Larvae bore into the tillers and cause "deadheart" at the vegetative stage and "whitehead" at the flowering stage of the crop.

Stem borers can be reared on cut rice stalks (Heinrichs *et al.* 1985), but the method is not practical for high volume screening that requires a large supply of larvae. Recently, Saxena *et al.* (1990) developed a simple method for year-round rearing of striped- or yellow stem borer. Seedlings of IR 8 or Rexoro for the striped stem borer, or of IR 62 for the yellow stem borer, are planted weekly in 34 x 25 x 10 cm plastic trays (Fig. 3). At mid-booting, a 2.5 cm long slit is made with a scalpel in the bulging middle portion of the leafsheath below the flag leaf. The incision is dilated to expose a small portion of the developing panicle. One to two 1st instar stem borer larvae are released onto the panicle and the incision is closed. At 25 to 30 days after infestation, when the larvae have pupated, plants are cut at 15 cm above the base (20 cm for the striped stem borer). The trays with stubbles are transferred to a 2 x 1 x 1 m screen cage for adult emergence. About 80% of the infested tillers produce moths.

Emerging moths, collected daily, are transferred to oviposition cages with wax paper strips for oviposition by the striped stem borer and 40-d-old TN 1 plants for egg laying by the yellow stem borer. Wax paper portions or leafcuts with egg masses are removed daily and stored in 15 x 1.5 cm test tubes or small bell jars. At 27 ± 2 °C, eggs hatch in about a week. Emerging larvae are used for varietal screening, basic studies, or for maintaining insect culture. If the larvae are not immediately needed, then their emergence can be delayed up to 10 days without affecting survival by holding the eggs at 15 °C (Viajante and Saxena 1990). The white stem borer can also be reared with slight modifications and use of IR 64, a susceptible variety.

The striped stem borer also has been reared on a modified southwestern cornborer diet (Davis 1976). To prepare 1 litre of diet one needs the following materials:

A. Distilled water (911 ml)
B. Agar (17.2 g)
C. Toasted wheat germ (21.1 g), vitamin-free casein (25 g), sucrose (25 g), Wesson salt mix (7 g), linseed oil (0.2 g), cholesterol (0.1 g), maize cob grits (autoclaved 37 g), methyl parabenzoate (1.5 g), sorbic acid (0.5 g), and corn starch (35.2 g)
D. Vanderzant vitamin mix (5.3 g), ascorbic acid (2.1 g), and 10% formaldehyde (4 g).

Agar is added to 545 ml boiled water in a blender and stirred until dissolved. The mixture of C is added to agar solution and blended at high speed for 3 min. Then, 366 ml of cold water is added and blended for 1 min. The diet, still liquefied, is poured at about 6 ml per 28 ml plastic rearing containers and kept behind an air curtain. When the diet in each cup has cooled and gelled, it is infested with two first-instar larvae, freshly emerged from eggs surface-sterilised with 5.25% sodium hypochlorite. Larvae at the desired age can be collected from the diet for various studies. To produce adults, pupae are transferred from the cups to petri dishes and allowed to emerge in oviposition cages. Honey-water solution (25%) is provided to enhance the survival of stem borer moths. Egg masses are collected on rice plants or on wax paper strips hung vertically in oviposition cages.

5. Leaffolders

Leaffolders are widespread in South and Southeast Asia. The larvae fold rice leaves by tying silk to leaf margins and feed within folded leaves, removing the green layer. Heavily damaged fields have a scorched appearance, particularly at flowering and crop maturity.

The insect can be reared efficiently in the greenhouse (Waldbauer and Marciano 1979). Initially, field collected larvae are used to infest susceptible TN 1 rice plants growing in earthen pots (30 cm diam.). Each pot is enclosed in a mylar-film, cylindrical cage having a side lever. Each pot holds 30 plants with about 125 tillers. The caged pots are held in the greenhouse in a metal tray with water about 12 cm deep. When adults emerge, they are collected using small glass vials and transferred to oviposition cages containing 60-d-old potted TN 1 plants, placed on

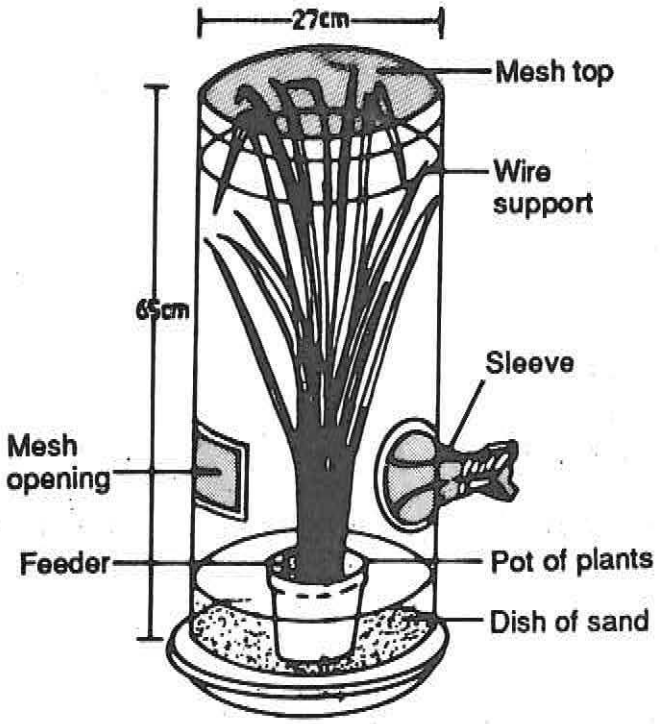


Fig. 4. Oviposition cage for rearing leaffolders (Waldbauer and Marciano 1979)

a shallow earthen tray (30 cm diam., 5 cm deep) filled with wet sand (Fig. 4). Moths are fed 25% honey water solution, using a feeder with cotton soaked in honey solution (changed every 2 days). Leaf portions with eggs laid singly or in rows are removed daily and the leafcuts are placed in moist filter paper-lined petri dishes. Each dish contains 300 to 400 eggs. The eggs hatch in about 4 days with 90% viability. The emerged larvae are used for varietal screening or for maintaining culture.

6. Caseworm

Caseworm is widespread in Asia, Africa, Australia, and South America. The larvae are aquatic and their attack is most serious when the crop is young and growing in standing water.

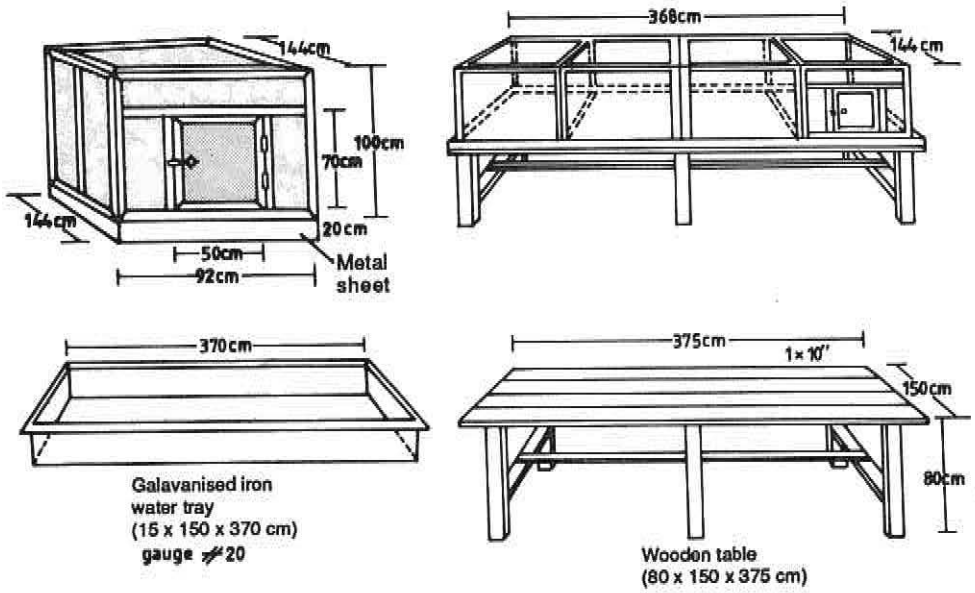


Fig. 5. Bottomless cage, water tray and table for rearing rice bugs (Heinrichs *et al.* 1985)

Caseworm moths are collected from infested rice fields and released in oviposition cages in the greenhouse (Bandong and Litsinger 1981). Each oviposition cage, comprising a mylar-film cylinder (55 cm high, 25 cm diam.) with nylon-mesh top with a hole for introducing moths, sits in a plastic basin containing water about 6 cm deep and 5 to 8 cm long leafcuts floating on water. About 30 males and females are released in each oviposition cage. Moths lay eggs on the underside of leafcuts. After 24 hr, the oviposition cage enclosing moths are removed and placed over another tray holding fresh rice leafcuts. The procedure is repeated for 5 days until females have spent egg-laying. The leafcuts bearing eggs are transferred to larval rearing trays, each with six pots; each pot containing 50 2-wk-old rice seedlings. The water level in the tray is about 1 to 2 cm above the level of the pots. As larvae emerge, they first feed on the underside of the cut leaves, then they move to potted plants and cut the leaf tip out of which they construct a case. The young larvae find it easier to make cases out of young leaves. After 17 to 20 days, the larvae pupate in the leafsheaths of the potted plants. The pots with pupae are transferred to a moth emergence cage and then to an oviposition cage to restart the culture cycle.

7. Rice Bugs

Rice bugs are common throughout Asia. They feed on the developing rice grains mostly at dawn and at dusk. Their long and sharp stylets penetrate the lemma and palea and the removal of milk produces chaffy grains. Feeding at the dough stage causes black spots on the milled rice and enhances grain breakage.

Rice bug culture is started with adults collected in the field using a sweepnet. They are released on potted, panicle-bearing IR 36 plants placed in a water-filled tray covered with a bottomless rearing cage (1.5 m high, 1.5 m long, 1.45 m wide) comprising nylon-mesh sidewalls and roof and a front door (Fig. 5). Leaf portions with rice bug eggs are removed daily and placed in moist, filter paper-lined petri dishes and kept in the laboratory for 5 days at 24–30°C. The petri dishes with eggs are then placed in a rearing cage at the base of potted plants. When nymphs emerge they move up and begin feeding within a couple of hours. Nymphs reach adulthood in one month. The food plants in the milky dough stage are changed weekly.

8. Storage Pests

i. Angoumois grain moth. It is major pest of stored rough rice. The infestation begins in the field even before harvest. Eggs are laid on or near grains and the larvae bore into the grain and feed on the contents; the active larval and pupal periods are spent inside the grain. The moth can be reared easily on rice, wheat, or maize kernels (Strong *et al.* 1967). Moths are collected from a warehouse and released in 25 pairs in one litre plastic or glass jars, each containing about 0.5 litre of rice, wheat or maize kernels (12 to 13% moisture content). The culture jars are covered with a lid provided with a screen and laid on the side in a rearing room. After one week, the jars are set upright. Old moths die. About 400 to 500 new moths emerge in each jar in about 6 weeks after oviposition. These moths are used to restart the culture or obtain eggs for evaluating cultivars. To obtain eggs, newly emerged moths are released for 4 days in oviposition cages containing folded wax paper. The wax paper with eggs is unfolded, eggs are counted, and areas with egg clusters are cut. Paper pieces with up to 200 eggs are placed in each of the 10 g samples of test varieties of rice. The oviposition papers are removed after 7 to 14 days and examined to determine the number of eggs hatched.

ii. Rice weevils, maize weevils, and granary weevils. These weevils seriously

damage stored rough rice and milled rice; the larvae and adults being internal feeders. The weevils are hardy insects and can be reared easily. To obtain pure cultures, the culture medium should be frozen at -15°C for about a week to exclude the possibility of subsequent contamination with unwanted storage pests. Wide-mouthed 1 litre jars containing about 0.5 litre of the culture medium and covered with screened metal lids are ideal for mass rearing the weevils.

Weevils collected from infested grain from a warehouse are used as the starters. About 0.5 cc of the rice and maize weevils and 8 cc of the granary weevil are enough in each of the two jars with fresh medium for oviposition. One week after oviposition, the used adults are discarded and destroyed and the medium with developing larvae is saved for the emergence of a new brood of the insects in about 6 weeks for the rice and maize weevils and about 7 weeks for the granary weevil.

To obtain a regular supply of insects of uniform age, it is advisable to rear them in multiples of two or more jars per week at 27 ± 2° C and 65 ± 5% r.h., 12:12 hour dark and light period. Cross contamination should be avoided either by rearing the insects in different rooms or changing the cultures on different days. Old cultures should be discarded by freezing them for 4 or more days. Proper sanitation can keep away invasion by mites (*Pymotes* spp.) which can totally destroy the cultures of stored grain pests.

iii. The lesser grain borer. This insect attacks both rough and milled rice. Both larvae and adults are internal grain feeders. Rearing procedures are similar to those used for rearing grain weevils, except that adult emergence takes longer, about 8 weeks.

Conclusion

In this paper, I have described methodologies for rearing selected species of insect pests affecting the rice crop and stored rice. Selection of the species for mass-rearing in a given area should be based on their relative importance and the damage they cause to the crop or stored grain. The techniques to be used also should take into account the available resources of manpower, facilities, and financing. Based on requirements, the techniques can be modified and programmed to mass produce insects efficiently and economically without sacrificing the "product" quality.

References

Arifin, K. and G. V. Vreden. 1977. Mass rearing of the rice gall midge *Orseolia oryzae* (Wood-Mason). Contrib. Cent. Res. Inst. Agric., Bogor, Indonesia, 32. 15 pp.

Bandong, J. P. and J. A. Litsinger. 1981. Method for mass-rearing the rice caseworm *Nymphula depunctalis*. *Int. Rice Res. Newsl.* 6: (1) 3–4.

Chang, T. T. 1989. The case for large collections, pp. 123–135. In: A. D. H. Brown, O. H. Frankel, D. R. Marshall and J. T. Williams (eds), *The Use of Plant Genetic Resources*. Cambridge University Press, Cambridge.

Cramer, H. H. 1970. Plant protection and world crop production. *Pflanzenschutz-Naher Bayer Leverkusen*, Germany, 20:1–524.

Davis, F. M. 1976. Production and handling of eggs of southwestern corn borer for host plant resistance studies. *Miss. Agric. For. Exp. Stn. Tech. Bull.* 74, 11 pp.

Domingo, I. T., E. A. Heinrichs and R. C. Saxena. 1983. Occurrence of brown planthopper on *Leersia hexandra* in the Philippines. *Int. Rice Res. Newsl.* 8(4): 17.

Heinrichs, E. A., F. G. Medrano and H. Rapusas. 1985. *Genetic Evaluation for Insect Resistance in Rice*. Int. Rice Res. Institute, Los Banos, Phil. 365 pp.

Heinrichs, E. A., S. Chelliah, S. L. Valencia, M. B. Arceo, L. T. Fabellar, G. B. Aquino and S. Pickin. 1981. *Manual for Testing Insecticides*. Int. Rice Res. Institute, Los Banos, Phil. 134 pp.

Kalode, M. B. 1980. The rice gall midge—varietal resistance and chemical control, pp. 173–193. In: Int. Rice Res. Institute and Chinese Academy of Agricultural Sciences. *Rice Improvement in China and Other Asian Countries*. Los Banos, Phil.

Khan, Z. R. and R. C. Saxena. 1990. Purification of biotype 1 of brown planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). *Insect Sci. Applic.* 11: 55–62.

Nugaliyadde, L. and E. A. Heinrichs. 1984. Biology of rice thrips, *Stenchaetothrips biformis* (Bagnall) (Thysanoptera: Thripidae) and a greenhouse rearing technique. *J. Econ. Entomol.* 77: 1171–1175.

Oka, I. N. 1979. Feeding populations of people versus populations of insects: The example of Indonesia and the rice brown planthopper. Paper presented at the 9th Int. Congress of Plant Protection. Washington, DC, 5–11 Aug. 1979. 27 pp. (mimeo).

Pathak, M. D. and R. C. Saxena. 1980. Breeding approaches in rice, pp. 421–455. In: F. G. Maxwell and P. R. Jennings (eds.), *Breeding Plants Resistant to Insects.* John Wiley and Sons, New York.

Prakasa Rao, P. S. 1975. Some methods of increasing field infestations of rice gall midge. *Rice Entomol. Newsl.* 2: 16–17.

Saxena, R. C. and Z. R. Khan. 1989. Factors affecting resistance of rice varieties to planthopper and leafhopper pests. *Agric. Zool. Rev.* 3: 97–132.

Saxena, R. C., F. G. Medrano and M. Sunio. 1990. Rearing yellow stem borer (YSB) for screening varietal resistance. *Int. Rice Res. Newsl.* 15(3): 15.

Saxena, R. C., F. G. Medrano and C. C. Bernal. 1991. Rapid screening for rice tungro resistance using viruliferous green leafhopper nymphs. *Int. Rice Res. Newsl.* 16(1): 10–11.

Strong, R. G., D. E. Sbur and G. J. Patrida. 1967. Rearing stored product insects for laboratory studies: lesser grain borer, granary weevil, rice weevil, *Sitophilus zeamais,* and *Angoumois* grain moth. *J. Econ. Entomol.* 60: 1078–1082.

Velusamy, R. and R. C. Saxena. 1990. Using rice nurseries to collect thrips for use in screening rice germplasm. *Int. Rice Res. Newsl.* 15(3):16–17.

Viajante, V. D. and R. C. Saxena. 1990. Effect of temperature and storage on yellow stem borer (YSB) egg hatchability and larval survival. *Int. Rice Res. Newsl.* 15(1): 13–14.

Waldbauer, G. P. and A. P. Marciano. 1979. Mass rearing of the rice leaf folder. *Int. Rice Res. Newsl.* 4(2): 7.

Parasitoid *in vitro* Rearing: Successes and Challenges

S. Bradleigh Vinson

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

Abstract

Successes in the in vitro *rearing of parasitic beneficial insects began in the 1950s and to date several parasitic Diptera and ecto-, pupal- and egg-parasitic Hymenoptera have been reared from egg to adult on artificial media. Although success with the endoparasitic forms is still elusive, progress is being made.*

In this review the different parasitoid life styles are described along with some of the related problems that are essential to any successful in vitro *rearing effort. These include methods of obtaining eggs for* in vitro *rearing, and aspects of larval and pupal development that must be considered.*

A review of the current successes in in vitro *rearing is presented followed by a discussion of the challenges that are presented to those interested in developing* in vitro *rearing techniques.*

Our understanding of the physiology and behaviour of insect parasitoids, as well as their use in biological control has lagged behind other areas of entomology because of their inconsistent availability. Early research utilised species readily available from the field or species available through laboratory rearing such as the more readily available parasitoids of household pests (Wylie 1971; Salt 1970; Doutt 1963), but since the 1980s there has been an expansion in the study of parasitoids, particularly those of the more readily available laboratory cultured phytophagous species (see articles in J. Insect Physiology, *Vol. 32(4), 1986 and* Archives of Insect Biochemistry and Physiology, *Vol. 13, 1990). However, the*

inability to mass rear parasitoids* in vitro *(without their host) remains a limiting factor for both basic research and development of new approaches to biological control (Vinson 1986). It is the inability to mass produce beneficials that has limited the augmentative approach to biological control (Ridgeway and Vinson 1977; Vinson 1986). The result is that biological control has been largely confined to the classical approach centering on the search for and introduction of exotic species, and the conservation of natural enemies (DeBach 1974; Huffaker 1969).

Many new approaches to biological control depend on the consistent availability of large numbers of reproductively competent parasitoids which is, in turn, dependent on* in vitro *production (Vinson 1986, 1988a). One of these approaches is what can be termed the manipulative (Vinson 1988a) which includes both behavioural (Vinson 1977; Tumlinson and Lewis 1991) and genetic (Bartlett 1984; Beckendorf and Hoy 1985; Sailer 1981; Weseloh 1984) management. Simmonds (1944) suggested that parasitic insects could be bred for biological control and he even suggested that they might be reared on an artificial media. But where do we stand with* in vitro *rearing and what are the obstacles to the development of* in vitro *rearing technology? I will begin with a review of the different life styles and developmental diversity of parasitoids, as these differences must be considered in any* in vitro *rearing programme. I will review the status of* in vitro *rearing followed by a discussion of the challenges that lay ahead.

I. Parasitoid Life Styles

Insect parasitoids occur primarily in three holometabolus orders: the Stripseptera; the Diptera containing two major parasitoid families, the Tachinidae and some species of Sarcophagidae; and a number of families of the Hymenoptera which contain the bulk of the parasitoid species.

The Stripseptera are considered by some authors to be specialised Coleoptera (Evans 1984) and others consider them only related to the Coleoptera (Borror and DeLong 1964). Females develop within a host, commonly Orthoptera, Homoptera or adult Hymenoptera (Askew 1971), where they spend both their larval and adult stages. The numerous eggs hatch within the female which is within the hosts' body whereupon the active triungulin larvae escape to either search for or ambush a new host. Males also develop within a host, but emerge as winged adults that seek females. Their biology has been described by Kathirithamby (1989), but little is known of their behaviour or physiology.

The parasitic Diptera tend to parasitise late instar larvae, pupae or adult insects. The parasitic Diptera lack a well developed, piercing ovipositor and many either larvaposit or eggs are laid apart from the host. In the latter case, the eggs are often consumed by the host, the larvae hatch and penetrate the host's digestive system. In the former case, the first instar larvae seek the host (van Emden 1950). However, a few species have evolved to oviposit on or through the host's cuticle (Sweetman 1963). Dipterous larvae have a well developed digestive system and a well developed cuticle. The Calliphoridae are generally saprophagous, the larvae killing the host and then feed on dead tissue (Clausen 1940). The Tachinidae are exclusively protelean parasites (Askew 1971), and the second or later instars often require more oxygen than is provided by the host haemolymph, the older larvae either associating with the host's tracheal system (Pantel 1910; Imms 1931) or reach air through an intersegmental membrane (Keilin 1944; Clausen 1940). Mature larvae always leave the host to pupate; adults lacking chewing mouthparts that are needed to escape host remains. Eggs must be fertilised to hatch and fertilisation gives rise to both sexes.

The parasitic Hymenoptera are characterised by a piercing ovipositor and associated accessory glands, haplo-diploid sex determination (unfertilised haploid eggs develop into males) providing some control over the allocation of sex, a blind digestive system as larvae, and chewing mouthparts as both larvae and adults. The parasitic Hymenoptera exhibit three major life styles. Many species are ectoparasitic and their eggs tend to be large due to the presence of yolk needed to sustain early development (Hagen 1964). Further, the chorion is generally thick to reduce desiccation and provide added protection. Because they feed externally these larvae develop in a microenvironment similar to the host and gas exchange is not a problem. Venom is usually injected into the host during oviposition and the host does not grow after oviposition (Vinson and Iwantsch 1980b). Ectoparasitoids of immature stages must deal with variable but finite resources. In such cases, the female either adjusts the sex or number of progeny oviposited or the progeny adjust their size to the resource (Vinson and Barbosa 1987). Those attacking pupae and adults have more consistent resources. The host in some of these relationships is killed as the larvae develop and the larvae feed on the remains.

The egg parasitoids feed internally within an insect host egg that provides a uniform and consistent environment. The eggs of egg parasitoids are small with a thin chorion and have no yolk. Insect eggs have evolved to allow for gas

exchange necessary for embryonic development (Hinton 1981). Developing egg parasitoids have successfully taken advantage of the host egg chorion's permeability and small distances involved in gas exchange (see references in Hagen 1964). The resource is uniform in amount, but may be variable in condition; more or less yolk to tissue ratio depending on the host's embryonic development. The resource is finite and many species carefully select their host, ensuring the proper amount of nutrition is available or the size or number of parasitoids is adjusted to the resource (Vinson 1988b; Vinson and Barbosa 1987). Further, some egg parasitoids appear to be capable of developing on dead eggs. For example, *Trichogramma* will develop on sterile eggs (Young and Hamm 1967; Lewis and Young 1972).

The last major group comprise the endoparasitoids that grow and develop within the haemocoel of their host. The eggs of these species are generally small with no yolk and have a thin chorion (Clausen 1940). The haemolymph of insects is generally an oxygen poor environment (Wyatt 1961), but the respiratory needs and how they are met in endoparasitoids is not well defined. Endoparasitoids tend to be more uniform in size, requiring a specific amount of resources. Thus, in many species after parasitism the host continues to grow and develop, the parasitoid both altering the host and responding to the various changes occurring within the changing environment (Vinson 1988b).

II. Developmental Considerations

In order to develop effective *in vitro* rearing programmes, efforts must not only be directed to the development of a nutritious artificial media in which to grow the immatures, but the other biological and physical factors needed to produce large numbers of adults must be considered. One of the first considerations is a ready source of viable parasitoid eggs. Other considerations include fertilisation and the physical and chemical conditions for embryonic development, larval development, and pupation.

A. Source of Eggs

Eggs are oviposited once a host is located and accepted, a process that involves a number of behavioural steps (Vinson 1986). For *in vitro* rearing the steps involved in host location can be circumvented as host and the parasitoid can be physically brought together. However, this is not to say that the host location steps should be ignored because to do so might lead to the development of strains less adapted

to these behaviours (see Vinson, in press), but from a practical *in vitro* rearing perspective only a ready source of eggs is needed. These may be obtained through: (1) stimulating females to oviposit in artificial substrates, (2) dissection of parasitised hosts and removal of eggs, or (3) by dissection of female parasitoids and removal of eggs. Each has its own benefits and problems.

The first approach may require the isolation and identification of the factors that are involved in host recognition and acceptance, but how is a host recognised? There appear to be both chemical and physical cues (Vinson 1976, 1985) involved. These cues tend to be non-volatile, Group III cues, (see Vinson 1991), that along with shape or movement stimulate females to probe with their ovipositor. For example, Hendry *et al.* (1973) identified heptanoic acid from *Pthorimaea operculella* that causes antennation followed by ovipositor probing in the braconid, *Orgilus lipidus*. Vinson and Piper (1986) reported a similar behaviour that was elicited when *Tetrastichus hagenowii* contacted calcium oxalate on a spherical surface which simulated a cockroach ootheca. In some species, shape alone may be sufficient. For example, *Trichogramma* not only respond to spherical objects (Marshal 1936; Salt 1934), but host egg size influences egg allocation (Schmidt and Smith 1987). Hagen and Tassen (1965) and Rajendram and Hagen (1974) developed a wax egg that elicited oviposition that was later used by Hoffmann *et al.* (1974) to rear *Trichogramma*.

However, host acceptance, and the release of eggs, appears to require another set of cues, Group IV cues (see Vinson 1991). A number of these cues have also been identified and are often amino acids, sugars or salts (Wu and Qin 1982b; Rajendram and Hagen 1974; Kainoh 1989; Xie *et al.* 1991; Ding *et al.* 1980b). For example, Arthur *et al.* (1972) reported that *I. conquisitor* would oviposit in a media of arginine, leucine, serine and $MgCl_2$. Later, Hegdekar and Arthur (1973) reported that the addition of trehalose increased the response. Nettles *et al.* (1982) reported that KCl_2 and $MgSO_4$ stimulated oviposition of *Trichogramma pretiosum* into wax eggs. Using these wax eggs several hundred *Trichogramma* eggs could be collected from each artificial egg for use in rearing studies. In China the use of small egg-like depressions of polyethylene film filled with media not only stimulates oviposition by the shape and presence of amino acid stimulants (Qin and Wu 1988), but supports the development of *T. dendroleme* (Li *et al.* 1988). House (1978) used an artificial host that consisted of encapsulated media to obtain oviposition and to rear *I. conquisitor*.

For most researchers involved in *in vitro* parasitoid rearing programmes, eggs have been obtained by the second method, superparasitising hosts which are then dissected (Greany 1986; Nettles *et al.* 1980; Lawrence 1988; Thompson 1981d; Grenier *et al.* 1975; Pennacchio *et al.* 1992b). This has allowed some researchers to begin their rearing with embryonated eggs (Pennacchio *et al.* 1992b) or even larvae (Nettles *et al.* 1980), which is important because some developmental constraints are bypassed (see below). For ectoparasitoids, Thompson (1975) was able to remove and use eggs oviposited on coddled (heat treated) larvae.

A third approach is to remove eggs directly from the ovary of females. For proovigenic and those synovigenic Hymenoptera (Flanders 1950) where large numbers of mature eggs are available at the time of dissection, this may have some potential. However, there are two problems that can occur when eggs are removed from the reproductive system of Hymenoptera. One is the failure to be fertilised and the second is their failure to develop unless they have passed through the ovipositor (Borstel 1960; Salt 1965; King and Rafai 1973).

B. Egg Activation and *in vitro* Fertilisation

Fertilisation is needed to get development of dipteran eggs and for the production of females of the parasitic Hymenoptera where unfertilised eggs are normally produced but only develop into males. Thus, the source of eggs is very important since eggs removed from the female prior to fertilisation or oviposition would either not develop (Diptera) or in the Hymenoptera would only produce males.

Went and Krause (1973, 1974) and Went (1982) working with Hymenoptera demonstrated the importance of mechanical deformation to initiate embryonic development. However, Vinson and Jang (1987) reported that hypertonic salines and cold shock could also activate *Campoletis sonorensis* (Ichneumonidae) eggs. Regardless of the mechanisms these results are encouraging since it appears that properly manipulated hymenopteran eggs removed from a female can be stimulated to develop. Another possibility that is presented using ovarian eggs from the Hymenoptera is the development of *in vitro* fertilisation techniques. The development of such techniques could lead to the control of the number of females cultured.

C. Embryonic Development

For all endoparasitic Hymenoptera studied, researchers have had to work with postgerm band eggs (Pennacchio *et al.* 1992b; Rotundo *et al.* 1988; Greany 1986); a problem not encountered with the other groups. Working with *Microplites croceipes* (Braconidae), Greany (1986) showed that egg hatch could occur *in vitro* but was dependent on host blood factors probably from the fat body. The factor appeared to be a protein with a molecular weight about 230 KDa (Greany *et al.* 1990). Ferkovich and Dillard (1990) showed that coincubated fat body stimulated protein biosynthesis in the eggs. Using a fat body-derived cell line conditioned media Ferkovich *et al.* (1991) showed that *M. croceipes* eggs would hatch.

D. Larval Development

The larvae of parasitoids differ dramatically (Hagen 1964). Some parasitic forms have free-living triungulin larvae capable of searching or ambushing hosts. Once the host is reached the larvae transform to more sedentary forms. Many dipterous larvae are of the planidial type which also transform once a host is reached. What factors cause these changes are unknown. Many first instar endoparasitic forms, particularly the solitary species, have sickle-shape mandibles which are used in the destruction of competitors (Salt 1932, 1961). This necessitates the rearing of these species separately, at least during the 1st instar. The sickle-shaped mandibles of most species are absent in the second instar which have more triangular shaped mandibles which do not appear to be very functional in combat. The parasitic Diptera lack mandibles but have oral hooks that tear tissue, a process that appears to be much more destructive of host tissue.

Ectoparasitoids normally feed through the cuticle and thus are exposed to air and relatively dry conditions. When such insects are placed on a liquid diet they drown. This led Thompson (1975) to try feeding larvae on 82 mesh silk bolting covered with a paralodin solution (1 g paralodin in 50 ml ether + 50 ml absolute-ethanol). However, the larvae slowly destroyed the membrane and became contaminated with diet and died. Nettles *et al.* (1980), working with tachinid larvae which also need contact with air, were successful with agar. Thompson (1975) and Yazgan (1972) found lipophilic Sephadex beads to be effective. Egg parasitoids appear to do fine in a liquid media but they must be removed to pupate (Strand *et al.* 1988; Xie *et al.* 1986a).

Rearing Parasitoids *in vitro*

The situation for endoparasitoids is not yet clear. The eggs of some species, for example *Blastothrix* spp. (Encyritidae), oviposit an egg with a stalk (pedical) which remains attached to the site of oviposition. As the larvae develop, they remain in contact posteriorly with the egg stalk thus maintaining contact with the atmosphere (Imms 1931). Many endoparasitic forms can move freely within the host's body cavity and appear to be able to develop to at least the 2nd instar in a liquid media (Lawrence 1988; Pennacchio *et al.* 1992b). Endoparasitic larval ichneumonids and braconids are often caudate (with a tail) or vesiculate (with an anal-vesicle) or both. The function of the tail and anal-vesicle is not clear. Ullyett (1944) suggested the "tail" found on some larvae could be involved in food absorption, serve as an egg buster, or may help balance the large head. Some authors (Fisher 1971) suggested a respiratory function for these organs, but others have failed to find a respiratory function (Thorpe 1932; Edson and Vinson 1976). The oxygen needs of some parasitic forms may be very low. For example, Flanders (1930) reported that parasitised eggs of *Trichogramma* would develop to the pupal stage under water and Edson and Vinson (1976) reported that *M. croceipes* appeared to be facultatively anaerobic.

The suggestion of food absorption by the anal vesicle has received some support by Edson and Vinson (1977) and Edson *et al.* (1977). Their results suggest that larvae absorb amino acids and sugars through their anal vesicle and that oral intake is not essential during development of the early and middle instar. These ideas are supported by the report of Thompson (1975) that the development of *Exeristes roborator* (Ichneumonidae) was better on a media containing free amino acids than protein. Although for most insects (Dadd 1973), including some pupal endoparasitoids (Thompson 1981d) and some parasitic Diptera (Nettles 1986b), protein is more important in the media than the presence of free amino acids; for some species free amino acids appear to be important (Thompson 1976b; Yazgan 1972). For ectoparasitoids extraoral digestion may be important in releasing the nutrients and increasing their availability. Thus, the ectoparasitoids might be expected to respond better to simple nutrients. Whether the endoparasitic Hymenoptera, which are bathed in a protein-amino acid rich environment have the necessary enzymes to feed on and digest complex nutrients is not known, but there is evidence that the endoparasitic larvae secrete several factors (Führer and Willers 1986) that could be involved in extraoral digestion. Führer *et al.* (1978) has shown that larvae do secrete factors into the surrounding media. These factors have been shown to be antifungal agents and similar suggestions have been made for secretions released from teratocytes (Führer and El-Sufty 1979). However,

these secretions could have some nutritional significance (Strand *et al.* 1986).

Some endoparasitoids such as *M. croceipes* and *Cotesia congregatus* (Braconidae) only feed within the hemolymph, egress to spin a cocoon and pupate leaving the host intact (Beckage and Riddiford 1983; Webb and Dahlman 1985). Other species such as *C. sonorensis*, *Venturia canescens* (Ichneumonidae) and *Cardiochiles nigriceps* (Braconidae) begin to feed on host tissues just before they egress from the host (Wilson and Ridgway 1975; Corbet 1968; Vinson and Barras 1970). Thus, for some species both the nutritional, as well as the composition and physical characteristics of the diet may change.

E. Pupation

The larvae of parasitoids pupate in many different situations. Ectoparasitoids, which adjust their size, after reaching a critical size may pupate if food is depleted but may continue to increase in size if food is available. What triggers pupation in the latter case is not clear. Pupation generally occurs near the host remains. When placed on less than "ideal" diets the larvae of some ectoparasitic species may become "bloated" or are unable to pupate. A similar situation occurs with egg parasitoids such as *Trichogramma* (Xie *et al.* 1986 a, b; Grenier and Bonnot 1986) and *Trissolcus basalis* (Scelionidae) (Volkolf *et al.* 1972); removing the larvae from the media stimulates pupation if they have not become bloated (Xie *et al.* 1986a; Strand *et al.* 1985). Most egg parasitoids pupate within the egg chorion although this does not appear to be essential (Volkolf 1990; Xie *et al.* 1986b). A few endoparasitoids and many pupal parasitoids pupate within host remains although most egress and construct silken cocoons (Hagen 1964) and proper conditions for cocoon formation may be necessary. What stimulates endoparasitoids to egress is not clear. Beckage and Riddiford (1982) reported that the injection of juvenile hormone stimulated *C. congregatus* to egress from their host although other authors have reported the opposite effect (Vinson 1974).

III. Current *in vitro* Rearing Progress

Since the early attempts at rearing of parasitoids on different insects (Simmonds 1944) and the work of Prell (1915), who attempted to rear a parasitic dipteran on non-insect material, the development in *in vitro* rearing has been slow, but encouraging. The first successful attempts were with the parasitic sarcophagid Diptera (House 1954a; 1966a,b) followed by successes with pupal endoparasitoids

(Bronskill and House 1957). Successes soon followed with ectoparasitoids (Yazgan and House 1970; Thompson 1975) and egg parasitoids (Hoffmann *et al.* 1975); the success with larval endoparasitic species remains elusive.

A. Parasitoid Diptera

Parasitic Diptera have been the subject of the earliest attempts to develop *in vitro* parasitoid rearing techniques. Prell (1915) reported that the tachinid *Parasitigena sitvestres (=segregata)* could be maintained on hen egg albumen as long as its spiracles were open to the air. However, it was House (1954a, 1966a,b) that reported some of the first successful attempts to rear a parasitoid on an artificial diet. Working with the sarcophagid fly, *Agria affinis* which is a parasitoid of the spruce budworm, House (1966a) was able to rear larvae to adults on a chemically defined semi-solid media. However, Grenier *et al.* (1974, 1975) attempted to rear *Phreyxe caudate* (Tachinidae) to the first instar on a liquid diet, but the larvae drowned. In many dipterous parasitoids the first instar larvae may respire cutaneously (Keilin 1944; Ziser and Nettles 1978), but as they develop they require spiracular contact with air (Keilin 1944). In these endoparasitic forms the tracheal system of the parasitoid becomes attached to that of its host. In artificial diets the spiracles need to be open to the air. Keeping this in mind Grenier *et al.* (1975) attempted to place larvae on a thicker diet, but the increased osmotic pressure was detrimental.

Grenier *et al.* (1978) was able to rear a pair of *Lixaphaga diatraeae* (Tachinidae) to adults by adding agarose which resulted in a solid support for the developing larvae that did not alter the osmotic pressure but reduced drowning. Nettles *et al.* (1980) developed a diet in which first stage larvae of *Eucelatoria bryani* (Tachinidae) would develop, but he encountered the same problem as Grenier *et al.* (1978). Nettles *et al.* (1980) found that the growth of 1st instar larvae was greatly improved when the media was shaken; presumably due either to the removal of waste product build up or to increased gas exchange. However, the 2nd instar larvae that were produced drowned. This led these authors to develop a technique that slowly led to a solid support on which the larvae could develop while exposing their spiracles to the air. Nettles *et al.* (1980) added 1 to 2.5% agar to the diet, adjusted the pH to 6.7–6.8 and poured the mixture into hot petri dishes. Once cooled the dishes were placed in a 50% r.h. for 24 hr to dry out the agar-based media. The liquid diet containing the developing 1st instar larvae was then layered over the hardened agar diet and the system was maintained at 90% r.h. As

the larvae developed in the liquid media, the liquid was also being absorbed into the agar so when the larvae were older they ended up on a solid support that held the spiracles of the older larvae above the liquid. However, both Nettles (1986b) and Bonnot *et al.* (1984) reported that *E. bryani* and *P. caudate*, respectively developed better if they fed on the host for 24 hr; suggesting some needed growth factors were missing. Nettles (1986a, 1987) found that asparagine was an essential ingredient and proline, tyrosine, and glycine, although not essential, were very beneficial. Further, improvements in rearing were obtained by the addition of bovine serum albumin and soybean flour (Nettles 1986b).

B. Ecto- and Pupal-Parasitoids

Bronskill and House (1957) were able to rear a limited number of the pupal endoparasitoid, *Pimpla turionella* (Ichneumonidae) to the adult on a non-insect diet of pork liver and saline. Bouletreau (1968, 1972) utilised a hanging drop slide containing host haemolymph to rear the pupal parasitoid *Pteromallus puparum* (Pteromalidae) to the adult stage. Hoffmann *et al.* (1973) reported similar data floating *P. puparum* eggs on heat-treated, centrifuged and filtered (0.3 g) insect plasma. They found that a 50% dilution of the plasma with sterile distilled H_2O also allowed for development and that adults could be obtained using a diet consisting of equal volumes of 10% yeastalate, fetal bovine serum and Graces tissue culture medium, although the success rate was nearly half that of the use of haemolymph. In all cases, the developed larvae were transferred to moist filter paper to pupate (Hoffmann and Ignoffo 1974). Yazgan and House (1970) developed a chemically defined semi-solid diet for the ichneumonid *Itoplectis conquisitor* based on the diet used to rear *A. affinis* (House 1966a,b).

The ectoparasitoid *E. robrator* was reared from egg to adult on a chemically defined semi-solid diet similar to that developed by Yazgan and House (1970) by Thompson (1975). Thompson (1975) found that Sephadex (lipophilic) LH-20 provided a semi-solid consistency that prevented larval drowning of early instars. Drowning, like the Diptera, appears to be a major problem for ectoparasitoids. Problem of contamination was accomplished by transferring larvae to new diet each day.

The pupal endoparasitoid *Brachymeria intermedia* (Chalcididae) was reared to the pupal stage on several diets where increased proteins increased survival. Larvae were also reared on a semi-solid agar (2%) diet (Thompson 1980) similar

to that used for *E. roborator* (Thompson 1975). The high osmotic pressure produced by the small molecular weight nutrients appeared to be detrimental and Thompson (1981d) used albumin in place of other thickening agents by placing the albumin in the diet and then heating to denature the media. In the soluble form albumin retarded the development of *B. intermedia*, but the larvae developed when the albumin thickened. Here the albumin served both as a source of amino nitrogen and as a physical substrate. However, *Pachycrepoideus vendemmiae* (Ichneumonidae) did not develop on this diet which led Thompson and Bednar (1983) to speculate that the problem was due to the osmotarity which he altered by replacing the albumin with a 15-poly amino acid mixture and the glucose with trehalose. Using these diets Thompson (1983b,c) was able to rear *Brachymeria lasus* (Chalcididae) on a variety of diets supplemented with different glucose and amino acid levels.

C. Egg Parasitoids

Research in developing *in vitro* rearing techniques for egg parasitoids began with diets consisting almost entirely of insect haemolymph used to rear *Trichogramma* (Hoffmann *et al.* 1975) and many efforts were directed to the development of artificial eggs (Grenier and Bonnot 1986; Dai *et al.* 1988; Li *et al.* 1988) and ovipositional stimulants (Xie *et al.* 1991; Qin and Wu 1988; Nettles *et al.* 1982, 1983) for this species. Efforts in the development of diets for *Trichogramma* have been directed to the reduction of the amount of haemolymph. Liu *et al.* (1979) used 35–40% insect haemolymph, chicken egg yolk, milk and fetal pig serum for the trichogrammatids, *Trichogramma dendrolimi*, *T. anstraticun*, *T. confusum*, *T. evanescens* and *T. japonicum*. Since their work, a number of authors have used varying amounts of insect haemolymph with chicken egg yolk, bovine milk and other factors to rear adults of both *T. dendrolimi* and *T. pretiosum* (Xie *et al.* 1986a,b; Strand and Vinson 1985; Grenier and Bonnot 1986). Strand and Vinson (1985) attempted to rear *T. pretiosum* on a more defined media consisting of various free amino acids, vitamins, salts, carbohydrates, lipid and proteins. They were unable to get pupation without the addition of 40% insect haemolymph. Similar results were obtained by Grenier and Bonnot (1986) for *T. dendrolimi* and *T. maidis*. Xie *et al.* (1986b) reported that pupation of *T. pretiosum* fed a chicken egg yolk and milk based diet required insect haemolymph for pupation while egg liquid was needed for adult emergence. Irie *et al.* (1987) isolated from haemolymph two carbohydrate containing fractions from a DEAE-HPLC column that were necessary for pupation. However, Wu *et al.* (1980, 1982), Wu and Qin (1982a), and Liu and Wu (1982) were able to rear *T. dendrolimi* to the adult stage on diets devoid of insect

derivatives. The composition of the basic medium of amino acids, salts, vitamins, sugars, and organic acids was supplemented with bovine serum, chicken embryo extract, calf fetal serum, hen egg yolk, yeast extract, and wheat germ, which produced adults (Qin and Wu 1988). However, this media did not prove successful for *T. pretiosum* (unpubl.).

In addition to the media there has been a lot of attention directed to the development of artificial eggs and ovipositional stimulants for the egg parasitoids. Rajendram and Hagen (1974) used an artificial wax egg consisting of 50% bees wax and 50% paraffin oil. This mixture is layered over the media to be encapsulated. The size of the beads (artificial eggs) is influenced by the temperature of the media and the size of the pipette used to form drops which are allowed to fall on wax paper or glass slides. The bead wall thickness is also influenced by the temperature and the composition of the paraffin oilbees wax mixture. Grenier and Bonnot (1986) used frozen drops of media coated with a paroxylylene shell that allowed for O_2 exchange and they alluded to the importance of shell thickness for oviposition by *Trichogramma*.

Li *et al.* (1988) utilised 32–65 µm thick polyethylene and 32–36 µm thick polypropylene films which are permeable to O_2 and found different species were more or less successful in ovipositing in the different thickness of film. Small 2 to 5 mm diameter depressions are made in the film by a glass rod or other suitable means and 3–5 µl of media was placed in the depression which was then covered by another sheet of plastic and heat sealed at the edges. For *T. dendrolimi* 20–30 µm thick polyethylene with a 2.5 mm depression has been successfully used (Li *et al.* 1988). Using this technique a species of *Anastatus* (Li *et al.* 1988; Liu *et al.* 1988) and a species of *Tetrastichus* (Ding *et al.* 1980a) have also been reared.

Hoffman *et al.* (1975) used filter paper moistened with the diet which was placed in a petri dish and hanging drop techniques have been used by several research groups (Guan *et al.* 1978, Hubei Res. Group 1979; Xie *et al.* 1986a). Xie *et al.* (1986a) were able to rear groups of 60 larvae together in microtitre plates containing 96 flat bottom wells (6.4 mm in 18 diameter) and over 24,000 larvae were able to develop in a Petri dish. Under such conditions there is an over abundance of media and the larvae must be removed, rinsed, and allowed to dry to pupate.

Trichogramma, *Tetrastichus* and *Anastatus* are gregarious while some egg para-

sitoids, such as the Scelionidae, are solitary. Both *Telenomus heliothidis* (Scelionidae) and *T. basalis* normally oviposit one egg per host and *T. heliothidis* preferentially accepts hosts with a volume of 0.1 µl (Strand and Vinson 1983). Because 1st instar larvae are aggressive, they had to be reared individually. Using the media of Xie *et al.* (1986b) containing w/v 40% *Manduca sexta* haemolymph, 35% chicken egg yolk, 23.5% bovine milk and 1.5% trehalose at a pH 6.8, and 390 mOsm/kg, Strand *et al.* (1988) were able to rear *T. heliothidis* to the adult stage. Strand *et al.* (1988) also reported that teratocytes were important to the development of *T. heliothidis* in the artificial media. However, 3rd instar larvae were only allowed to feed for 12 hr at which time they had to be removed and rinsed of excess media in order to pupate. Using a similar media Volkolf *et al.* (1972) reared *T. basalis* from eggs to 3rd instar larvae or they could rear 2nd instar to adults, but they were unable to rear this species from egg through to the adult on the same media.

D. Endoparasitoids

It has been suggested that the *in vitro* culture of endoparasitic idiobionts (Halselbarth 1979) may be the most difficult (Greany 1986; Thompson 1986 a,b). There are several reasons for this view. One is that endoparasitoids develop within a living and changing environment that provides both resources and developmental triggers at specific times during the parasitoid's developmental period. One of the major concerns has been the suggestion that parasitoids utilise the hormonal balances of their host to optimise their own growth and development (Lawrence 1986a). In the case of the endoparasitic braconids *Opius concolor* and *O. malleus*, the larvae do not moult until their dipteran hosts pupate (Cals Usciati 1969, 1975; Lathrop and Newton 1933). Cals Usciati (1975) found that parasitoid larvae did not moult in host larvae developmentally suspended by γ-radiation, but would moult if these hosts were injected with ecdysone. Lawrence (1982, 1986b) reported similar results with *Biosteres longicaudates* (Braconidae) using ligated larvae. She further demonstrated that larvae would not moult if removed to artificial medium prior to host ecdysone release, but was stimulated by the addition of 20-hydroxyecdysone to moult (Lawrence 1986b). Lawrence (1988) used 36–48 hr embryonated eggs placed in Goodwin's IPL-52B medium (K. C. Biological Products, Lenexa, KS), with and without fetal calf serum (20%), and with and without 20-hydroxyecdysone. Growth was good in all media used but moulting only occurred in the 20- hydroxyecdysone supplemented media; with the media containing the fetal calf serum being significantly better (Lawrence 1988).

Greany (1980) reported growth of first instar *Cotesia (Apanteles) marginiventris* (Braconidae) in Graces media co-cultured with host fat body and containing bovine serum albumin, fetal bovine serum and whole egg ultrafiltrate. Greany (1981) found that egg development improved if L-glutamine was added to the medium just before use. The presence of teratocytes appeared to improve larval growth, but moulting to second instar did not occur. Similar results were obtained with *M. croceipes* using Goodwin's IPL-52B media supplemental with L-glutamine (2 mg/ml), 50 mg/ml Gentamicin and adjusting to pH 6.4 with 10% NaOH and to 320 mOsm using glycerol and beginning with post-germband eggs (Greany 1980, 1986). The growth promoting properties of fetal bovine serum added to the media was reduced with charcoal absorption indicating some growth promoting factor may be necessary, but other serum proteins, foeterin and albumin, also promoted growth of the first instar larvae (Greany 1986; Greany *et al.* 1989). Pennacchio *et al.* (1992b) working with a basic medium containing a complex of nutrients (Table 1) was able to rear post-germband eggs of *C. nigriceps* to first instar and obtained moults to the second instar by the addition of fetal bovine serum when the first instar larvae had reached a critical size. Teratocytes did not appear to improve growth and failed to develop in the medium where the parasitoid larvae moulted.

Rotundo *et al.* (1988) attempted to rear the aphid parasitoid *Lysiphebus fabarum* (Braconidae) on an artificial media. They began with 2nd instar larvae with and without their sister teratocytes in a cell culture media developed for the host and modified by Cavalloro (1981). Mortality was high (over 50%) the first day following the transfer from the host, but a couple of adults were obtained. Teratocytes did not do well on the media and did not appear to improve the survivorship of larvae.

IV. Challenges

A. Nutritional

The determination of the dietary needs of animals has involved the identification of the nutrient composition of the food, an analysis of the waste products produced along with a determination of the digestive capabilities, the biochemical processes, the vitamin, and cofactor needs of the organism. With parasitic species this approach is a challenge because the food is often a living host with its own biochemical processes that can be influenced by the parasitoid. Instead, many

Table 1. Composition (mg/100 ml) of various media utilised to rear insect parasitoids *in vitro* over the last two decades

Reference/Components	1	2	3	4	5	6	7	8
Alanine	129	312.5	300.0	100.0	50.0	–	125.0	100.0
Arginine	Hcl 53	381.2	Hcl 240.0	125.0	Hcl 40.0	Hcl 48.0	125.0	160.0
Aspartic Ac.	153	400.0	-ate 600.0	–	ate 100.0	78.0	–	30.0
Cysteine	18	81.25	Hcl 60.0	8.0	Hcl 10.0	6.0	8.0	13.0
Glutamic Ac.	274	636.25	-ate 600.0	–	100.0	90.0	–	200.0
Glycine	35	487.5	300.0	80.0	50.0	12.0	80.0	120.0
Histidine	Hcl 35	150.0	480.0	75.0	80.0	FB 12.0	100.0	180.0
Hydroxyproline	24	106.25	240.0	–	40.0	80.0	–	30.0
Isoleucine	153	325.0	240.0	80.0	40.0	45.0	80.0	100.0
Leucine	140	481.25	420.0	100.0	70.0	–	130.0	150.0
Lycine Hcl	Hcl 88	362.5	420.0	150.0	2Hcl 70.0	42.0	150.0	250.0
Methionine	94	187.5	120.0	30.0	20.0	60.0	40.0	40.0
Phenylaline	140	418.75	240.0	70.0	40.0	60.0	100. 0	80.0
Proline	100	468.75	240.0	50.0	40.0	30.0	75.0	60.0
Serine	176	418.75	240.0	50.0	40.0	DL 24.0	100.0	100.0
Threonine	94	337.5	240.0	70.0	40.0	12.0	70.0	90.0
Tryptophan	47	87.5	60.0	40.0	10.0	6.0	40.0	30 0
Trysosine	82	218.75	240.0	70.0	–	15.0	70.0	25.0
Valine	165	368.75	720.0	100.0	120.0	42.0	100.0	150.0
β-Alanine	–	–	–	10.0	–	18.0	10.0	–
Glutamine	–	–	–	200.0	–	60.0	225.0	100.0
L-Asparagine	–	–	250.0	170.0	–	78.0	200.0	100.0
Ornathine	–	–	250.0	–	–	–	–	–
Haemolymph	–	–	–	–	–	40% F	–	–
Albumin	–	–	–	lacto 900	BSA 15,000	BSA 20,000	–	BSA 20.0 lact 5.0
Egg yolk	–	–	–	–	–		–	10%

☛

Table 1. Continued

Reference/Components	1	2	3	4	5	6	7	8
Yeastlate	–	–	–	200.0	–	–	–	–
Bacto-Peptone	–	–	–	500.0	–	–	–	–
Protein	–	–	–	–	–	–	–	FBS 20% milk 20%
Agar	750	–	–	–	–	–	1500.0	–
Soy flour	–	–	–	–	–	–	2600.0	–
Sucrose	–	–	–	–	–	990.0	–	–
Trehalose	–	2000.0	–	1000.0	–	4800.0	1000.0	600.0
Glycerol	–	–	–	–	450.0	450.0	–	–
Glucose	–	–	2000.0	50.0	4000.0	150.0	50.0	800.0
Maltose	200	–	–	–	–	60.0	–	–
Glutathione	–	–	–	10.0	–	–	10.0	–
Adenine	–	–	–	6.0	–	–	6.0	–
Thymine	–	–	–	6.0	–	–	6.0	–
Guanine	–	–	–	6.0	–	–	6.0	–
Cytosine	–	–	–	3.0	–	–	3.0	–
Uracil	–	–	–	6.0	–	–	6.0	–
ATP	–	–	–	20.0	–	–	20.0	–
Cholesterol	100	65.63	30.0	10.0	10.0	100.0	10.0	–
Triolein	–	–	–	50.0	–	–	50.0	–
Linoleic	–	44.63	50.0	–	–	–	–	–
Linolenic	–	20.99	50.0	–	–	–	–	–
Palmitic	–	81.37	123.0	–	–	–	–	–
Palmitoleic	–	2.63	25.0	–	–	–	–	–
Oleic	–	112.87	123.0	–	–	–	–	–
Stearic	–	–	25.0	–	–	–	–	–
Tween 80	25	168.95	–	150.0	–	–	150.0	–
Interlipid	–	–	–	–	–	–	–	–

Table 1. Continued

Reference/Components	1	2	3	4	5	6	7	8
Egg yolk phospholipid	–	–	–	–	240.0	240.0	–	–
Soy oil	–	–	–	–	2000.0	2000.0	–	–
Disterayl phosphotidyl choline	–	–	–	–	0.1	–	0.1	–
Dimyristayl phosphotidyl choline	–	–	–	–	0.1	–	0.1	–
Phosphoethanolanine	–	–	–	–	5.0	–	5.0	–
Ca Phosphotydyl choline	–	–	–	129.0	–	–	129.0	–
Succinic Ac	–	–	–	–	–	0.3	–	–
Malic Ac	–	–	–	30.0	–	3.0	30.0	–
α-ketoglutaric Ac	–	–	–	45.0	–	2.0	45.0	–
Fumaric Ac	–	–	–	50.0	–	0.3	50.0	–
Citric Ac	–	–	–	25.0	–	–	–	–
Pyruvic Ac	–	–	–	30.0	–	–	–	–
Vit C	–	8.16	–	5.0	–	–	5.0	–
GABA	–	–	–	5.0	–	–	5.0	–
Biotin	0.025	0.04	0.06	0.05	0.06	23.0	0.05	0.016
Choline	cl 100	cl 189.47	cl 200.0	–	cl 200.0	cl 49,200	–	180.0
Cyanocobala-mine	–	–	0.02	–	0.02	–	0.0035	0.1
Folic acid	5	0.11	0.1	1.0	0.1	7.0	1.0	0.12
Inositol	100	13.12	15.0	5.0	15.0	myo 384	myo 5.0	10.0
Niacin	–	–	–	–	–	10.0	–	–
Nicotinamide	–	4.37	5.0	–	5.0	160.0	2.0	2.0
Pantothenic Ac	ca 15	ca 2119.0	ca 2.0	ca 4.0	2.0	ca 50.0	ca 4.0	1.6
Pyridoxine	15	cl 0.29	cl 0.3	0.1	cl 0.3	cl 31.0	0.1	0.02
Riboflavin	15	1.02	1.0	1.0	1.0	30.0	1.0	0.016
PABA	15	0.15	–	–	–	20.0	–	–
ß Carotene	–	–	–	–	–	1000.0	–	–
B-12	–	0.015	–	–	–	26.0	–	–
Thiamine Hcl	5	–	0.2	0.1	0.2	10.0	0.1	0.016
Nicotinic acid	15	–	–	2.0	–	–	–	–

Table 1. Continued

Reference/Components	1	2	3	4	5	6	7	8
Retinol	–	–	–	0. 5	–	–	0 .5	–
α cocooherol	–	–	–	0.5	–	–	0.5	–
$CaCl_2$	–	30.57	30.0	–	15.0	–	–	66.0
Ca-succinate	–	–	–	67.0	–	–	–	–
Co Cl $6H_2O$	–	7.13	5.0	–	2.5	2.0	–	0.005
$Co(CH_3CO_2)_2$ $4H_2O$	–	–	–	0.02	–	–	0.02	–
Cu $Cl_2.2H_2O$	–	–	–	–	–	3.0	–	0.02
Cu SO_4 . $5H_2O$	–	5.60	5.0	–	2.5	–	–	–
$Cu(CH_3CO_2)_2.H_2O$	–	–	–	0.2	–	–	0.02	–
Fe $Cl_3.6H_2O$	–	17.99	20.0	–	10.0	–	–	–
Fe $SO_4.7H_2O$	–	–	–	–	–	30.0	–	0.055
Fe $(NO_3)_3.9H_2O$	–	–	–	8.9	–	–	8.9	–
K_2HPO_4	–	375.12	370.0	51.0	180.0	–	51.0	–
KH_2PO_4	–	–	–	40.0	–	–	40.0	–
KCl	–	–	–	59.0	–	72,000	59.0	260.0
KI	–	–	–	0.02	–	–	0.02	–
$MgCl_2.6H_2O$	–	–	–	319.0	–	–	319.0	–
$MgSO_4.7H_2O$	–	131.54	120.0	123.0	60.0	55,000	123.0	92.0
$MnCl_2$. $4H_2O$	–	–	–	–	–	12.0	–	0.002
$MnSO_4$. H_2O	–	0.40	1.0	–	0.5	–	–	
$Mn(CH_3CO_2)_2$ $.4H_2O$	–	–	–	5.5	–	–	5.5	–
$(NH_4)MO_7O_{24}$. $4H_2O$	–	–	–	–	–	2.0	–	0.004
NaCl	–	–	–	261.0	–	–	261.0	–
$NaHCO_3$	–	–	–	–	–	–	35.0	–
Na_2HPO_4 $7H_2O$	–	51.83	50.0	–	25.0	–	–	–
NaH_2PO_4-H_2O	–	–	–	–	–	70,000	–	116.0
Zn Cl_2	–	–	5.0	–	2.5	2.0	–	0.006
Zn (acetate)	–	–	–	–	–	–	2.0	–
$Zn(NO_3)_2$-$6H_2O$	–	–	–	2.0	–	–	–	–
K (acetate)	–	–	–	8.3	–	–	83.0	–

Table 1. Continued:
1 = House (1954a) Dipteran, pupal parasitoid (used 200 mg USP salt mixture No. 2 and 100 mg RNA).
2 = Yazgan (1972) ichneumonid, pupal parasitoid.
3 = Thompson (1975) ichneumonid, pupal parasitoid.
4 = Nettles *et al.* (1980) dipteran, larval endoparasitoid.
5 = Thompson (1981b) chalcidid, pupal endoparasitoid.
6 = Strand and Vinson (1985) scelionid, egg parasitoid.
7 = Nettles (1986a,b) dipteran, larval endoparasitoid.
8 = Pennacchio *et al.* (1992 a,b), braconid larval endoparasitoid (only to 2nd instar).

researchers have approached the rearing of beneficial insects on the results of the vast data available for free-living forms (Singh 1984; Slansky and Rodriguez 1987). For example, after the successful rearing of *A. housei*, House (1972) concluded that the parasitic sarcophagids depended on the same nutrients needed by other insects. House (1977) later suggested that the same nutrients needed by most insects would be needed by most parasitoids. This view has not been radically altered, although a new essential nutrient has been found to be required by *E. bryani* (Nettles 1986a) and there are indications that other essential factors are yet to be identified in other species (Irie *et al.* 1987; Grenier and Bonnot 1986). These results led Nettles (1990) to suggest that certain chemicals present in the host may have become essential dietary components as a result of mutations and other genetic changes. These genetic changes being harmless to the developing parasitoid because they are available and present in the host, but are essential and must be identified and added to artificial diets to get optimal growth (Nettles 1990). However, in addition to essential nutrients, there are some that may not be essential but may be very beneficial. Further, when developing artificial diets, it is not just the presence of essential nutrients that must be considered but the presence of sufficient levels of the basic nutrients in the proper balance and form. Toxic compounds should be avoided. Also, compounds that have no nutritional value but increase the osmotic pressure, cause other needs to develop or interfere with digestion must be avoided.

Amino acids and proteins. Thompson (1974, 1976a) found that *E. roborator* required not only the 10 essential amino acids in its diet, but needed the presence of a mixture of several of the non-essential amino acids as well. However, *Brachymeria lasus, Brachymeria ovata,* and *P. vendemiae* only demonstrated the requirement for the essential 10 amino acids (Thompson 1981a). Increasing the protein content of the media increased the survival of *B. intennedia,* but adults were not obtained and the larvae did not do well when proteins were the only source

of amino nitrogen (Thompson 1980). Nettles (1986a,b; 1987) working with *E. bryani*, found that the deletion of any one of the 10 amino acids found to be essential resulted in larval death even in a protein containing media. In addition to the 10 essential amino acids, asparagine was essential to *E. bryani* and the presence of tyrosine, proline and glycine were beneficial (Nettles 1986a,b; 1987). Apparently, *E. bryani* ingest proteins slowly resulting in the need for free amino acids in the media (Nettles 1990). In contrast Thompson (1980) found that *E. roborator* did well on protein as the only source of amino nitrogen. The addition of proteins to the media has had a beneficial effect for many species (Thompson 1983a; Greany 1986; Pennacchio *et al.* 1992 a, b), although as reported by Thompson (*op. cit*), *B. intermedia* does not develop on a high protein diet. Even the polyamine prutrecine, which can stimulate protein synthesis (Moray and Tarnoy 1978), had no effect on *B. intermedia* or *B. insus* (Thompson 1980, 1981d).

Thompson (1974) reported that *E. roborator* incorporated carbohydrate into amino acids, but comparing the rate of incorporation of synthesised amino acids into body protein indicated that much of that incorporated was dietary. Greenblatt *et al.* (1982) reported that *B. intermedia* and *P. (Coccygomimus) turionellae* were inefficient at nitrogen conversion and suggested, along with Thompson (1986a), that the low rate of amino acid synthesis may represent an adaptation to the parasitic habit of feeding in an amino acid rich environment.

Lipids. As observed by Thompson (1986b) the fatty acid composition of both hymenopteran and dipteran parasitoids, in contrast to "free-living" species, reflects the corresponding fatty acid composition of their host (Bracken and Barlow 1967; Delobel and Pageaux 1981; Thompson and Adams 1976; Thompson and Barlow 1970). Furthermore, when a parasitoid was reared on different hosts, its fatty acid pattern was qualitatively a "duplicate" of its host. Although quantitative differences in fatty acid titres occurred (Thompson and Barlow 1974) the change in the parasitoid's fatty acid composition could not be accounted for by direct deposition of dietary fats (Barlow and Bracken 1971). Thompson and Barlow (1976) demonstrated that *E. roborator* could synthesise fatty acids, although the parasitoid tissue levels were lower than host reared individuals (Thompson and Johnson 1978). Jones *et al.* (1982) working with *Exeristes, Itoplectis, Aphaereta, Brachymeria* and *Hyposotor* reported low rates of *de novo* glyceride synthesis and concluded that triglyceride synthesis was by the esterification of partially digested host glycerides. Thompson and Barlow (1983) suggested that a high-fat nutritional diet would allow the parasitoid to rapidly synthesise glycerol esters at

a reduced cost by utilising its own fatty acids and the host's partially digested glycerides. Yazgan (1972) suggested some fatty acid needs may be low for one generation due to stores carried over in the egg. However, lipids appear to improve growth if they can be homogenised into the media. Usually this requires emulsifying agents such as lecithin, tween 80 or lauryl sulfate. But care should be taken in the use of these agents as they have been shown to be toxic for some species (Grenier *et al.* 1974; Thompson 1977).

Sterols. The sterol nucleus is essential to insects (Dadd 1985) and parasitoids do not appear to be an exception (Thompson 1981b). Further, most insects appear capable of utilising most steroids to meet this need including some parasitoids (Thompson 1981b). However, there is some question as to whether some endoparasitoids may specifically require ecdysone, either as an essential nutrient or as a trigger to initiate certain developmental events (Lawrence 1991; Cals-Usciati 1975; Nenon 1972). Lawrence (1988) suggested that parasitoid apolysis was initiated by endogenous ecdysone and could not proceed without exogenous ecdysone from the host. Parasitoid larvae were found to accumulate ecdysteroids from the media (Lawrence and Hagedorn 1986) and the low level of ecdysteroid in *in vitro* reared larvae (Lawrence 1988) led her to suggest (Lawrence 1986b) that the endogenous ecdysone is supplemented by the host. Dahlman *et al.* (1990) did not observe any relation between the larval moults of *M. croceipes* as the host ecdysteroid titre changed and Pennacchio *et al.* (1992b) reported similar data with *C. nigriceps.* Further, Pennacchio *et al.* (1992a) obtained a moult from first to second instar *in vitro* by the addition of fetal bovine sera which may contain a steroid contamination, but not ecdysteroids. Because of the precise timing of ecdysteroid and juvenile hormone release these agents may be more important as triggers for developmental synchronisation than as an important nutritional consideration. However, the presence of a steroid in the media is probably essential.

Carbohydrates. In general, carbohydrates are not considered to be essential but necessary (Dadd 1985; Reinecke 1985). Carbohydrate did not appear to be essential to *E. roborator,* but the growth of larvae increased with the addition of glucose daily (Thompson 1979). Increasing both glucose (2%) and AA (4%) also increased the growth of *B. lasus* (Thompson 1983a). Most diets (Table 1) have not utilised trehalose, the insect blood sugar (Mullins 1985). For ectoparasitoids or egg parasitoids this may not be important since it is assumed that ectoparasitoids ingest and can digest the more complex carbohydrates to glucose and glycogen is the more important carbohydrate in eggs. However, for endoparasitoids develop-

ing within an environment where trehalose predominates this may not be the case. Edson and Vinson (1977) investigated the role of the anal vesicle in *M. croceipes* and reported that the vesicle may be involved in the absorption of nutrients, particularly trehalose and amino acids. It was also reported (Dahlman and Vinson 1976) that the trehalose level of the haemolymph increased in parasitised *M. croceipes*. Such an increase would facilitate the absorption of trehalose by the anal vessicle and may be related to the way carbohydrates are absorbed in insects (Turunen 1985); the anal vesicle is derived from hind gut tissue (Edson *et al.* 1977).

Most of the studies of glucose have been conducted by Thompson (1979, 1981c) in regard to lipogenesis. He reported increased lipid synthesis in response to glucose and the dietary glucose mediated fatty acid synthetase activity. Further, in contrast to other animals, the increased dietary carbohydrate increased the polyunsaturated fatty acids (Thompson 1979, 1981c).

Vitamins and other specific biochemicals. In general, most media formulated have contained most of the B vitamins (Table 1). Some have used fat soluble vitamins, citric acid cycle components, and some of the nucleic acids (Grenier *et al.* 1974; House 1954a, b; Yazgan 1972). However, their importance has not been investigated and most are added as a precaution. Strand *et al.* (1988) did report that the presence of β-carotene improved larval growth for *T. heliothidus*.

Salt. The addition of salts has been based primarily on vertebrate work (Clark 1958; House and Barlow 1965; House 1974) although attempts to increase the potassium and reduce the sodium as occurs in plant feeding insect haemolymph (Florkin and Jenniaux 1964) has been done (Table 1). Salt requirements have not been studied since the work of House (1954 a, b, 1977), but usually a source of Ca, Co, Cu, Fe, K, Mg, Mn, Na, and Zn have been added. These cations have been added as salts of the chloride, sulfate, nitrate, phosphate or acetate anion (Table 1).

B. Physical Factors

House (1974, 1977) suggested that all insects require about the same kinds and numbers of amino acids, lipids, carbohydrates, vitamins and salts in some sort of ordinary proportional relationship. But, despite the proper nutrition, to be successful an artificial diet must also meet the insect's feeding requirements. Feeding requirements are those chemical and physical factors that induce feeding, may influence behaviour, or allow for development (House 1977). For example, House

(1966b) reported that *A. housei* readily fed and developed on slabs of pork liver or on a defined artificial diet (Table 1). But, *P. turionella* did not develop on pork liver unless it was coagulated and then ground into a fine-paste in saline (House 1966a). As House (1977) noted, diets for *Musca domestica* and *A. housei* differ only in agar concentration. No problems in development of *A. housei* occurred in 0.75% agar nutrient media, but *M. domestica* larvae submerged themselves and drowned. However, a 1.5% agar diet permitted air to follow the borings made by *M. domestica*, thus preventing drowning. In contrast, Yazgan (1972) reared *I. conquistor* on a diet containing 0.5% agar to reduce the accumulation of undigestible material that might cause problems in the blind gut of Hymenoptera. But to induce feeding, Yazgan (1972) had to grind the semi-jelled diet into a slurry. Thompson (1975) found that *E. roborator* would not feed on an agar-jelled diet but would develop on a diet applied to a cotton dental wick or Sephadex that held the larvae up off of the liquid media. Such results support the idea that diet success can depend on diet form (Vanderzant 1974).

Nettles *et al.* (1980) encountered the same problem with *Eucelaloria* but was faced with a change in the biology of the parasitoid from an aquatic existence as a first instar to a situation that is similar to an ectoparasitoid since it has a direct connection via trachea to air and feeds through a sheath. Nettles *et al.* (1980) solved this problem by layering the liquid media, in which the 1st instar larvae developed, over dry agar diet that slowly absorbed the liquid diet so that, as the larvae developed, the rearing diet changed from a liquid to a semi-solid that allowed the 2nd instar larvae to contact air. However, other factors may be involved. Some endoparasitic forms develop in the haemolymph but just prior to egression, and in some species such as *C. nigriceps* even after egression (Lewis and Vinson 1968), feed on solid host tissues. This change in feeding behaviour may require a change in the form of the diet presented.

Osmotic pressure is another serious physical consideration. As the complexity of the media is increased with small molecules the osmotic pressure increases. This problem can be reduced by adding complex molecules such as proteins or complex carbohydrates, but then the question is whether these complex molecules can be assimilated or digested by the developing parasitoid. The osmotic pressure of various media used to *in vitro* rear has varied. The more saprophagous species such as *A. affinis*, *E. roborator* and *I. conquisitor* have been reared on media having an osmotic pressure of over 1700 mOsm/Kg (House and Barlow 1956; Yazgan 1972; Thompson 1975) while the osmotic pressure of the media used for *B. lasus*

(Thompson 1983a) was between 500–700 mOsm/kg. Egg parasitoid and tachinids do not develop in mOsm/kg over 450 which is similar to the haemolymph of lepidopterous larvae (Grenier *et al.* 1986). The osmotic pressure may be even more critical for endoparasitoids.

Another consideration is the pH of the media, particularly as the larva develops. Generally, the pH is near natural to slightly acid (Nettles 1986b; Thompson 1980) or adjusted between 6.5 to neutral by NaOH or KOH when formulated, but how the pH of the media changes with time or as the larvae develop has not generally been evaluated. Access to O_2 and elimination of CO_2 can also be a factor in developing *in vitro* rearing methods. As discussed above, many dipterous species must have access to air and readily drown in liquid media (Grenier *et al.* 1978; Nettles *et al.* 1980) and ectoparasitoids are also subject to drowning (Thompson 1975). Such species appear to develop if placed so they are prevented from being submerged in the rearing medium. Such techniques may also work for species such as the chalcids that remain attached to their egg stalk (Imms 1931). In the egg parasitoid, *Trichogramma*, the use of artificial diets enclosed in membranes that are permeable to O_2 has been important to the successful rearing of these species (Li *et al.* 1988; Guan *et al.* 1978). However, the situation in the endoparasitic species is less clear. The haemolymph of most insects is considered to be low in oxygen (Florkin and Jenniaux 1964; Mullins 1985) and studies with *M. croceipes* indicated the larvae were facultatively anaerobic (Edson and Vinson 1976). If some species are facultatively anaerobic, the presence of high O_2 tension could be detrimental. However, at the time of egression these larvae may be susceptible to drowning.

C. Changing Conditions

When developing *in vitro* rearing methods it is important to consider the changes that the parasitoid is exposed to during its development and the effects these changes may have on the parasitoid's development. This is particularly important for insect parasitoids for two reasons. One is that insect development is discontinuous, i.e., there are distinct stages that are most well developed in the Holometabolus orders (Agrell 1964). Second is that insect parasitoids ultimately kill their host so that the interrelationship that occurs between parasitoids and their host (Vinson and Iwantsch 1980a) is less important, the host serving more as a container to be fully exploited (Vinson 1988b). The host places limits and conditions on the developing parasitoid which must conform (Lawrence 1990),

but the parasitoid is also provided the opportunity to alter the biochemical and physiological processes and mobilise the resources of the host to its advantage without regard to the long-term survival of the host, i.e., host regulation (Vinson 1975; Vinson and Iwantsch 1980a). A good example of the latter situation is provided by the egg parasitoid, *T. heliothidis* where the female injects a substance into the host along with her egg that stops egg development (Strand *et al.* 1986). Host eggs exposed to only the injected substance never developed and host eggs in which the parasitoid larvae hatch was found to rapidly decompose due to enzymes produced by teratocytes, cells associated with the parasitoid (Strand *et al.* 1986). In a real sense, the host is nothing more than a preserved food source and several egg parasitoids successfully attacked sterile or eggs killed by irradiation or freezing (Salt 1938; Hu and Xu 1988; Young and Hamm 1967). Many of the ectoparasitoids inject venom which paralyses the host (Vinson and Iwantsch 1980b), and in many cases these hosts are also killed, the parasitoid larvae feeding on the remains.

A common assumption is that there is a negative correlation between the amount of resource available to the progeny and the parasitoid's fitness (Skinner 1985). Gregarious parasitoids which parasitise a non-growing host often adjust their clutch size with host size (Charnov and Skinner 1985; Waage and Godfrey 1985), with an optimal clutch size that results in the maximum progeny fitness for a particular resource. Although hymenopteran growth is indeterminate (Charnov *et al.* 1981), the survival of suboptimal clutch sizes of some endoparasitoids also decreases (Godfrey 1987; Strand 1986). This Alee effect suggests there is both an upper and lower size limit and that excess food consumption can have just as a profound effect on development as to little food. Studies with *Trichogramma* both *in vivo* (Klomp and Terrink 1978) and *in vitro* (Li *et al.* 1988; Strand and Vinson 1985; Grenier and Bonnot 1986; Xie *et al.* 1986a) result in the development of oversized or overfed larvae. Normally within the host, the larvae consume the resource completely and pupate within a day or two. Whether the trigger to pupate is due to the absence of additional nutritional input, changes in moisture conditions or other factors is not known. Insect moulting in insects can be stimulated by a number of different factors (Donner and Laufer 1983). In *in vitro* rearing this problem has been solved in two ways. Some authors have reared larvae within artificial eggs that limit the available resource (Li *et al.* 1988), while others have removed them from the media when they obtain a comparable size to *in vivo* reared insects (Strand *et al.* 1988). Changes in the availability of nutrients may be important to endoparasitoids as well. For example, Pennacchio *et al.* (1992a)

obtained a moult to the 2nd instar only by the addition of proteins to the media when the larvae had obtained the proper size.

In many parasitoid-host relationships the survival of the host is essential to the parasitoid's growth and development. Such parasitoids have been referred to as koinobionts (Halselbarth 1979), which refers to the fact that the parasitised host continues to grow and develop after parasitism. One extreme are egg-larval parasitoids where the parasitoid egg is deposited within the host egg, but development is completed in late larval stages (Sweetman 1963). In such cases, the nutritional and physical environment may change in many ways. Although no growth of insect eggs generally occur (the eggs of some endoparasitic species being an exception), there is a transformation from stored yolk to active protoplasm, yet both young and older eggs containing embryos just a few hours from hatching are often suitable as hosts for some species (Ables and Vinson 1982).

The haemolymph becomes the total environment for the development of the earlier stages of most endoparasitic species which must compete with tissues for the available and incoming resources. Because immature host insect growth is discontinuous, there are periodic changes in the internal environment of the host as the hormonal balance changes and as the nutritional composition of the haemolymph changes in response to these developmental changes. As the host approaches the adult these changes become more important as the host begins to store resources for its adult development and reproductive needs (Agrell and Lundquist 1973). These changes are even more pronounced in holometabolus insect species. For example, a number of proteins increase their titre during the last larval instar and drop dramatically just prior to pupation (Wyatt and Pan 1978; Levenbook 1985). Thus, the internal environment of a first instar host may differ dramatically from the same environment of a final larval instar. The parasitoid either evolves to respond to these changing conditions or alters them. For example, *C. nigriceps* 1st instar parasitoid larvae remain as a 1st instar longer when parasitism occurs in a 1st instar host and the length of the host larval period (from parasitism to parasitoid emergence or host pupation of non-parasitised hosts) is identical (Pennacchio *et al.* 1992a). On the other hand, parasitism of host 4th instars results in a shortened 1st instar parasitoid larval period, but a prolonged host larval stage. The ecdysteroid levels are altered in parasitised larvae, but the protein levels are not affected until a point past the normal host larval period (Pennacchio *et al.* 1992a). Further, the last day before the parasitoid emerges from the host it begins to feed on host tissue remains which it continues to do for a day

after it emerges (Lewis and Vinson 1968; Vinson and Barras 1970). Thus, the change in available protein levels, for example, between the haemolymph of a 1st instar host and a 5th instar host may be important and may necessitate one type of media for young larval development, another for late larval development, and a third for the last day of larvae development.

Further, as noted above, a parasitoid may alter the host including the endocrine balance (Beckage and Riddiford 1978) and this alteration is often accomplished by the release of various factors into the host by the parasitoid (Vinson 1988b; Vinson and Iwantsch 1980a). Understanding these factors may also be important to rearing, but more in regard to understanding what the needs of the developing parasitoid are, rather than the factors themselves being necessary components of an *in vitro* rearing media. For example, polydnavirus and venom of *C. nigriceps* reduce the ecdysteroid titre of the last larval host instar, where most parasitoid growth occurs, to undetectable levels (Tanaka and Vinson 1991), but the virus and venom would not be required for this purpose in an artificial diet.

The changes in the host, however, may be very important as triggers that control the growth and development of the parasitoid. Just as free living forms respond to certain environmental cues, a parasitoid would be expected to respond in a similar way to the changing cues in its environment. For example, the role of hormones as discussed in Section E below.

D. Morphological Factors

There are three morphological considerations. First is the presence of sickle shaped mandibles in the 1st instar larvae of many solitary endoparasitoids that precludes gregarious rearing in close quarters. This is primarily a problem for 1st instars, whether combining 2nd instar larvae is practical is not known.

Second is the blind gut that occurs in larval Hymenoptera (Flanders 1978). Because of the blind midgut, bulk waste products are accumulated and are only eliminated at pupation. If the diet contains undigestible bulk, these accumulate in the gut and prevent further feeding and digestion. Thus, care must be taken not to incorporate non-digestible materials in the diet that these larvae can feed on. This problem appears to be less serious in the parasitic Diptera since Nettles *et al.* (1980) was able to rear *E. bryani* on a high agar-based diet.

The third morphological consideration is the anal vesicle found on some larval endoparasitoids (Hagen 1964). The function of this organ is not clear. Earlier researchers suggest a respiratory function (Tothill 1922; Thorpe 1932; Fisher 1971), but the technique used and the interpretation of the responses can be questioned. The studies by Edson and Vinson (1977) and Edson *et al.* (1977) suggest the organ may function in absorption of nutrients and may secrete some products into the media. Further, El-Sufty and Führer (1981) and Willers *et al.* (1982) also suggested that larvae secrete factors into the haemolymph that may function as antimicrobials. The source of these factors possibly being the hind gut (Führer and Willers 1986). The anal vesicle of braconids may have some similar functions.

E. Developmental Triggers

Schoonhoven (1962) reported that the development and growth of the tachinid, *Encarcelia rutilla* was triggered when its host, the pine looper moth, *Bupalus piniarius*, broke diapause. He suggested that the prothoracicotropic hormone released from the brain of the host stimulated the prothoracic glands to synthesise ecdysone which initiated the parasitoid's moult. The possible role of the host's endrocrine system in controlling the growth and development of the internal larvae parasitoids has received a lot of attention (Mellini 1983; Beckage 1985; Lawrence 1990). For some species there is good evidence of an important role for the hormones, particularly ecdysone. Molting of *O. concolor* occurred when its host, *Ceratitis capitata*, was injected with ecdysone and similar results have been reported for both *B. longicadatus* (Lawrence 1986b) and the tachnid, *Gonia cinerascens* (Baronio and Sehnal 1980). Lawrence (1986b) reported that ecdysteroid titres were similar in parasitised and non-parasitised hosts through the 1st instar and that the moult of 1st instar parasitoids always occurred on or after the host's larval-pupal apolysis. However, the moult also depended on the parasitoid body size, the larvae having to have reached a minimal size to moult (Lawrence 1986a). Cals-Usciati (1969) also reported that a similar species, *O. concolor* only moults from 1st to 2nd instar in hosts that have pupated. Similarly, other parasitic larvae may not show much developmental activity until late in the hosts development. For example, *Pathyplectes curculionis* and *B. anurus* (Ichneumonidae) do not show much development until the host spins a cocoon (Bartell and Pass 1978) and *C. nigriceps* only moults from 1st to 2nd instar in last instar larvae following what in non-parasitised hosts is the release of a small amount of ecdysone, i.e., the commitment peak. However, in *C. nigriceps* the commitment peak was not detected in parasitised hosts and the release of ecdysone that leads to pupation

never occurs (Pennacchio *et al.* 1992a; Tanaka and Vinson 1991). Similar results were reported for *M. croceipes* (Dahlman *et al.* 1990).

The tachinid *P. caudata* responds to the annual diapause of its host *Thaumetopoea pityocampa* (Lepidoptera) (Geri 1980) but shows no such developmental arrest when reared on *Galleria mellonella* (Lepidoptera) and will develop *in vitro* to the last instar without any hormone supply (Grenier *et al.* 1975). Thompson (1980) also demonstrated that development of *B. intermedia* was not affected by the presence or absence of β-ecdysone. However, in some species, as demonstrated by Lawrence (1988), Nenon (1972) and Cals-Usciati (1975), ecdysone is necessary in the media.

Juvenile hormones have also been implicated in stimulating parasitoid aggression from the host (Beckage and Riddiford 1982), although JH may reduce parasitoid development in other systems (Vinson 1974).

F. Associated Factors

There are two groups of endoparasitoids, the Scelionidae and certain braconids, that release cells that comprise the embryonic membrane of the host as the larvae hatches. These cells, referred to as teratocytes, desociate and grow in size but not number. Various functions have been attributed to these cells (Vinson and Iwantsch 1980a; Dahlman 1990), and, therefore, must be considered in *in vitro* rearing programmes. Strand *et al.* (1985, 1986) found that teratocytes of *T. heliothidis* produced lytic enzymes that may account for host decomposition as observed by Gerling and Orion (1973) with another *Telenomus*. Teratocytes were also found to improve *T. heliothidis* development *in vitro* when present in the media (Strand *et al.* 1988). Greany (1986) also reported that the development of first instar *M. croceipes* improved in the presence of teratocytes although the importance of these cells in the later development of either *M. croceipes* or *C. nigriceps* is unknown. Roturndo *et al.* (1988) reared a braconid from 1st instar larvae with and without teratocytes also taken from the hosts on an artificial diet developed for host adult tissues and obtained no significant improvement in growth of larvae in the presence of teratocytes. Although the role of teratocytes is not clear and they appear to be very important to *in vivo* development of parasitoid larvae, their importance *in vitro* is problematical. If the teratocytes major role within hosts is to alter host conditions to allow for parasitoid growth, the development of an optimal media may preclude the requirement for these cells. If, however, these cells produce and release specific products needed by the developing parasitoid,

then these cells or the isolation, identification and incorporation of these factors may be essential.

Parasitoid Hymenoptera often inject venom and other factors into their hosts (Vinson and Iwantsch 1980a). These maternally released factors have many effects on the host (Vinson and Iwantsch 1980 a, b; Stoltz 1986). Whether any of these factors cause the production or release of specific factors required by the developing parasitoid is unknown. While such a possibility cannot be ruled out, many of the effects of these factors are to alter the host's response to the parasitoid (Vinson 1988b). If any of these maternal produced factors are needed, most can probably be eliminated in *in vitro* rearing efforts once the important factors are understood and can be added to the media.

G. Contamination

When considering *in vitro* rearing of insect parasitoids there are two forms of contamination that should be considered. The first is the contamination of the media by microorganisms which compete for the nutrients and alter the media composition, as well as releasing toxicants into the media. In addition, diseases of the parasitoid may occur. The second problem is the contamination of the media with waste products and the build-up of unused products as the useful ones are absorbed and used.

Microbials. Contamination by microorganisms is a serious problem in the development of *in vitro* rearing and there is nothing more beneficial than cleanliness and use of sterile conditions. Even with these precautions and use of laminar flow hoods, microorganisms are often associated (Shapiro 1984) with insects making it difficult to eliminate the problem (Sikorowski 1984). Thompson (1975) reported 10% loss due to contamination and loss of some experiments. Similar problems were reported by Xie *et al.* (1986a), Grenier and Liu (1991), and Li *et al.* (1988).

Initially the hosts or parasitoid eggs are surface sterilised. For many ectoparasitoids the eggs are enclosed in a thick chorion and can be more easily handled. Thompson (1977, 1981b) rinsed the parasitoid eggs in $HgCl_2$ for 2 min. and rinsed in sterile water. Nettles *et al.* (1980) rinsed 1st instar tachinid larvae in 0.1 ml of a 2% formaldehyde solution and immediately blotted; repeating the process. The eggs of most egg parasitoids and endoparasitoids have a very thin chorion, are very small, and rinsing such eggs may not be practical. Most

researchers have rinsed the host in a disinfectant before the removal of eggs. Hoffman *et al.* (1975) rinsed the host egg in 0.125% sodium hypochlorite (15 min.) followed by neutralisation in 10% sodium thiosulfate (15 sec.) and rinsed in distilled H_2O (30 sec.). Strand and Vinson (1988) used a 2% solution of sodium hypochlorite (30 sec.) followed by rinsing in Hink-TNH-FH media, supplemented with 1% penicillin, 1% streptomycin and 50 g/ml gentamicin. Greany (1986) dipped host larvae in 95% ethanol (10 sec.) followed by 70% ethanol (5 min), then placed in sterile H_2O to dissect out the parasitoid eggs. Lawrence (1988) used 5% hypochlorite (1 min.) followed by 70% ethanol and deionised water. While all these methods reduce contamination, it is rarely eliminated.

When the media is formulated, it is generally filter sterilised using a 0.22, μ filtering system. However, once some of the more complex macromolecules and lipids are added, filter sterilisation becomes difficult. Most researchers add antimicrobials to the rearing media, however, some parasitoids appear to be susceptible to some of these agents. Grenier *et al.* (1974, 1975, 1978) reported that *P. caudate* and *L. diatraeae* were sensitive to plastics and antibiotics. Bacterial contamination is generally easier to control with penicillin, streptomycin or kanamycin added to the rearing media but fungi appear to be a more serious problem (Li *et al.* 1988). Grenier (1977) found that endoparasitic tachinids were particularly susceptible to antifungal agents. *Trichogramma* also appears to be more susceptible to sorbic acid, methyl-p-hydroxy benzoate, sodium propionate, merthiolate and sodium benzoate (Grenier and Liu 1991) than *P. caudate* or *A. housei* (Singh and House 1970). Grenier and Liu (1991) found that the most effective fungicides were toxic to *Trichogramma* although Geneticin, Amphotericin B (Fungizone^R) and Nystatin (Mycostatine^R) if used carefully can be beneficial.

Waste products. The build up of waste products through their elimination by larval Hymenoptera is not generally expected since in all of the suborder *Apocrita* the mid-gut is not joined to the hind-gut, the release of waste material occurring at pupation. In fact, this does not appear to be a problem with the ectoparasitic Hymenoptera or egg parasitoids since they only defecate at pupation. Further, no problem has been expressed by those working with the parasitic Diptera. This may reflect the high tolerance to waste products exhibited by some Diptera. The situation with the endoparasitic Hymenoptera is not yet clear. In some of these species the hindgut is everted to form an anal vesicle (Edson *et al.* 1977), the function of which while not entirely clear, appears to both absorb certain nutrients (Edson and Vinson 1977) and may release some nitrogen waste products (Edson and Vinson 1976). Further, there is increasing evidence that parasitoid larvae

(Führer and Willers 1986) and teratocytes associate with them (Dahlman 1990; Tanaka and Wago 1990) release products into the host. The function of these secretions is unclear but their accumulation in a media could be detrimental.

Summary

Some of the media used to rear several parasitoids is provided in Table 1. Although not all of these have been successfully used to rear adults, since endoparasitoid kainobionts have not been successfully reared to the adult on an artificial diet, they may be useful to those attempting to develop new diets. Although the development of the *in vitro* rearing of beneficial insect parasitoids has been slow, the improved understanding of the physiology of parasitoids has resulted in the steady improvement in *in vitro* rearing techniques. Once the techniques and diets are perfected *in vitro* rearing will become increasingly important as a component of biological control and pest management (Vinson 1986, 1988a).

Acknowledgements

Approved as TA 30710 by the Director of the Texas Agricultural Experiment Station. Supported in part by a Texas Higher Education Board Advanced Technology Program Grant No. 999902-166.

References

Ables, J. R. and S. B. Vinson. 1982. Regulation of host larval development by the egg larvae endoparasitoid *Chelonus inssilaris*. *Entomophaga* 26: 453–458.

Agrell, I. 1964. Physiological and biochemical changes during insect development, pp. 91–148. In: M. Rockstein (ed.), *The Physiology of Insecta*, Vol. 1. Academic Press.

Agrell, I. and A. M. Lundquist. 1973. Physiological and biochemical changes during insect development, pp. 159–249. In: M. Rockstein (ed.), *The Physiology of Insecta*, Vol. 1. Academic Press.

Arthur, A. P., B. M. Hegdekar and W. W. Batsch. 1972. A chemically defined synthetic medium that induces oviposition in the parasite *Itoplectis conquisitor*. *Canad. Ent.* 104: 1251–1258.

Askew, R. R. 1971. *Parasitic Insects.* American Elsevier Press, New York.

Barlow, J. S. and G. K. Bracken. 1971. Incorporation of Na 1- ^{14}C-acetate into the fatty acids of two insect parasites reared on different hosts. *Canad. J. Zool.* 49: 1297–1300.

Baronio, P. and F. Sehnal. 1980. Dependence on the parasitoid *Gonia cinerascens* on the hormones of its lepidopterous hosts. *J. Insect Physiol.* 26: 619–626.

Bartell, D. B. and B. C. Pass. 1978. Effects of *Bathyplectes curculionis* and *Bathyplectes anuris* (Ichneumonidae) on the growth and development of *Hypera postica* (Col. Curculionidae). *Entomophaga* 23: 281–284.

Bartlett, A. C. 1984. Genetic changes during insect development, pp. 2–8. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA-ARS, US Gov. Printing Office.

Beckage, N. E. 1985. Endocrine interactions between endoparasitic insects and their hosts. *Annu. Rev. Entomol.* 30: 371–413.

Beckage, N. E. and L. M. Riddiford. 1978. Effects of parasitism by *Apanteles congregatus* on the endocrine physiology of the tobacco hornworm, *Manduca sexta*. *Gen. Comp. Endocr.* 47: 308–322.

Beckage, N. E. and L. M. Riddiford. 1982. Effects of methoprene and juvenile hormone on larval ecdysis, emergence, and metamorphosis of the endoparasitic wasp, *Apanteles congregatus*. *J. Insect Physiol.* 28: 329–334.

Beckage, N. E. and L. M. Riddiford. 1983. Growth and development of the endoparasitic wasp, *Apanteles congregatus:* dependence on host nutritional status and parasite load. *Physiol. Entomology* 8: 231–241.

Beckendorf, S. K. and M. A. Hoy. 1985. Genetic improvement of arthropod natural enemies through selection, hybridization or genetic engineering techniques,

pp. 167–187. In: M. A. Hoy and D. C. Herzog (eds.), *Biological Control in Agricultural IPM System*. Academic Press, New York.

Bonnot, G., B. Delobel and S. Grenier. 1984. Elevage, croissance et developpement de *Phyrxe caudata* (Diptera: Tachinidae) sur son hôte de substitution *Galleria mellonnella* (Lepidoptera: Pyralidae) et sur milieu artificial. *Bull. Soc. Linneenne, Lyon* 53: 313–320.

Borror, D. J. and D. M. DeLong. 1964. *An Introduction to the Study of Insects*. Revised Edition. Holt, Rinehart and Winston Pub., NY, 819 pp.

Borstel, R. C. 1960. Sulla natura della letalita dominante indotta dalle radiazioni. *Atti. Assoc. Genet. Ital.* 5: 35–50.

Bouletreau, M. 1968. Premiers résultats de l'élevage des larves d'un Hyménoptère chalcidien *(Pteramalus puparum)* sur hémolymphe de Lépidoptère. *Entomophaga* 13: 217–222.

Bouletreau, M. 1972. Developpement et croissance larvaires en conditions semi-artificielles et artificielles chez un Hyménoptère entomophage: *Pteromalus puparum* (Chalc.). *Entomophaga* 17: 265–273.

Bracken, G. K. and J. S. Barlow. 1967. Fatty acid composition of *Exeristes comstockii* reared on different diets. *Can. J. Zool.* 45: 57–61.

Bronskill, J. and H. L. House. 1957. Notes on rearing a pupal endoparasite, *Pimpla turionellae* (Hymenoptera: Ichneumonidae), on unnatural food. *Can. Entomol.* 89: 483.

Cals-Usciati, J. 1969. Influence de l'état physiologique de l'hôte *Ceratitis capitata* (Diptera) sur le développement du parasite *Opius concolor* (Hymenoptera). *C. R. Acad. Sci. Paris* 269D: 242–244.

Cals-Usciati, J. 1975. Repercussion de la modification du cycle normal de *Ceratitis capitata* Wied. (Diptere: Trypetidae), per irradiation γ et injection d'ecdysone, sur le developpment de son parasite *Opius concolor* Szepl. (Hyménoptère, Braconidae). *C.r. hedb. Seanc., Acad. Sci. Paris, Ser. D.* 281: 275–278.

Cavalloro, R. 1981. Establishment of continuously *in vitro* growing cell lines of med-fly (*Ceratitis capitata* Wied.). *Rev. Canad. Entomol.* 40:181–186.

Charnov, E. L. and S. W. Skinner. 1985. Complimentary approaches to the understanding of parasitoid oviposition decisions. *Environ. Entomol.* 14: 383–391.

Charnov, E. L., R. L. Hartogh, W. T. Jones and J. van den Assem. 1981. Sex ratio evolution in a variable environment. *Nature* 289: 27–33.

Clark, E. W. 1958. A review of the literature on calcium and magnesium in insects. *Ann. Entomol. Soc. Am.* 51: 142–154.

Clausen, C. P. 1940. *Entomophagous Insects*. McGraw-Hill, New York. 844 pp.

Corbet, S. A. 1968. The influence of *Ephestia kuhniella* on the development of its parasite *Nemeritis canescens*. *J. Exp. Biol.* 48: 291–294.

Dadd, R. H. 1973. Insect nutrition: Current developments and metabolic implications. *Annu. Rev. Entomol.* 18: 381–420.

Dadd, R. H. 1985. Nutrition: Organisms, pp. 313–390. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 4. Pergamon Press, NY.

Dahlman, D. L. 1990. Evaluation of teratocyte functions: An overview. *Arch. Insect Biochem. Physiol.* 13: 159–166.

Dahlman, D. L. and S. B. Vinson. 1976. Trehalose level in the hemolymph of *Heliothis virescens* parasitized by *Campoletis sonorensis*. *Ann. Entomol. Soc. Amer.* 69: 523–524.

Dahlman, D. L., D. L. Coar, C. N. Koller and T. J. Neary. 1990. Contributing factors to reduced ecdysteroid titers in *Heliothis virescens* parasitized by *Microplitis croceipes*. *Arch. Insect Biochem. Physiol.* 13: 29–40.

Dai, K., Z. Liangwu, M. Zhijean, Z. Liansheng, Z. Jixiu, C. Aihua, X. Kejing, L. Qun and G. Yignang. 1988. Research and utilization of artificial host egg for

propagation of parasitoid *Trichogramma*, pp. 311–318. In: J. Voegele, J. Waage, and J. van Lenteren (eds.), Trichogramma *and Other Egg Parasitoids*, 2nd Int. Symp.,Les Colloques de INRA Vol. 43, Paris.

DeBach, P. 1974. *Biological Control by Natural Enemies*. Cambridge University Press, New York.

Delobel, B. and J. F. Pageaux. 1981. Influence de l'atementation sur la composition en acides gras tutaux de diptères tachenaires. *Entomol. Exp. Appl.* 29: 281–288.

Ding, D. C., H. G. Qiu and C. B. Hwang. 1980a. *In vitro* rearing of an egg-parasitoid *Tetrastichus schoenobii* (Hymenoptera: Tetrastichidae). *Contr. Shanghai Inst. Entomol.* 1: 55–59.

Ding, D. C., T. P. Zhang and Y. K. Zhong. 1980b. Studies on *Tetrastichus schoenobii* (Hymenoptera: Tetrastichidae): oviposition into artificial media. *Contr. Shanghai Inst. Entomol.* pp. 59–61.

Donner, R. G. and H. Laufer. 1983. *Endrocrinology of Insects*. Alan R. Liss, NY, 707 pp.

Doutt, R. L. 1963. Pathologies caused by insect parasites, pp. 393–422. In: E. A. Steinhaus (ed.), *Insect Pathology, an Advanced Treatise*, Vol. 2. Academic Press, New York.

Edson, K. M. and S. B. Vinson. 1976. The function of the anal vesicle in respiration and excretion by the braconid *Microplitis croceipes*. *J. Insect Physiol.* 22: 1037–1043.

Edson, K. M. and S. B. Vinson. 1977. Nutrient absorption by the anal vesicle of the braconid, *Microplitis croceipes*. *Insect Physiol.* 23: 5–8.

Edson, K. M., W. N. Norton and S. B. Vinson. 1977. The ultrastructure of the anal vesicle of the parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). *Int. J. Insect Morph. Embryol.* 154: 253–257.

El-Sufty, R. and E. Fuhrer. 1981. Wechselbezie-hungen zwichen *Pieris brassicae* L. (Lep. Pieridae), *Apanteles glomeratus* L. (Hym. Braconidae) und dem Pilz

Beauveria bassiana (Bals.) Vuill. Z. *angew. Ent.* 92: 321–329.

van Emden, F. I. 1950. Dipterous parasites of Coleoptera. *Ent. Month. Mag.* 86: 182–206.

Evans, H. 1984. *Insect Biology.* Addison-Wesley Pub. Redding, MA, 436 pp.

Ferkovich, S. M. and C. R. Dillard. 1990. Effects of female wasp accessory secretions, host fat body, and host hemolymph on protein synthesis and egg viability in *Microplitis croceipes* (Braconidae). *Arch. Insect Biochem. Physiol.* 14 : 111–120.

Ferkovich, S. M., C. Dillard and H. Oberlander. 1991. Stimulation of embryonic development in *Microplitis croceipes* (Braconidae) in cell culture media preconditioned with a fat body cell line derived from a nonpermissive host, gypsy moth, *Lymantria dispar. Arch. Insect Biochem. Physiol.* 18: 169–175.

Fisher, R. C. 1971. Aspects of the physiology of endoparasitic insects. *Biol. Res.* 46: 243–278.

Flanders, S. E. 1930. Notes on *Trichogramma minutum* Riley and observations on the natural and artificial parasitism of the codling moth egg. *Trans. 4th Int. Congr. Ent.* 2: 110–130.

Flanders, S. E. 1950. Regulation of ovulation and egg disposal in the parasitic Hymenoptera. *Canad. Entomol.* 82: 134–140.

Flanders, S. E. 1978. Maximum reproductivity by the hymenopterous female, an effect of larvae nutrient conservation incidental to the delayed midgut-hindgut connection. *Ann. Entomol. Soc. Am.* 71: 715–717.

Florkin, M. and C. Jenniaux. 1964. Hemolymph: Composition, pp. 109–152. In: M. Rockstein (ed.), *The Physiology of Insecta,* Vol. 3. Academic Press, NY.

Führer, E., R. El-Sufty and D. Willers. 1978. Antibiotic effects of entomophagous endoparasites against microorganisms within the host body. 4th Int. Congr. Parasitol. F6, Warsaw, Poland.

Führer, E. and R. El-Sufty. 1979. Produktion fungistatischer Metabolite durch Teratocytes von *Apanteles glomeratus* L. (Hym.: Braconidae). *Z. Parasit.* 59: 21–25.

Führer, E. and D. Willers. 1986. The anal secretion of the endoparasitic larva *Pimpla turiotellae*: sites of production and effects. *J. Insect Physiol.* 32: 361–367.

Geri, C. 1980. Application des methodes d'etudes demecologigiques aux insectes defoliateurs forestiers. Ph.D. Dissertation, Univ. Paris, France.

Gerling, D. and T. Orion. 1973. The grant cells produced by *Telenomus remus*. *J. Invert. Path.* 21: 164–171.

Godfrey, H. C. J. 1987. The evolution of clutch size in parasitic wasps. *Am. Nat.* 129: 221–233.

Greany, P. 1980. Growth and development of an insect parasitoid *in vitro*. *Am. Zool.* 20: 946.

Greany, P. 1981. Culture of hymenopteran endoparasites *in vitro*. *In vitro* 17: 270 (Abstract).

Greany, P. D. 1986. *In vitro* culture of hymenopterous larval endoparasitoids. *J. Insect Physiol.* 32: 409–419.

Greany, P. D., S. B. Vinson and W. J. Lewis. 1986. Insect parasitoids: Finding new opportunities for biological control. *BioScience* 34: 690–696.

Greany, P. D., S. M. Ferkovich and W. R. Clark. 1989. Progress towards development of an artificial diet and an *in vitro* rearing system for *Microplitis croceipes*. *Southwestern Entomol. Suppl.* 12: 89–94.

Greany, P. D., W. R. Clark, S. M. Ferkovich, J. H. Law and R. O. Ryan. 1990. Isolation and characterization of a host hemolymph protein required for development of the eggs of the endoparasite, *Microplitis croceipes*. 306 pp. In: H. H. Hagedorn, J. G. Hildebrand, M. G. Kidwell and J. H. Law (eds.), *Molecular Insect Science*. Plenum Press, New York and London.

Greenblatt, J. A., P. Barbosa and M. E. Montgomery. 1982. Hosts diet effects on nitrogen utilization efficiency for two parasitoid species: *Brachymeria intermedia* and *Coccygomimus trionellae*. *Physiol. Ent.* 7: 263–267.

Grenier, S. G. 1977. Effects nocifs de la Nipagine M sur le parasitoide *Phryxe caudata* (Dipt.: Tachinidae). *Entomophaga* 22: 223–236.

Grenier, S. and G. Bonnot. 1986. Development of *Trichogramma dendrolimi* and *T. maidis* (Hymenoptera: Trichogrammatidae) in artificial media and artificial host eggs, pp. 319–326. In: J. Voegele, J. Waage and J. van Lenteren, (eds.) Trichogramma *and Other Egg Parasitoids*. 2nd Int. Symp. Les Colloques de INRA Vol. 43, Paris.

Grenier, S. and W. Liu. 1991. Mold control and safe levels of antifungals in artificial media for egg parasitoids (Hymenoptera), pp. 141–144. In: E. Wajnberg and S. B. Vinson (eds.), Trichogramma *and Other Egg Parasitoids*, 3rd Int. Symp. Les Colloques de INRA Vol. 56, Paris.

Grenier, S., G. Bonnot and B. Delobel. 1974. Definition et mise au point de milieux artificiels pour l'elevage *in vitro* de *Phryxe caudata* Rond. (Diptera: Tachinidae): I—Survie de parasitoide sur milieu dont la composition est basee sur celle de l'hemolymph de l'hote. *Ann. Zool. Ecol. Anim.* 6: 511–520.

Grenier, S., G. Bonnot and B. Delobel. 1975. Definition et mise au point de milieux artificiels pour l'elevage *in vitro* de *Phryxe caudata* Rond. (Diptera: Tachinidae): II Croissance et mues larvaires de parasitoide milieux definis. *Ann. Zool. Ecol. Anim.* 7: 13–25.

Grenier, S., B. Delobel and G. Bonnot. 1986. Physiological considerations of importance to the success of *in vitro* culture: an overview. *J. Insect Physiol.* 32: 403.

Grenier, S., G. Bonnot, B. Delobel and P. Laviolette. 1978. Developpement en milieu artificiel du parasitoide *Lixophaga diatraeae* (Diptera: Tachinidae). Obtention de l'imago a partir de l'oeuf. *C. R. Acad. Sci. Paris.* 287D: 535–538.

Guan, X. C., Z. X. Wu, T. X. Wu and H. Feng. 1978. Studies on rearing *Trichogramma dendrolimi* Matsumura *in vitro*. *Acta Entomol. Sinica*. 21: 122.

Hagen, K. S. 1964. Developmental stages of parasites, pp. 168–246. In: P. DeBach (ed.), *Biological Control of Insects, Pests and Weeds*. Chapman & Hall, London.

Hagen, K. S. and R. L. Tassen. 1965. A method of providing artificial diets to *Chrysopa* larvae. *J. Econ. Entomol.* 58: 999–1000.

Halselbarth, E. 1979. Zur Parasitierung des Puppen von Forleule, Kiefernspannes and Heidelbeerspanner in Bayerischen Kieferniwaldern. *Angew. Entomol.* 87: 186–202, 311–333.

Hegdekar, B. M. and A. P. Arthur. 1973. Host hemolymph chemicals that induce oviposition in the parasite *Itoplectis conquisitor*. *Canad. Ent.* 105: 787–793.

Hendry, L. B., P. D. Greany and R. J. Gill. 1973. Kairomone mediated host-finding behavior in the parasitic wasp *Orgilus lepidus*. *Entomol. Exp. Appl.* 16: 471–477.

Hinton, H. E. 1981. *The Biology of Insect Eggs*. Vol. 1. Pergamon Press, Oxford.

Hoffman, J. D. and C. M. Ignoffo. 1974. Growth of *Pteromalus puparum* in a semisynthetic medium. *Ann. Entomol. Soc. Am.* 67: 542–625.

Hoffman, J. D., C. M. Ignoffo and S. H. Long. 1973. *In vitro* cultivation of an endoparasitic wasp, *Pteromalus puparum*. *Ann. Entomol. Soc. Am.* 66: 633–634.

Hoffman, J. D., C. M. Ignoffo and W. A. Dickerson. 1974. *In vitro* rearing of the endoparasitic wasp, *Trichogramma pretiosum*. *Ann. Entomol. Soc. Am.* 68: 335–336.

Hoffman, J. D., C. M. Ignoffo and W. A. Dickerson. 1975. *In vitro* rearing of the endoparasitic wasp, *Trichogramma pretiosum*. *Ann. Entomol. Soc. Am.* 68:335.

House, H. L. 1954a. Nutritional studies with *Pseudosarcophaga affinis* (Foll.), a dipterous parasite of the spruce budworm, *Charistonura fumiferana* (Clem.). I. A chemically defined medium and asceptic-culture technique. *Canad. J. Zool.* 32: 331–341.

House, H. L. 1954b. Nutritional studies with *Pseudosarcophaga affinis* (Foll.), a dipterous parasite of the spruce budworm, *Charistonura fumiferana* (Clem.). IV.

Effects of ribonucleic acid, glutamine, dextrose, salt mixture, cholesterol, and fats. *Canad. J. Zool.* 32: 358–365.

House, H. C. 1966a. Effects of varying the ratio between the amino acids and the other nutrients in conjunction with a salt mixture on the fly *Agria affinis*. *J. Insect Physiol.* 12: 299–310.

House, H. L. 1966b. Effects of vitamins E and A on growth and development, and the necessity of vitamin E for reproduction in the parasitoid *Agria affinis* (Falen) (Diptera: Sarcophagidae). *J. Insect. Physiol.* 12: 409–417.

House, H. L. 1972. Insect nutrition, pp. 513–573. In: R. N. Finnes (ed.), *Biology of Nutrition* Vol. 17. Pergamon Press, NY.

House, H. L. 1974. Nutrition, pp. 1–62. In: M. Rockstein (ed.), *The Physiology of Insecta*, Vol. 5. Academic Press, New York.

House, H. L. 1977. Nutrition of natural enemies, pp. 105–182. In: R. L. Ridgeway and S. B. Vinson (eds.), *Biological Control by Augmentation of Natural Enemies*. Plenum Press, NY.

House, H. L. 1978. An artificial host: Encapsulated synthetic medium for *in vitro* oviposition and rearing the endoparasitoid *Itoplectis conquisitor* (Hymenoptera: Ichneumonidae). *Canad. Entomol.* 110: 331–333.

House, H. L. and J. S. Barlow. 1956. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). V. Effects of various concentrations of amino acid mixture, dextrose, potassium ion, the salt mixture, and lard on growth and development, and a substitute for lard. *Canad. J. Zool.* 34: 182–189.

House, H. L. and J. S. Barlow. 1965. Effects of a new salt mixture developed for *Agria affinis* (Fallen) (Diptera: Sarcorphagidae) as the growth rate, body weight, and protein content of the larvae. *J. Insect Physiol.* 11: 915–918.

Hu, Z. and Q. Xu. 1988. Studies on frozen storage of eggs of rice moth and oak silkworm, pp. 327–338. In: J. Voegele, J. Waage and J. van Lenteren (eds.),

Trichogramma *and Other Egg Parasitoids*, 2nd Int. Symp. Les Colloques de INRA Vol. 43, Paris.

Hubei Research Group. 1979. Studies on the artificial host egg of the endoparasitic wasp, *Trichogramma*. *Acta Entomol. Sinica* 22: 301.

Huffaker, C. B. 1969. *Biological Control*. Plenum Press/Rosetta Ed., New York, NY. 511 pp.

Imms, A. D. 1931. *Recent Advances in Entomology*. J. and A. Churchill, London, 374 pp.

Irie, K., Z.-N. Xie, W. C. Nettles, R. K. Morrison, A. C. Chen, G. M. Holman and S. B. Vinson. 1987. The partial purification of a *Trichogramma pritiosum* pupation factor from hemolymph of *Manduca sexta*. *Insect Biochem*. 17: 269–275.

Jones, D., J. S. Barlow and S. N. Thompson. 1982. *Exerestes, Itoplectus, Aphaereta, Brachymeria* and *Hyposotor* species: *in vitro* glyceride synthesis and the regulation of fatty acid composition in insect parasites. *Exp. Parasit*. 54: 340–351.

Kainoh, Y. 1989. The role of chemical stimuli in the host-finding and oviposition behavior of *Ascogaster reticulatus* Watanabe. *Agr. and Forest Sci*. 5: 115–162.

Kathirithamby, J. 1989. Review of the order Strepsiptera. *Syst. Entomol*. 14: 41–92.

Keilin, D. 1944. Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. *Parasitology* 36: 1–66.

King, P. E. and J. Rafai. 1973. A possible mechanism for initiating the parthogenetic development of eggs in a parasitoid hymenopteran, *Nasonia vitripennis* Walker (Pteromalidae). *Entomologist* (London) 106: 118–120.

Klomp, H. and B. J. Terrink. 1978. Elimination of supernumerary larvae of the gregarious egg-parasitoid, *Trichogramma embryophagium*, in eggs of the host *Ephestia kuehniella*. *Entomophaga* 23: 153–159.

Lathrop, F. H. and R. C. Newton. 1933. The biology of *Opeius melleus* Gahan, a parasite of the blueberry maggot. *J. Agric. Res*. 46: 143–160.

Lawrence, P. O. 1982. *Biosteres longicaudatus:* Developmental dependence on host *(Anastrepha suspensa)* physiology. *Exp. Parasitol.* 53: 396–405.

Lawrence, P. O. 1986a. Host-parasite hormonal interactions: An overview. *J. Insect Physiol.* 32: 295–298.

Lawrence, P. O. 1986b. The role of 20-hydroxyecdysone in the molting of *Biosteres longicaudatus*, a parasite of the Caribbean fruit fly, *Anastrepha suspensa. J. Insect Physiol.* 32: 329–338.

Lawrence, P. O. 1988. *In vivo* and *in vitro* development of first instars of the parasitic wasp, *Biosteres longicaudatus*, pp. 351–366. In: V. Gupta (ed.), *Advances in Parasitic Hymenopteran Research.* E. J. Brill Co., Leiden, N.Y.

Lawrence, P. O. 1990. The biochemical and physiological effects of insect hosts on the development and ecology of the insect parasites: An overview. *Arch. Insect Biochem. Physiol.* 13: 217–228.

Lawrence, P. O. 1991. Hormonal effects on insects and other endoparasites *in vitro. In Vitro Cell & Dev. Biol.* 27A: 487–496.

Lawrence, P. O. and H. H. Hagedorn. 1986. Relationship between the ecdysteroid titers of a parasitic wasp and those of its host. *Insect Biochem.* 16: 163–166.

Levenbook, L. 1985. Insect storage proteins, pp. 307–346. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry, and Pharmacology,* Vol. 10. Pergamon Press, Oxford.

Lewis, W. J. and S. B. Vinson. 1968. Egg and larvae development of *Cardiochiles nigriceps* Viereck. *Ann. Entomol. Soc. Am.* 61: 561–564.

Lewis, W. J. and J. R. Young. 1972. Parasitism by *Trichogramma evanescens* of eggs from tepa-sterilized and normal *Heliothis zea. J. Econ. Entomol.* 65: 105–108.

Li, Li-ying, Liu Wen-hui, Chen Chao-shian, Han Shi-tyow, Shin Jia-chi, Du Han-sung and Feng Shn-yi. 1988. *In vitro* rearing of *Trichogramma* spp. and *Allastatus* sp. in artificial eggs and methods of mass production, pp. 339–352. In: J.

Voegele, J. Waage, and J. van Lenteren (eds.), Trichogramma *and Other Egg Parasitoids*, 2nd Int. Symp. Les Colloques de INRA, Vol. 43, Paris.

Liu, W. H., Z. N. Xie, G. F. Xiao, Y. F. Zhou, D. H. Ou Yang and L. Y. Li. 1979. Rearing of *Trichogramma dendrolimi* in artificial diets. *Acta Phyto. Sinica* 6: 17–24.

Liu, W. H. and Z. X. Wu. 1982. Recent results in rearing *Trichogramma pretiosum* on an artificial media devoid of insect derivatives. *Acta Entomol. Sinica* 25: 160–164.

Liu, Z., Z. Wang, Y. Sun, J. Liu and W. Yang. 1988. Studies on culturing *Anastatus* sp. a parasitoid of Litchi stink bug, with artificial host eggs, pp. 253–360. In: J. Voegele, J. Waage, and J. van Lenteren (eds.), Trichogramma *and Other Egg Parasitoids*, 2nd Int. Symp., Les Colloques de INRA Vol. 43, Paris.

Marshal, P. 1936. Recherches sur la biologie et le developpement des Hymènoptéres parasites: les *Trichogrammes*. *Ann. Epiphyties*, Paris 2 (n. ser.) pp. 447–550.

Mellini, E. 1983. L'ipotesi della dominazione armonale, esercitata dagli ospiti sui parassitoidi, alla luce della recenti scoperte nella endocrinologia degli insetti. *Boll. Ent. Bologna* 38: 135–166.

Moray, P. and K. Tarnoy. 1978. Molting hormone level during the last instar of *Galleria mellonella* larvae. *J. Insect Physiol.* 24: 325–327.

Mullins, D. E. 1985. Chemistry and physiology of the hemolymph, pp. 355–400. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 3. Pergamon Press, NY.

Nenon, J. P. 1972. Culture *in vitro* des larves d'um Hymenoptera endoparasite polyembryonnaire: *Ageniaspis fuscicollis*: Role des hormones de synthese. C. *rend. hebd. Seanc. Sci. Paris*, 274: 3409–3412.

Nettles, W. C., Jr. 1986a. Asparagine: a host chemical essential for the growth and development of *Eucelatoria bryani*, a tachinid parasitoid of *Heliothis* spp. *Comp. Biochem. Physiol.* 85A (4): 697–701.

Nettles, W. C., Jr. 1986b. Effects of soy flour, bovine serum albumin, and three amino acid mixtures on growth and development of *Eucelatoria bryani* (Diptera:

Tachinidae) reared on artificial diets. *Environ. Entomol.* 15: 1111–1115.

Nettles, W. C., Jr. 1987. Amino acid requirements for growth and development of the tachinid *Eucelatoria bryani*. *Comp. Biochem. Physiol.* 86A: 349–353.

Nettles, W. C., Jr. 1990. *In vitro* rearing of parasitoids: Role of host factors in nutrition. *Arch. Insect Biochem. Physiol.* 13: 167–175.

Nettles, W. C., Jr., C. M. Wilson and S. W. Ziser. 1980. A diet and methods for the *in vitro* rearing of the tachinid, *Eucelatoria* sp. *Ann. Entomol. Soc. Am.* 73: 180–184.

Nettles, W. C., R. K. Morrison, Z. N. Xie, D. Ball, C. A. Shenkis and S. B. Vinson. 1982. Synergistic action of potassium chloride and magnesium sulfate on parasitoid wasp oviposition. *Science* 218: 164–166.

Nettles, W. C., Jr., R. K. Morrison, Z. N. Xie, D. Ball, C. A. Shenkis and S. B. Vinson. 1983. Effect of cations, anions, and salt concentration on oviposition by *Trichogramma pretiosum* in wax eggs.

Pantel, J. 1910. Recherches sur les Diptères à entomobies. Caracteres parasitiques aux points de vue biologique, ethologique et histologique. *Cellule* 26: 27–216.

Pennacchio, F., S. B. Vinson and E. Tremblay. 1992a. Preliminary *in vitro* rearing of the endoparasitoid *Cardiochiles nigriceps* from egg to second instar. *Entomol. Exp. Appl.* (in press).

Pennacchio, F., S. B. Vinson and E. Tremblay. 1992b. Growth and development of *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) larvae and their synchronization with some changes of the hemolymph composition of their host, *Heliothis virescens* (F.). *Arch. Insect Biochem. Physiol.* (in review).

Prell, H. 1915. Zur Biologie der Tachinen *Parasetigena segregata* Rdi und *Panzeria rudis*. *Z. angew. Entomol.* 2: 57–148.

Qin, J. and Z. Wu. 1988. Studies on cultivation of *Trichogramma in vitro*: ovipositional behaviour and larval nutritional requirement of *T. dendrolimi*, pp. 379–388. In:

J. Voegele, J. Waage and J. van Lenteren (eds.), Trichogramma *and Other Egg Parasitoids.* 2nd Int. Symp.,Les Colloques de INRA, Vol. 43.

Rajendram, G. F. and K. S. Hagen. 1974. *Trichogramma* oviposition into artificial substrates. *Environ. Ent.* 3: 99–101.

Reinecke, J. P. 1985. Nutrition: Artificial diets, pp. 241–277. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* Vol. 4. Pergamon Press, NY.

Ridgeway, R. L. and S. B. Vinson. 1977. *Biological Control by Augmentation of Natural Enemies:Insect and Mite Control with Parasites and Predators.* Plenum Press, New York. 480 pp.

Rotundo, G., R. Cavalloso and E. Trembley. 1988. *In vitro* rearing of the aphid endoparasitoid *Lysiphebus fabarum* (Marsh.). *Entomophaga* 33: 7–12.

Sailer, R. I. 1981. Possibilities for genetic improvement of beneficial insects, pp. 295–303. In *Germplasm Resources.* Amer. Assoc. Adv. Sci., New York.

Salt, G. 1932. Superparasitism by *Collyria calcitrator. Bull. Ent. Res.* 23: 211–216.

Salt, G. 1934. Experimental studies in insect parasitism. II. Superparasitism. *Proc. R. Soc. Lond.*, Ser. B 114: 455–476.

Salt, G. 1938. Experimental studies in insect parasitism. VI. Host suitability. *Bull. Entomol. Res.* 29: 223–246.

Salt, G. 1961. Competition among insect parasitoids. *Symp. Soc. Exp. Biol.* 15: 96–119.

Salt, G. 1965. Experimental studies in insect parasitism. XIII. The hemocytic reaction of a caterpillar to eggs of the silkworm, *Hyalophora cecropia. Dev. Biol.* 22: 249–263.

Salt, G. 1970. Experimental studies in insect parasitism. XV. The means of resistance of a parasitoid larvae. *Proc. R. Soc. Lond.* 176B: 105–114.

Schmidt, J. M. and J. J. B. Smith. 1987. Measurement of host curvature by the parasitoid wasp *Trichogramma minutum* and its effect on host examination and progeny allocation. *J. Exp. Biol.* 129: 151–164.

Schoonhoven, L. M. 1962. Diapause and the physiology of host-parasite synchronization in *Bupalus pinarius* L. (Geometridae) and *Eucarcalia rutilla* VIII. (Tachinidae). *Arch. Néerl. Zool.* 15: 111–174.

Shapiro, M. 1984. Microorganisms as contaminants and pathogens in insect rearing, pp. 130–142. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA-ARS, US Gov. Printing Office.

Sikorowski, P. 1984. Microbial contamination in insectaries: occurrence prevention and control, pp. 143–154. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA-ARS, US Gov. Printing Office.

Simmonds, F. J. 1944. The propagation of insect parasites on unnatural hosts. *Bull. Ent. Res.* 35: 219–226.

Singh, P. 1984. Insect diets, historical development, recent advances, and future prospects, pp. 32–44. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA-ARS, US Gov. Printing Office.

Singh, P. and H. L. House. 1970. Antimicrobials "safe" levels in a synthetic diet of an insect, *Agria affinis*. *J. Insect Physiol.* 16: 1769–1782.

Skinner, S. W. 1985. Clutch size as on optimal foraging problem for insects. *Behav. Ecol. Sociobiol.* 17: 231–238.

Slansky, F. O. and J. G. Rodriguez. 1987. *Nutritional Ecology of Insects, Mites and Spiders*. John Wiley and Sons, NY.

Stoltz, D. B. 1986. Interaction between parasitoid-derived products and host insects: an overview. *J. Insect Physiol.* 32: 339–346.

Strand, M. R. 1986. The physiological interactions of parasitoids with their hosts and their influence on reproductive strategies, pp. 97–136. In: J. Waage and D. Greathead (eds.), *Insect Parasitoids*. Academic Press, NY.

Strand, M. R. and S. B. Vinson. 1983. Factors affecting host recognition and acceptance in the egg parasitoid, *Telenomus heliothidis*. *Environ. Entomol.* 12: 1114–1119.

Strand, M. R. and S. B. Vinson. 1985. *In vitro* culture of *Trichogramma pretiosum* Riley on an artificial medium. *Entomol. Exp. Appl.* 39: 203–209.

Strand, M. R., J. M. Quarles, S. M. Meola and S. B. Vinson. 1985. Cultivation of teratocytes of the egg parasitoid *Telenomus heliothidis*. *In vitro* C21: 361–367.

Strand, M. R., S. M. Meola and S. B. Vinson. 1986. Correlating pathological symptoms in *Heliothis virescens* eggs with development of the parasitoid *Telenomus heliothidis*. *J. Insect Physiol.* 32: 389–402.

Strand, M. R., S. B. Vinson, W. C. Nettles and Z.-N. Xie. 1988. *In vitro* culture of the egg parasitoid *Telenomus heliothidis*: The role of teratocytes and medium consumption in development. *Entomol. Exp. Appl.* 46: 71–78.

Sweetman, H. L. 1963. *Principles of Biological Control*. Wm. C. Brown Co., Dubridge, IA.

Tanaka, T. and H. Wago. 1990. Ultrastructural and functional maturation of teratocytes of *Apanteles kariyi*. *Arch. Insect Biochem. Physiol.* 13: 187–198.

Tanaka, T. and S. B. Vinson. 1991. Depression of prothoracic gland activity of *Heliothis virescens* by venom and calyx fluids from the parasitoid *Cardiochiles nigriceps*. *J. Insect Physiol.* 37: 139–144.

Thompson, S. N. 1974. Aspects of amino acid metabolism in the ectoparasitoid wasp, *Exeristes*. *J. Insect Physiol.* 20: 1515–1528.

Thompson, S. N. 1975. Defined meridic and holidic diets and aseptic feeding procedures for artificially rearing the ectoparasitoid *Exeristes roborator*. *Ann. Entomol. Soc. Am.* 68: 220–226.

Thompson, S. N. 1976a. The amino acid requirements for larval development of the hymenopterous parasitoid *Exeristes roborator* (Hymenoptera: Ichneumonidae). *Comp. Biochem. Physiol.* 53A: 211–213.

Thompson, S. N. 1976b. Effects of dietary amino acid level and nutritional balance on larval survival and development of the parasite *Exeristes roborator*. *Ann. Entomol. Soc. Am.* 69: 835–838.

Thompson, S. N. 1977. Lipid nutrition during larval development of the parasitic wasp, *Exeristes roborator*. *J. Insect Physiol.* 23: 579–583.

Thompson, S. N. 1979. Effect of dietary glucose on *in vitro* fatty acid metabolism and *in vitro* synthetase activity in the insect parasite *Exeristes roborator* (Fabricius). *Insect Biochem.* 9: 645–651.

Thompson, S. N. 1980. Artificial culture techniques for rearing larvae of the chalcidoid parasite, *Brachymeria intemedia*. *Entomol. Exp. Appl.* 27: 133–134.

Thompson, S. N. 1981a. Essential amino acid requirements of four species of parasitic Hymenoptera. *Comp. Biochem. Physiol.* 69A: 173–174.

Thompson, S. N. 1981b. *Brachymeria lasus* and *Pachycrepoideus vindemmiae:* sterol requirements during larval growth of two hymenopterous insect parasites reared *in vitro* on chemically defined media. *Exp. Parasitol.* 51: 220–235.

Thompson, S. N. 1981c. Effects of dietary carbohydrate and lipid on nutrition and metabolism of metazoan parasites with special reference to parasitic Hymenoptera, pp. 215–257. In: S. Friedman and J. G. Rodriguez (eds.), *Current Topics in Insect Endocrinology and Nutrition*. Plenum Press, New York.

Thompson, S. N. 1981d. *Brachymeria lasus:* culture *in vitro* of a chalcid insect parasite. *Exp. Parasitol.* 32: 414–418.

Thompson, S. N. 1983a. The nutritional physiology of *Trichoplusia ni* parasitized by the insect parasite, *Hyposotor exiquae*, and the effects of parallel-feeding. *Parasitology* 87: 15–28.

Thompson, S. N. 1983b. Larval growth of the insect parasite *Brachymeria lasus* reared *in vitro*. *J. Parasitol.* 69: 425–427.

Thompson, S. N. 1983c. *Brachymeria lasus:* effects of nutrient level on *in vitro* larval growth of a chalcid insect parasite. *Exp. Parasitol.* 55: 213–319.

Thompson, S. N. 1986a. The metabolism of insect parasites (Parasitoids): An overview. *J. Insect Physiol.* 32: 421–423.

Thompson, S. N. 1986b. Nutrition and *in vitro* culture of insect parasitoids. *Ann. Rev. Entomol.* 31: 197–219.

Thompson, S. N. and J. D. Adams. 1976. Characterization of selected lipids of the parasite *Exeristes roborator* (Fabricius). *Comp. Biochem. Physiol.* 55B: 591–593.

Thompson, S. N. and J. S. Barlow. 1970. The change in fatty acid pattern of *Itoplectis conquisitor* (Say) reared on different hosts. *J. Parasitol.* 56: 845–846.

Thompson, S. N. and J. S. Barlow. 1974. The fatty acid composition of parasitic Hymenoptera and its possible biological significance. *Ann. Entomol. Soc. Am.* 67: 627–632.

Thompson, S. N. and J. S. Barlow. 1976. Regulation of lipid metabolism in the insect parasite, *Exeristes roborator* (Fabricius). *J. Parasitology* 62: 303–306.

Thompson, S. N. and J. S. Barlow. 1983. Metabolic determination and regulation of fatty acid composition in parasitic Hymenoptera and other animals, pp. 73–106. In: T. E. Mittler and R. E. Dadd (eds.), *Metabolic Aspects of Lipid Nutrition in Insects.* Westview Press, Boulder, CO.

Thompson, S. N. and L. Bednar. 1983. Artificial culture of the insect parasite, *Pachycrepordeus vindemiae. Entomol. Exp. Appl.* 33: 121–122.

Thompson, S. N. and J. Johnson. 1978. Further studies on lipid metabolism in the insect parasite, *Exeristes roborator* (Fabricius). *J. Parasitology* 64: 731–740.

Thorpe, W. H. 1932. Experiments on respiration in the larvae of certain parasitic Hymenoptera. *Proc. R. Soc. London* B109: 450–471.

Tothill, J. D. 1922. The natural control of the fall webworm (*H. cunea)* in Canada. *Tech. Bull.* Canad. Dep. Agric.

Tumlinson, J. H. and W. J. Lewis. 1991. Chemically mediated foraging behavior of parasitoids: New implications for biocontrol, pp. 161–178. In: S. B. Vinson and

R. L. Metcalf (eds.), *Entomology Serving Society: Emerging Technologies and Challenges,* Entomological Soc. Am., Lanham, MD.

Turunen, S. 1985. Absorption, pp. 241–277. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* Vol. 4. Pergamon Press, NY.

Ullyett, G. C. 1944. On the function of the caudal appendage in primary larvae of parasitic Hymenoptera. *J. Entomol. South Africa* 6: 65–80.

Vanderzant, E. S. 1974. Development, significance, and application of artificial diets for insects. *Annu. Rev. Entomol.* 19: 139–160.

Vinson, S. B. 1974. Effect of an insect growth regulator on two parasitoids developing from treated tobacco budworm larvae. *J. Econ. Entomol.* 67: 335–336.

Vinson, S. B. 1975. Biochemical coevolution between parasitoids and their hosts, pp. 14–48. In: P. W. Price (ed.), *Evolutionary Strategies of Parasitic Insects and Mites.* Plenum Press, New York. 480 pp.

Vinson, S. B. 1976. Host selection by insect parasitoids. *Annu. Rev. Entomol.* 21: 109–133.

Vinson, S. B. 1977. Behavioral chemicals in the augmentation of natural enemies, pp. 237–279. In: R. L. Ridgeway and S. B. Vinson (eds.), *Biological Control by Augmentation of Natural Enemies.* Plenum Press, New York.

Vinson, S. B. 1985. The behavior of parasitoids, pp. 417–469. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* Vol. 9. Pergamon Press.

Vinson, S. B. 1986. The role of behavioral chemicals for biological control, pp. 75–87. In: J. M. Franz (ed.), *Biological Plant and Health Protection,* Fortsch. Zool. 32. G. Fischer Verlag, Stuttgart.

Vinson, S. B. 1988a. The potential use of synomones, kairomones, and pherotnones in the management of enemies of cotton pests. *Beltwide Cotton Production*

Research Conferences Proceedings, pp. 217–223.

Vinson, S. B. 1988b. Physiological studies of parasitoids reveal new approaches to the biological control of insect pests. *ISI Atlas of Science: Animal and Plant Science* 1988: pp. 25–32.

Vinson, S. B. 1991. Chemical signals used by parasitoids. *Redia, Giornale di Zoologia* 124: 15–42.

Vinson, S. B. and P. Barbosa. 1987. Interrelationship of nutritional ecology of parasitoids, pp. 673–693. In: F. Stansky, Jr. and J. G. Rodriguez (eds.), *Nutritional Ecology of Insects, Mites and Spiders.* John Wiley & Sons, Inc., New York.

Vinson, S. B. and D. J. Barras. 1970. Effects of the parasitoid, *Cardiochiles nigriceps*, on the growth, development and tissues of *Heliothis virescens*. *J. Insect Physiol.* 16: 1329–1338.

Vinson, S. B. and G. F. Iwantsch. 1980a. Host regulation by insect parasitoids. *Q. Rev. Biol.* 55: 143–165.

Vinson, S. B. and G. F. Iwantsch. 1980b. Host suitability for insect parasitoids. *Annu. Rev. Entomol.* 25: 397–419.

Vinson, S. B. and H. S. Jang. 1987. Activation of *Campoletis sonorensis* eggs by artificial means. *Ann. Entomol. Soc. Am.* 80: 486–489.

Vinson, S. B. and G. L. Piper. 1986. Source and characterization of host recognition kairomones of *Tetrastichus hagenowii*, a parasitoid of cockroach eggs. *Physiol. Entomol.* 11: 459–468.

Volkoff, A. N., S. B. Vinson, Z. W. Wu and W. C. Nettles, Jr. 1972. *In vitro* rearing of *Trissolcus basalis* (Hymenoptera: Scelionidae) an egg parasitoid of *Nezara viridula* (Heteroptera: Pentatomidae). *Entomophaga* 37:141–148.

Volkoff, A. N. 1990. Recherches de base pour l'elaboration d'un milieu artificial brut assurane le developpement de *Trissolcus basalis* (Woll.) (Hymenoptera, Scelionidae) parasitoid oophage de *Nezara viridula* L. (Hemiptera: Pentatomidae). Dissertation Thesis: De L'Institute National Polytechnique de

Toulouse, France, Nov. 1990.

Waage, J. K. and H. J. C. Godfrey. 1985. Reproductive strategies and population ecology of insect parasitoids, pp. 449–470. In: R. M. Sibly and R. H. Smith (eds.), *Behavioral Ecology*. Blackwell Scientific Pub. Oxford, London.

Webb, B. A. and D. L. Dahlman. 1985. Developmental pathology of *Heliothis virescens* larvae parasitized by *Microplitis croceipes:* parasite mediated host developmental arrest. *Arch. Insect Biochem. Physiol.* 2: 131–143.

Went, D. F. and G. Krause. 1973. Normal development of mechanically activated, unlaid eggs of an endoparasitic hymenopteran. *Nature* (London) 244: 454–455.

Went, D. F. and G. Krause. 1974. Egg activation in *Pimpla turionellae* (Hym.). *Naturvessenschaften* 61: 407–408.

Went, D. F. 1982. Egg activation and parthenogenetic reproduction in insects. *Biol. Rev. Cambridge Philos. Soc.* 57: 319–344.

Weseloh, R. M. 1984. Selecting *Cotesia (Apanteles) melanoscelus* to attack large gypsy moth larvae. 17th Int. Congr. Entomol. Abstract. p. 802. Hamburg, Fed. Rep. Germany.

Willers, D., H. Lehmann-Danzinges and E. Fuhrer. 1982. Antibacterial and antimycotic effect of a newly discovered secretion from larvae of an endoparasitic insect, *Pimpla turionellae* L. (Hym.). *Arch. Microbiol.* 133: 225–229.

Wilson, D. D. and R. L. Ridgeway. 1975. Morphology, development and behavior of the immature stages of the parasitoid, *Campoletis sollorensis. Ann. Entomol. Soc. Am.* 68: 191–196.

Wu, Z. X., X. P. Zhang, T. X. Li and D. M. Liu. 1980. Artificial media devoid of insect additives for rearing larvae of the endoparasitoid wasp *Trichogramma. Acta Entomol. Sin.* 21: 232–234.

Wu, Z. X. and J. Qin. 1982a. Culturing *Trichogramma dendrolimi in vitro* with media devoid of insect material. *Proc. Chinese Acad. Sci. US National Acad. Sci. Joint Symposium on Biological Control.* pp. 431–435.

Wu, Z. and J. Qin. 1982b. Ovipositional response of *Trichogramma dendrolimi* to the chemical content of artificial eggs. *Acta Entomol. Sin.* 25: 363–372.

Wu, Z., J. Qin, T.-X. Li, Z.-P. Chang and T.-M. Liu. 1982. Culturing *Trichogramma in vitro* with artificial media devoid of insect materials. *Acta Entomol. Sin.* 25: 128–135.

Wyatt, G. R. 1961. The biochemistry of insect haemolymph. *Annu. Rev. Entomol.* 6: 75–102.

Wyatt, G. R. and M. L. Pan. 1978. Insect plasma proteins. *Annu. Rev. Biochem.* 47: 779–817.

Wylie, H. G. 1971. Oviposition restraint of *Muscidifurax zaraptor* on parasitized housefly pupae. *Canad. Ent.* 103: 1537–1544.

Xie, Z.-N., W. C. Nettles, Jr., R. K. Morrison, K. Irie and S. B. Vinson. 1986a. Three methods for the *in vitro* culture of *Trichogramma pretiosum* Riley. *Entomol. Sci.* 21: 133–138.

Xie, Z.-N., W. C. Nettles, Jr., R. K. Morrison, K. Irie and S. B. Vinson. 1986b. Effect of ovipositional stimulants and diets on the growth and development of *Trichogramma pretiosum in vitro*. *Entomol. Exp. Appl.* 42: 119–124.

Xie, Z., W. C. Nettles and S. B. Vinson. 1991. Identification of ovipositional stimulants for *Anastatus japonicus*, pp. 101–104. In: E. Wajnberg and S. B. Vinson (eds.), Trichogramma *and Other Egg Parasitoids*, 3rd Int. Symp., Les Colloques de INRA, Paris.

Yazgan, S. and H. L. House. 1970. A hymenopteran insect, the parasitoid *Itoplectis conquisitor*, reared axenically on a chemically defined diet. *Can. Entomol.* 102: 1304–1306.

Yazgan, S. 1972. A chemically defined synthetic diet and larvae nutrition requirements of the endoparasitoid *Itoplectis conquisitor*. *J. Insect Physiol.* 18: 2123–2142.

Young, J. R. and J. H. Hamm. 1967. Reproduction of *Trichogramma fasciatum* in eggs

from Tepa-sterilized fall armyworms. *J. Econ. Entomol.* 60: 723–724.

Ziser, S. W. and W. C. Nettles. 1978. The larval development of *Eucelatoria* sp. in the host, *Heliothis virescens*. *Ann. Entomol. Soc. Am.* 71: 383–388.

B. Methods of Laboratory Colonisation of Insects

Methods of Colonisation and Quarantine of Insects, *G. Hill* 107

Methods of Colonisation of Tsetse Flies, *H. K. Bandah* 119

Methods of Colonisation and Quarantine of Insects

Garry Hill

IIBC Kenya Station
P.O. Box 76520,
Nairobi, Kenya

Introduction

The main purpose of colonisation and quarantine procedures in the establishment of insect cultures is to ensure that only the organism of interest is present in the culture. The researcher must ensure that the culture of the organism in question is completely free from pathogens, predators, parasites, parasitoids and "passenger" material carried phoretically. Predators and parasitoids are relatively easy to remove from insect cultures using simple observation and manual removal (predators); or by rearing the material through one generation and removing any emerging parasitoids. Pathogens, parasites and extraneous "passenger" material can be more difficult.

Insect cultures can only be certified parasite and pathogen-free after the parents from which they originate have been killed, subjected to a rigorous microscopic examination, and pronounced free from pathogens and parasites. The presence of parasites and diseases may sometimes be detected by macroscopic or behavioural symptoms, and their expression in the culture may be expedited by stressing the insects. Unwanted passenger, the most potentially troublesome being spores of plant pathogens, may be removed by surface-sterilising egg or pupal stages of the culture insect.

Many potential problems associated with the colonisation process may be circumvented by choosing an appropriate life stage with which to start the culture. This is often the egg or pupal stage. Guidelines for quarantine procedures and buildings are briefly discussed.

Definitions

The two critical words, Quarantine and Culture, are here defined to clarify the scope of this chapter.

Colonisation

The establishment of a laboratory culture of an insect using material collected from the field.

Quarantine

The containment of live plants, animals or microorganisms in a state of isolation from which they are unable to escape into the surrounding environment.

In the context of insect rearing, quarantine refers in most cases to the containment of a particular insect or mite species immediately after its introduction into a new country.

Most such introductions are for the purposes of biological control, which is the use of a parasitoid, parasite, predator or pathogen to control a pest or weed species.

The purpose of the containment is normally to remove unwanted pathogens, parasitoids, parasites, predators or other extraneous living organisms from the target insect or mite.

This ensures that only specifically identified and desirable beneficial insects and mites are introduced into the laboratory.

Factors Which Need to be Considered for Both Quarantine and Colonisation Purposes

There are five factors which have to be considered when setting up a culture or bringing a new insect into quarantine:

1. Detection of predators, parasitoids, pathogens, parasites and passengers.
2. Removal of predators, parasitoids, pathogens, parasites and passengers.

3. The appropriate life stage with which to start the culture
4. The initial size and origin of culture
5. Secure containment (Quarantine only)

Each of these is discussed in turn:

1. Detection of Predators, Parasitoids, Pathogens, Parasites and Passengers

1.1 Predators

These can be most easily detected by careful visual inspection of cultures. There are several examples where the predator is about the same size or even smaller than its adult prey (e.g. Phytoseiid mite predators of Tetranychid mite pests and the hysterid beetle *Teretriosoma nigrescens*, a predator of the larger grain borer) and must be searched for carefully, ensuring that the entire culture is checked.

1.2 Parasitoids

Three factors should be considered here: 1. search the literature, so that you know what parasitoids are likely to be present in your target insect and from which life stage they are likely to emerge; 2. visually inspect the cultures for ecto-parasitoids, as you would for detecting predators; and 3. observe the culture daily for endo-parasitoids emerging from host material or for the death of hosts which may be parasitised.

1.3 Pathogens and Parasites

Macroscopic Symptoms

In general, macroscopic symptoms of pathogen and parasite attack are uncommon until the insect is close to death. However, one should always be suspicious of unexplained deaths of insects in newly acquired cultures. The following is a general guide to some common macroscopic symptoms exhibited by different pathogens when their hosts are dead or near to death (C. Prior, pers. comm.).

Viruses— behavioural changes (e.g. negative geotaxis, cessation of feeding); disintegration of internal tissues leading to loss of body turgor (many lepidopterous

larvae become "limp"); change in cuticle coloration (e.g. "dark cheeks" disease of locusts); excretion of copious milky fluid (baculoviruses).

Protozoa—behavioural changes (lethargy, loss of appetite). Generally there are few macroscopic symptoms of protozoan diseases.

Bacteria — behavioural changes (cessation of feeding, sluggish, lack of co-ordination); diarrhoea; regurgitation; change of colour (e.g. "milky" disease of scarab beetles). Following death, most insects turn black, which is the characteristic colour of bacterial decomposition.

Fungi — behavioural changes (e.g. negative geotaxis "summit disease" of crickets); sluggish; loss of appetite. Insects infected with *Metarhizium* and *Beauveria* often die with their legs extended and fall from plants. Following death, fungal hyphae may appear through the integuments of the cuticle and sporulation of the fungus may occur.

Nematodes — there are few macroscopic symptoms of nematode infection. However, heterorhabditid infections often turn the insect red after death.

Microscopic Symptoms

As a matter of routine, a sample from a shipment of insects newly introduced to the laboratory should be subjected to microscopic examination. A reasonable proportion (say 10% of the population) should be examined on arrival so that diseases may be detected as soon as possible.

However, a shipment of insects cannot be guaranteed disease-free until every individual has been microscopically examined. This can only be carried out once a satisfactory number of progeny have been produced so that the original colonists can be killed and microscopically examined. A full description of the methods of detection of diseases is beyond the scope of this chapter, but a brief discussion for each major group of pathogen follows (see also Poinar and Thomas 1984; Wigley 1980):

Viruses — Viruses forming inclusion bodies (Baculoviruses (e.g. NPV), Entomopoxviruses and Reoviruses (e.g. CPV)) can be detected by staining methods

such as Feulgen Schiff, slow Giemsa and iron haematoxylin which reveal the polyhedral inclusion bodies (Wigley 1980).

Protozoa — Spores of many microsporidians are characteristic and can be seen easily under phase contrast in wet mounts of fat body or malpighian tubule tissue. Gregarines may occur in the gut, but these are not usually harmful.

Bacteria —There are a range of standard staining techniques available for bacteria identification. The crystals of *Bacillus thuringiensis* can be revealed by staining with Naphthalene Black.

Nematodes — Nematodes can be easily observed under low- to medium-power microscopes. However, specific identification is difficult and specialised. It is important to remember that there are many saprophytic nematode species and therefore the presence of large numbers of nematodes in a dead insect does not necessarily mean that they are pathogenic. Cross-infectivity studies are necessary to confirm this.

Expression of Latent Infections Using Stress

Some insect diseases and protozoan parasites are present at a low level in apparently healthy insect cultures. They only express themselves when the insects are stressed. This is particularly true of virus diseases. Artificial stressing of the population will promote the expression of the disease in the culture. Stressing may be achieved by subjecting the insects to food shortage, overcrowding or high temperatures.

1.4 Passengers

"Passengers" on insects may include other insects or mites using the insect for phoresy (dispersal by attachment to another animal). There are many examples of mite species using the dispersal stage of insects (usually adults and predominantly Lepidoptera and Coleoptera) for phoresy.

For example a genus of mites (*Hemisarcoptes* spp.) is specifically adapted to living beneath the elytra of ladybird beetles (Coccinellidae) during the dispersal phase of its life cycle.

Spores of plant-pathogenic fungi or bacteria may also be carried passively on insects. These obviously do not affect the colonisation of insects into culture, but they may be critically important for quarantine.

An example of this is the presence of the coffee fungal pathogen coffee berry disease (*Colletotrichum coffeanum*) on coffee berry borer parasites (*Heterospilus* spp.) (Nemeye *et al.* 1990). Detection of these spores is difficult, and if there is any possibility of the presence of such passively carried pathogens, it is sensible to take steps to remove the threat (see below) rather than spend time trying to detect the spores.

2. Removal of Predators, Parasitoids, Pathogens, Parasites and Passengers

2.1 Predators

These can be most easily removed by hand following detection by visual inspection. However, the manual removal of some small predators would be very difficult (for example, removal of predatory phytoseiid mites from cultures of their tetranychid prey). In this case, it would be best to start several small colonies of the tetranychid mite from individuals removed from the main culture and held in isolation.

2.2 Parasitoids

Removal of ecto- and endo-parasitoids can be effectively achieved by maintaining the insect culture in a secure environment until is has passed through one complete generation. After the completion of a generation, the insect culture can be certified free from parasitoids providing that it has been inspected daily and all dead insects and emerging parasitoids have been removed.

2.3 Pathogens and Parasites

These are more difficult to remove from a culture. The method of elimination depends upon the mode of transmission of the disease between generations of the insects.

Trans-ovarially Transmitted Diseases

This condition occurs where the disease or parasite is transmitted from one generation to the next within the fertilised egg. Examples are some protozoan parasites (e.g. *Nosema*) and some viruses. The literature should be consulted to determine whether a disease which has been discovered is known to be trans-ovarially transmitted.

Removal of trans-ovarially transmitted diseases and parasites can only be achieved with certainty by line-breeding. This is the maintenance of many small colonies, each isolated from the others.

The smallest and most convenient starting unit for such a colony is a single pair of insects. The progeny of each colony can be certified disease-free once the adults have been thoroughly examined and both have been shown to be disease-free. The progeny from several lines of disease-free parents can be amalgamated to start the clean nucleus culture.

Fortunately, most diseases and parasites are not trans-ovarially transmitted. However, if there is some doubt as to the mechanism of transfer between generations, trans-ovariolar transmission should be assumed, and line-breeding commenced.

Non Trans-ovarially Transmitted Diseases

Diseases and parasites of this nature (which is the majority) can be eliminated relatively easily by sterilising the outer surface of the eggs. Providing that this is done rigorously, under conditions of strict hygiene, this will break the transmission of the disease or parasite.

The best sterilising agent is sodium hypochlorite solution. Eggs should be tested for viability after immersion in the solution and some experimentation may be needed to ensure that the exposure is sufficient to kill the pathogens while retaining egg viability.

2.4 "Passengers"

Careful examination of specimens under a microscope can be used to detect the presence of insects and mites using the culture insect for phoresy. Phoresy is

usually associated with the dispersal stage of the insect, which is most often the adult. It is particularly important to look carefully on and under the elytra of beetles.

2.5 General Comments on the Elimination of Diseases and Parasites

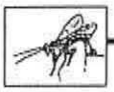
It is important not to over-emphasise the dangers of pathogens in insect cultures. Many pathogens have a universal distribution (e.g. non-specific bacteria and protozoa) and many are of low virulence (Helms and Raum 1971). In general, the removal of pathogens from insect cultures may be restricted to known, highly virulent pathogens unless there is a specific reason to remove more obscure organisms.

In general, the decision on whether to eliminate the pathogen should be based upon i. the known virulence of the pathogen, and ii. the intended use of the culture.

For instance, it would be important to ensure that a culture of the African armyworm, *Spodoptera exempta*, being raised for virus production, is completely free from its specific nuclear polyhedrosis virus (NPV), at the time of rearing; but not to worry if the culture contains some benign protozoan diseases (e.g. gut gregarines) which have no significant effect upon the reproductive ability of the culture insects.

On the other hand, when rearing locusts for bioassays to assess e.g. their reaction to fungal pathogens, it is important to ensure that the culture is uniformly clean of any organism which could potentially alter the performance of individual insects, no matter how benign it may appear to be.

Many insects harbour microorganisms which act as symbionts, not pathogens. Recent studies of viruses in the reproductive tract of female Ichneumonids (parasitic Hymenoptera) have shown that they appear to act as symbionts, assisting in the suppression of host immune responses to ichneumonid eggs and larvae (Whitfield 1990). Clearly, the presence of these viruses is beneficial (if not essential) to the well being of the insect. Many wood-boring beetles harbour gut symbionts which assist with cellulose digestion, these too are clearly beneficial.



A prior knowledge of what pathogens and parasites to expect in an insect species being cultured is very useful, and it is very important to search the literature for information on the common pathogens of the insect.

It is also very important to seek advice from an insects pathologist if necessary.

3. What Stage of the Life Cycle Should Be Used to Start the Culture?

The risks of importing a pathogen, parasitoid or other foreign material with a new insect introduction can be minimised by importing the insects in a specific life stage which is known to be free from parasitoids or disease or phoretic organisms.

Phoresy is usually restricted to dispersal stages, mostly adults. Using immatures to start the culture will eliminate most risks of introduction of phoretic species.

The example of non trans-ovarially transmitted diseases being the norm has already been given. Thus a good starting point for many lab cultures is surface-sterilised eggs from wild collected adults or larvae.

A further example is the elimination of the threat of hyper-parasitoids in cultures of hymenopterous parasitoids in the genus *Cotesia* by electing to field-collect only larvae of its host.

Several members of this large genus of parasitoids have been used very successfully as biological control agents around the world on a variety of pest lepidopteran species. The parasitoid lays eggs in small to medium-sized larvae and mature parasitoid larvae emerge from larger host larvae prior to pupation. The parasitoid pupae are commonly attacked by hyper-parasitoids.

With this knowledge of the life history of the insect it is a simple matter to eliminate the possible presence of hyper-parasitoids in the culture by field-collecting larval hosts of the *Cotesia* species and collecting the *Cotesia* sp. cocoons as they emerge from the host larvae in the laboratory. These will be guaranteed free from hyper-parasitoids and may be used to start a clean culture.

4. The Optimal Starting Size for a Laboratory Culture

This subject is somewhat conjectural. It has been argued, and in some cases shown, that insects held in culture for a long period suffer a decline in genetic diversity compared with a wild population (Bartlett 1985; Mackauer 1980).

This may lead to the appearance of undesirable traits in the laboratory population or of unnatural behaviour patterns. One reason for the narrow genetic base of the laboratory population is the number of individuals used to start the culture.

The obvious solution is to start the culture with as many individuals as possible and to maintain a large culture throughout the period of laboratory rearing. It has been suggested that a minimum of 200–500 individuals be used to start a culture, and that the insects be collected from several widely scattered sites. Care should also be taken to ensure that the sex ratio of a founding population is roughly equal.

The importance of the maintenance of genetic diversity in the culture is clearly dependent upon the use to which the culture is put.

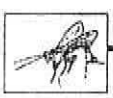
5. Quarantine Conditions

Containment of insects and mites in secure quarantine conditions prior to their release into the laboratory or the environment is a well established practice. Special facilities have been designed to minimise the risk of insects escaping, and strict quarantine procedures have been worked out to guide technicians working in the rooms (Ooi 1986; Girling 1990; Leppla and Ashley 1978; Jones *et al.* 1985).

5.1 The Physical Design of Quarantine Buildings

Standard designs of insectaries to contain insect cultures under quarantine conditions typically have the following features:

- An ante-chamber between the outside and the quarantine rooms which can be used to trap insects which escape from the quarantine room. This chamber is often painted black, to minimise the attraction of insects from the room, and may have a trapping device to kill insects which enter the chamber.



- Controlled temperature and humidity quarantine rooms which have a closed air circulation or filters to trap any escaping insects or pathogens. These rooms also have doors sealed with rubber seals to prevent the escape of insects.

- All windows are sealed.

- An incinerator or autoclave within the building to sterilise all material coming from the quarantine rooms.

- A water-filled channel running around the building to act as a physical barrier for crawling insects, especially ants.

- Protective clothing, lab coats, shoes and hats, which are worn by technicians working in the quarantine rooms.

5.2 Quarantine Operating Rules

Reliable quarantine conditions can only be maintained if technicians adhere to strict operating standards (Ooi 1986). These usually include rules requiring that technicians

- always wear protective clothing in the quarantine rooms,

- never remove any material from the rooms which has not been sterilised or burnt,

- sterilise all surfaces with alcohol or disinfectant after use and maintain a high standard of cleanliness on all surfaces within the rooms,

- never allow unauthorised persons into the room.

References

Bartlett, A.C. 1985. Guidelines for genetic diversity in laboratory colony establishment and maintenance, pp. 7–19. In: P. Singh and R.F. Moore (eds.), *Handbook of Insect Rearing* Vol. 1.

Girling, D. (ed.). 1990. *Biological Control Training Manual.* CAB International.

Helms, J.T. and E.S. Raum. 1971. Perennial laboratory culture of disease-free insects, pp. 639–654. In: H.D. Burgess and N.W. Hussey (eds.), *Microbial Control of Insects and Mites.* Academic Press.

Jones, W.A., J.E. Powell and E.G. King. 1985. Stoneville Research Quarantine Facility: A national centre for support of research on biological control of arthropod and weed pests. *Bull. Entomol. Soc. Am.* 31: 20–26.

Leppla, N.C. and T.R. Ashley (eds.). 1978. Facilities for insect research and production. *USDA Technical Bulletin* no. 1576.

Mackauer, M. 1980. Some aspects of quality and quality control of biological control agents during insectary propagation, pp. 207–220. In: *Proceedings of 5th International Symposium on Biological Control of Weeds.* E.L. Del Fosse, (ed.). CSIRO, Australia.

Nemeye, S., D.M. Moore and C. Prior. 1990. Potential of the parasitoid *Heterospilus prosopidis* (Hymenoptera:Braconidae) as a vector of plant-pathogenic *Colletotrichum* spp. *Ann. Appl. Biol.* 116: 11–19.

Ooi, P.1986. Quarantine and biological control. *Biocontrol News and Info.* 4: 203–226.

Poinar, G.O. and G.M. Thomas. 1984. *Laboratory Guide to Insect Pathogens and Parasites.* Plenum Press.

Whitfield, J. 1990. Parasitoids, polydnaviruses and endosymbiosis. *Parasitology Today* 6: 381–384.

Wigley, P.J. 1980. Diagnosis of virus infections—staining of insect inclusion body viruses, pp. 35–39. In: J. Kalmakov and J. Longworth (eds.), *Microbial Control of Insect Pests, DSIR Research Bull.* 228. DSIR New Zealand.

Methods of Colonisation of Tsetse Flies

Hastings Kiilu Bandah

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

1. Introduction

Insects are biological organisms that live within ecosystems that have been part and parcel of their evolution. Much scientific work has to be done in specified laboratories in order for control and management strategies to be developed. It is therefore important to bring insects from the natural ecosystem into the laboratory for experimental work. Where such insects are brought into the laboratory for propagation i.e. rearing, the process is referred to as colonization. In many cases the laboratory colonies are limited in size and are used for laboratory experiments. In certain cases, it is important to alter the laboratory colony so as to produce control material (e.g. sterilised insects for Sterile Insect Technique (SIT) or infected insects for mass release). Such colonies are produced in large scale and the process referred to as mass rearing.

2. Tsetse Flies and Their Economic Importance

Tsetse flies inhabit approximately 10M km^2 of tropical Africa. The African trypanosomiases are among Africans' most devastating diseases. The sleeping sickness (human disease) and nagana in cattle are caused by trypanosomes, protozoan parasites transmitted by tsetse flies (Dransfield 1991). The inhabited area would be affected mainly in three ways: land use in the inhabited area would be limited, the infected people would not be able to provide labour, and the animals infected with nagana would not provide enough meat for consumption.

3. Methods of Establishing Tsetse Colony

To establish a tsetse colony which is free from infection is possible by trapping of wild flies from the field to harvest pupae or by importation of pupae from an established colony elsewhere. In this paper I intend to discuss the methodology of establishing a clean colony from the field until that colony is self sustaining.

4. Steps in Colonisation of Tsetse Flies

A. Laboratory Preparation

The following provisions must be adequately made, for one to be able to undertake a successful tsetse collection in the field i.e. traps, cages, cokes, collection cages, fly tubes, rubber bands, cotton wool, attractants, panga, slashers, grease, polystyrene or cardboard box, trough, blanket, gum boots, rabbits and rabbit pellets enough to keep one the period of trapping and a vehicle.

B. Transportation to the Field

A vehicle would be necessary to carry various items, if the trapping area is far from the operational base. When the area and the laboratory are close, most items would not be transported to the trapping site, only the necessary items would be taken to the site e.g. panga, slasher, grease, bait, rubber bands, traps and the collection cages.

C. Operational Area

A basic knowledge of the area of operation is essential for a successful trapping of tsetse. The presence of the required species must be known, location — whether in a national park for security arrangements or the nature of the soil during rainy season to be able to arrange for rescue transport in case of being stuck.

D. Trapping

Tsetse trapping is an exercise which requires careful planning to ensure success, every item must be present.

Traps

Animal traps fit into two categories; those that catch at random and those that catch after having attracted animals in some way. Tsetse traps belong in the last category (Owaga, 1982). Traps are initially designed for a particular species, whose behaviour and habits in a particular habitat type are known. Different species will respond differently to various types of traps. Tsetse traps have been in use for a long time since the 1930s, and have undergone numerous developmental stages by different designers and researchers. Some types of traps which have been in use are listed below.

(i) *Animal model traps*: These traps are designed to resemble slightly the vertebrate hosts of tsetse. They consist of a box or screen supported so that it does not touch the ground; there is a collecting cage above the box or screen. Flies land on the screen and enter the cage from which they cannot escape. The screens and covers to the boxes are often made of hessian or similar material.

Examples:

Harris trap:	developed for *G. pallidipes*, it was the first one devised for catching tsetse.
Swynnerton trap:	developed for *G. pallidipes* and *G. fuscipes*.
Jack trap:	developed for *G. morsitans*, *G. pallidipes* and *G. brevipalpis*.
Morris trap:	developed for *G. palpalis* and *G. tachinoides*.
Langridge trap:	developed for *G. pallidipes*.
Moloo trap:	developed for *G. pallidipes* and *G. fuscipes*.

These traps are suitable for catching large numbers of flies quickly and cheaply, especially females. Baiting the traps with animal dung or carbon dioxide (from a cylinder) may increase the catches. But the traps are large and a few at a time can be carried in a vehicle. They can be used for detecting low densities, to study distribution and help in post spray surveys.

(ii) *Biconical (Challier & Laveissiere) trap:* This trap catches species of all the three species groups, i.e., *fusca, palpalis, morsitans* species which are active at dawn and dusk (crepuscular species); the trap is less efficient, cheap, collapsible, easy to carry by man and vehicle.

(iii) *Moving vehicles as traps:* Vehicles moving at approximately 10 km/h with open windows often act as good tsetse traps. Hungry flies are normally attracted and can be collected by stopping the vehicle from time to time and use a hand net for catching the flies. *G. morsitans, G. brevipalpis, G. longipennis* and *G. medicorum,* which do not readily approach fly rounds can be caught through this means. This method is limited to motorable tracks and type of fuel being used.

(iv) *Sticky traps:* These are a semi-liquid glue painted onto a surface. Dark blue screens are especially effective in attracting *G. pallidipes* and *G. palpalis.* Can be used to discover resting sites or detect flies in thinly populated areas.

(v) *Electric traps:* In these traps, a grid consisting of many fine parallel wires is powered by a car or torch battery. Low current electric traps (using a torch battery) are lighter and safer and so more suitable for collecting flies over an ordinary fly round route. Their efficiency is reduced if an insect gets caught across the wire.

These traps are used when (a) investigating the physiology and behaviour of tsetse in the field more exactly than any other method and (b) to check on the efficiency of other kinds of traps (which may be more convenient to use than electric traps) and of fly rounds using hand nets, screens, and bait animals.

Trap Siting

The placing of traps on a right site is very important, (Harris 1930), attributed the success of his trap to be due to peculiar combinations of light and shade which were responsible for bringing tsetse into traps and the site. The trap must be visible and light relationship to it is very important. However, recognition of a good site is possible with a comprehensive knowledge of the habits of the tsetse fly. One species can behave differently under different circumstances and in different seasons. Nash (1930) noted that *Glossina morsitans* in Tanzania mainland, when hungry, tended to follow a path, and a concentration of tsetse occurred where paths converged especially game path. While ICIPE staff were trapping *G. pallidipes* for colonisation at Lambwe Valley, western Kenya, this phenomena was

apparent especially along the buffalo path or drinking sites. The area should be cleared off any grass and branches of trees to prevent the insects from climbing, landing on the trap and in case of fire outbreaks, the traps would be secure. The traps should be a few metres from the thicket and the pole supporting the trap should be greased at the bottom to prevent ants from climbing to eat the insects. The collection cage should be firmly held with rubber bands otherwise, it would be blown off by wind or other animals.

E. Tsetse Attractants

Different odours were shown in the past to enhance trap catches for different species of tsetse. Among active natural odours were those emanating from live animals or derivatives such as hair and skin scrappings, sweat, dung and urine. Animal baits especially oxen, have been successfully used in tsetse surveys and sampling (Harley 1967; Pilson and Pilson 1967). Nash (1969) states that, up to 1949, baits of animals and human beings were the only known means of attracting tsetse. Since then various workers have shown that scent without the live animal can attract tsetse (Langridge 1961), used emulsions containing benzene extracts of pig hair and skin scrapping. Recent work by (Owaga *et al.* 1988) on attraction of tsetse to buffalo urine showed that the natural odours from animals are effective attractants. (Freizil and Carnevale 1976) improved trap catches of *G. fuscipes quanzensis* in West Africa by using carbon dioxide.

Tsetse attractants enhance catches which are very important when colonising tsetse flies.

F. Emptying of Traps

Tsetse flies for colonisation are very sensitive materials and require great care right from the field upto the laboratory. Emptying of trapped flies at the right time is an essential element of this process. Tsetse should not be left for more than one hour in the traps, depending on weather conditions. Normally tsetse flies require 25°C and relative humidity of 70–80%. A hot day with temperatures above 25°C would be injurious to the insects. Overcrowding in the collection cage, which could be due to delay in emptying would cause high mortality. A wet cotton wool should be inserted in the collection cage to maintain humidity and overcome the effect of dehydration, which is caused by high activity because the flies are crowded. The collection cage is removed and replaced with another immediately

to allow continued collection, the flies are transferred from collection cages to the normal holding cage by a fly tube and then coked to prevent flies from escaping. Depending on the size of cages, the small type can hold a maximum of twenty-five (25) insects.

G. Handling and Feeding in the Field

The tsetse flies should be kept away from insecticides, fresh paints, smoke, bleach, strong chemicals, petrol and diesel fumes and direct sunlight. Under field conditions, the flies should be kept in such a way that they do not get too hot, i.e., temperatures above 25°C. A cool box would provide such conditions or cardboard box with proper packing materials. When flies are caught in the field, a blood meal should be provided on the same day either in the field or in the laboratory by using rabbits. Enough rabbits should be used to avoid heavy losses of animals if more than 1000 insects are used on one animal. To minimize this loss, insects can be sexed and males killed.

H. Precautions for Colonising Personnel

It is important to realise that wild flies could be infected with both human and animal diseases. Right from the start care should be taken to avoid transmission. The use of hand gloves to avoid bites from the tsetse flies is crucial. Animals used for feeding wild flies *must* be kept away and separate from those used for feeding clean colonies. The wild flies must be handled in a separate laboratory, all doors should be double and automatic locking. After rabbits have been used on feeding wild flies and no other flies are being brought from the field such animals must be destroyed.

I. Transportation of the Flies from the Field and Packing

Before sending flies to a distant place, they should be fed, labelled properly. Cages should be carefully packed in a cardboard or cool box. A good arrangement is to surround the cages with plenty of clean materials and placed within a polystyrene box. A polystyrene box is useful because it is a good insulator and very light in weight. Cardboard box would be satisfactory if there is plenty of clean packing material. This method can be used to bring flies from the field to the colonising laboratory.

5. The Colonising Laboratory

A. Design – The colonising laboratory should be designed in such a way that the flow of various activities in the laboratory are enhanced.

– Rooms holding wild flies and the feeding area should be if possible interconnected. The doors should be automatic and double doors. The conditions should be similar to the field conditions depending on the species.

– The rooms for wild flies must be completely separate from those holding clean colonies and if possible no through passage.

B. Handling and Feeding

In the same way wild flies should be separately handled and fed. Care should be exercised not to open cages holding wild flies to avoid escapees. The animals must be kept away from those used in the clean colony.

C. Precautions Against Contamination

Because it is difficult to differentiate a wild fly from laboratory bred flies or infected fly from non-infected by looking, it is advisable to kill all straying flies whether found in the holding rooms of either type. Pupae larviposited from the wild flies should be weighed and transferred to the clean colony side to emerge from there.

The number of flies to be caught in the field depends upon the survival rate and fecundity of such flies. The *in vitro* system of feeding would be most ideal, because the infection and re-infection and loss of animals factor would be eliminated although it is not possible to feed the flies in the field using this method.

6. Conclusion

Tsetse colonisation is therefore identified as a key component for development of Integrated Pest and Vector Management Strategies. The species reared in the laboratories should be as competitive as the wild flies and the laboratory colonies should be replenished to keep them as natural as possible.

Acknowledgements

I wish to acknowledge the help of various people who helped in one way or the other. Dr. J.P.R. Ochieng'-Odero for his corrections, advice and encouragements, Drs. Steve Mihok and Madubunyi of Livestock Pests Research Programme and Mrs. Mary Owaga for providing advice and slides of traps and Mrs. Opiyo for typing the paper.

References

Dransfield, R.D. 1991. Control of tsetse flies and trypanosomiasis—myth or reality. *Parasitology Today* 7: 287–291.

Freizil, J.L. and P. Carnevale. 1976. Utilization de la Caboglace pour la capture de *Glossina fuscipes quanzensis*. *Ent. Med. Parasit.* 14: 225–233.

Harley, J.M. 1967. The influence of sampling method on the trypanosome infection rate of catches of *G. pallidipes* and *G. fuscipes*. *Entomol. exp. appl.* 10: 240–252.

Harris, R.H. T.P. 1930. Report on the bionomics of tsetse fly (*G. pallidipes* Aust.); and a preliminary report on a new method of control, presented to the Provincial Admin. of Natal Fol. 75 pp. Pietermaritzburg.

Langridge, W.P. 1961. Scent attractants for tsetse flies. Proc. 8th Meeting Int. Sci. Comm. Tryp. Res. Tech. Coop. Afr. S. Sahara Jos. 1960 Publ. No. 63: 235–241.

Nash, T.A.M. 1930. A contribution to our knowledge of the bionomics of *Glossina morsitans*. *Bull. Ent. Res.* 21: 201.

Nash, T.A.M. 1969. *African Bane: The Tsetse Fly*. Collins London.

Owaga, M.L.A. 1982. Tsetse trapping devices and methods. ICIPE Publications. *Dudu* No. 23: 4–5.

Owaga, M. L. A., A. Hassanali and P. G. MacDowell. 1988. The role of 4-cresol and 3-n-propylphenol in the attraction of tsetse flies to buffalo urine. *Insect Sci. Applic.* 9: 95–100.

Pilson, R.D. and B. M. Pilson 1967. Behaviour studies of *Glossina morsitans* Westw. in the field. *Bull. Ent. Res.* 57: 227.

C. Principles in the Development of Insect Rearing Methodology

Basic Requirements for Artificial Rearing of Insects, Mites and Spiders, *J.P.R. Ochieng'-Odero* **131**

Principles for Insect Colony Management, *J.P.R. Ochieng'-Odero* **139**

Basic Principles of Diet Development, Establishment and Feeding of Phytophagous Insects on Artificial Diet, *J.P.R. Ochieng'-Odero* **159**

Ethological Aspects of Rearing Insects, *S.A. Lux* **173**

Basic Requirements for Artificial Rearing of Insects, Mites and Spiders

J. P. R. Ochieng'-Odero

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

Introduction

Efficient artificial rearing of insects, mites or spiders begins with the definition of clear objectives. The purpose for rearing (e.g. bio-control, plant screening for resistance, pheromonal studies, virus production etc.) will determine the type of resources (material, financial, spatial and personnel) needed. The resources in turn will depend on the scale of the rearing operation (whether experimental, laboratory rearing, mass rearing or "factory" production). This paper suggests "bare minimum" requirements for rearing. The requirements will basically support experimental or laboratory rearing but not mass rearing. The requirements, however, can be expanded to suit large scale rearing needs. The suggestions in this paper do not include detailed measurements or specification as these will vary depending on the rearing objectives. Review and discussion papers on resource planning for artificial rearing should be consulted for more details (e.g. Leppla and Ashley 1978; Owens 1984; Wolf 1984; Griffin 1984; Harrell and Gant 1984; Goodenough 1984; Burton and Perkins 1984; Fisher and Leppla 1985; Goodenough and Parnell 1985). This paper also lists common requirements for rearing on artificial diets.

Laboratory Design

The rearing laboratory is a restricted area. There should be no provision for the ready access of non-rearing staff. Continuous rearing requires two basic areas of operation:

(1) Handling and manipulation area; and
(2) Holding and storage area

(1) Handling and manipulation requires an area where workers will be able to handle different species and their rearing demands (e.g. diets, containers etc.). The environmental conditions should be maintained at ambient with air conditioners or fans for the comfort of the rearing personnel. Windows should be filtered by fine mesh. The floors, walls and tables should be designed to allow for total disinfection and for wet washing.

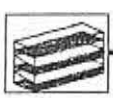
The handling and manipulation area should be further split into:

(a) A sterile area to be used for all artificial diet preparation, diet conditioning, diet inoculation etc. The area should be closed from the main laboratory and supplied with an overhead ultra violet (UV) lamp. The UV light should be left on, every night, after the last worker leaves. Within this area, all workers must use gloves and laboratory coats. The area should be supplied with adequate cupboards and shelves for the storage of sterile equipment and containers. Equipment and containers stored in the sterile area should be only those that are regularly used within the area;

(b) Harvest and cleaning area to be used for the general cleaning and repair of all containers and the disposal of contaminated material. The area also serves for species recovery and handling (e.g. pupal harvest, preparation of oviposition units etc.). Steel shelving for wet containers, ovens for drying and an autoclave can be located in this area. Large sinks should be provided for the overnight disinfection of dirty containers; and

(c) Service area to be used in attending to non-rearing personnel presenting orders, delivering or collecting different materials. A service window can be used to stop the entry of non-rearing personnel into restricted areas.

(2) Holding and storage areas are temperature- photoperiod- and relative humidity- regulated and are used for the incubation/holding of different developmental stages of species reared. The areas are restricted with air tight



doors. The areas should also be provided with adequate shelves and benches for holding different sized rearing containers. The shelves, walls and tables need to be waterproof to facilitate cleaning and disinfection. UV lights can also be installed to assist in sterilisation. Holding and storage areas should have some form of screened air flow system. It is essential that the air is not re-circulated to avoid microbial loading.

Containerisation in Rearing

Containers are selected to suit a particular species rearing needs. The selection should also depend on availability, durability and cost. In certain cases containers may need to be designed specifically to suit the rearing of a particular species (for example mating and oviposition units, pupation chambers etc.). Disposable containers are useful only in controlling microbial contamination but should generally be discouraged due to the cost they incur. It is important that all containers used be available locally. Burton and Perkins (1984) exhaustive review on the subject should be consulted before initiating any work.

Rearing Personnel

Brooks (1984) has discussed academic training needs for rearing personnel. Knowledge of medical and agricultural entomology is a basic requirement for rearing practitioners. Emphasis should be placed on evolutionary genetics, behavioural ecology, invertebrate pathology and microbiology, physiology and nutrition. A successful rearing practitioner, however, is one who has a cultivated interest in the subject, dedication and the keen eye of an observer. Rearing operations require 7 day/week commitment and demands unparalleled attention and involvement. Personnel who cannot sacrifice such a commitment should never be involved in rearing operations at any level.

Requirements for Rearing

Minimum equipment needed for artificial rearing

Type of equipment	Quantity (Nos. or pairs)
Autoclave	1
Humidifier	2–5
Sensitive temperature and relative humidity recorder (e.g. Thermo-hygrograph)	2–3
Electric time switch (for photoperiod control)	5–10
Deep freezer	1–2
Refrigerator	1–2
Electric cooker (4 plate + oven)	1
Ultra-blender	1
Electric blender (1–2 litre)	2–3
Centrifuge	1
Balance (to measure up to 10 kg)	1
Sensitive balance (to measure up to 150 g)	1
Electronic balance (to measure down to 0.0001 g	1
Sensitive pH meter	1
Clock timer	2–3
Thermometer	2–5
Cooking pot (non-stick surface, 1.5–2.0 litre)	4–6
Manual egg beater	1–2
Serving spoons	2–3
Table spoons	5–6
Teaspoons	5–6
Kitchen knife	1–2
Incubator (temperature regulated)	3–4
Incubator (1 temperature and 1 photoperiod regulated/24 hr)	2–3
Incubator (2 temperature and 2 photoperiod regulated/24 hr)	1
Growth chamber (complete regulation)	2
Soft-nose forceps	20
Forceps (pointed)	10
Scissors (large)	4
Scissors (small)	3
Camel brushes (large)	4
Camel brushes (fine)	10

Other important laboratory ware includes:
Gloves (heat resistant, waterproof or disposable), Stationery: labels (various sizes), marker pens (fine and non permanent)

Common ingredients used in formulating artificial diets

(1) *Protein bases*
Casein (technical and hydrolysate)
Wheatgerm
Wheat or rice bran
Various bean powders

(2) *Essential vitamins, salts and organic acids*
Vanderzant vitamin mix
Wessons salt mix
Brewers yeast (hydrolysate and ordinary)
Ascorbic acid
Cholesterol
Choline chloride
Folic acid
Linoleic acid
Riboflavin
Tocopherol

(3) *Antioxidants, stabilisers and pH regulators*
Sorbic acid (+ its K and Na salts)
Absolute ethanol
95% Ethanol
Citric acid
Methyl hydroxybenzoate alkyl esters (parabens)
Hydrochloric acid
Potassium hydroxide
Propionic acid (+ its Na and Ca salts)
Acetic acid
Benzoic acid
Epoxides

(4) *Antibiotics*
Penicillin
Streptomycin
Tetracyclin
Aureomycin
Chloromphenicol

(5) *Fungicides*
Benlate (Benomyl)
Fumidil B
Prochloraz (Octave W.P.)
Formaldehyde

(6) *Binding, bulking and gelling agents*
Alphacel
Gelatin
Agar
Cellulose

(7) *Phagostimulants*
Glucose
Starch
Sucrose
Inositol
Sodium chloride
Potassium chloride
Linseed oil
Lecithin

(8) *Amino acids*
Isoleucine
Tyrosine
Tryptophan
Leucine
Lysine
Methionine
Phenylalanine
Threonine
Valine
Arginine
Histidine
Asparagine
Glutamine
Proline
Alanine
Serine
Glycine
Cysteine

Common substances used for sterilisation and disinfection

Lysol
Sodium hypochlorite
Chlorodux/Chlorox
Formalin
Ethanol
Methylated spirit
Detergents

References

Brooks, M. A. 1984. Academic training for insect rearing, pp. 261–262. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Burton, R. L. and W. D. Perkins. 1984. Containerization for rearing insects, pp. 256–260. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Fisher, W. R. and N. C. Leppla. 1985. Insectary design and operation, pp. 167–183. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing*, Vol. I. Elsevier Science Publishers, Amsterdam.

Goodenough, J. L. 1984. Materials handling in insect rearing, pp. 77–86. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

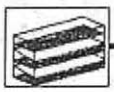
Goodenough, J. L. and C. B. Parnell. 1985. Basic engineering design requirements for ventilation, heating, cooling and humidification of insect rearing facilities, pp. 137–155. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing*, Vol. I. Elsevier Science Publishers, Amsterdam.

Griffin, J. G. 1984. General requirements for facilities that mass rear insects, pp. 70–73. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

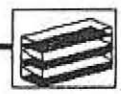
Harrell, E. A. and C. W. Gant 1984. Automation in insect rearing, pp. 74–76. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Leppla, N. C. and T. R. Ashley (eds.). 1978. Facilities for Insect Research and Production. *USDA Tech. Bull.* 1576. 86pp.

Owens, C. D. 1984. Controlled environments for insects and personnel in insect-rearing facilities, pp. 58–63. In: E. G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.



Wolf, W. W. 1984. Controlling respiratory hazards in insectaries, pp. 64–69. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.



Principles for Insect Colony Management

J. P. R. Ochieng'-Odero

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

Introduction

Rearing of insects has increasingly assumed importance as a primal necessity to research in entomology, more so as biological methods of pest control have superseded the use of only pesticides (Moore 1984; Knipling 1984). Concurrent with the importance of rearing is the question of quality and performance of laboratory or mass reared insects (Moore *et al.* 1985).

Insectary production and its organisation has been discussed by Knipling (1966), Leppla *et al.* (1982), Leppla (1984a), and Fisher (1983, 1984). Insect Rearing Management (IRM) was first introduced as a concept by Singh and Ashby (1985) and was defined as the efficient utilisation of resources for the production of insects of standardised quality to meet programme goals. IRM was discussed as the central core of any successful rearing programme around which revolved the seven component elements that determined the operation of the system. IRM was seen as an integration factor that determined the efficiency of any rearing system.

1.0 Objectives

Understanding the objectives of rearing any particular species is important for the future appraisal of the efficiency of production and for quality control (Singh and Ashby 1985).

2.0 Colony Establishment

Knipling (1966, 1984), Moore *et al.* (1985) and Bartlett (1985) discuss various inputs needed at the colonisation and establishment phase of a rearing programme (e.g. genetic variability, disease free parental stock, quarantine protocol).

3.0 Rearing Laboratory

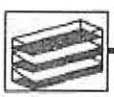
The rearing laboratory is in effect the new "habitat" for the insects and should have environmental inputs that are conducive to their development. The laboratory should also allow for worker comfort and not be hazardous to human health (Etkind 1976; Owens 1984). For insect life cycle manipulation the environment should be under some system facilitating different temperatures. Owens, Wolf and Griffin (1984) discussed exhaustively the engineering needs for a successful rearing project. Harrell and Gant, Goodenough (1984) reviewed automation and material handling in insect rearing; Batiste and Olson (1973) developed unique controlled environment rearing units in the mass production of codling moth while Goodenough and Parnell (1985) and Leppla and Ashley (1978) discussed the requirements of design and operation of insectaries.

4.0 Research and Development of Techniques

Research for rearing methods should be emphasised in rearing systems in order to develop the necessary tools and techniques for production. Research is also the basis of all quality evaluation to be subsequently undertaken.

4.1 Artificial Diets

Literature review is a valuable source of information regarding the current status of artificial diets for the insect to be reared (e.g. Dadd 1977; Singh 1977 and Singh and Moore's *Handbook of Insect Rearing* [Volumes I and II], 1985). Singh (1985) described multiple-species diets as those where a number of species can be reared for one complete or several continuous generations. These diets have the advantage of rapidly facilitating the introduction of new species into the laboratory. The general purpose diet, GPD (Singh 1983) is one such diet. Davis (1972) and Moore (1985) discussed sequential steps to be followed in order to improve multiple-species rearing diets for any given species.



4.2 Adult Diets

Adult diets have received little attention in developing rearing methods for Lepidoptera mainly because adults need only water for sustenance (necessary nutrition is gained during larval stages). Simple solutions of carbohydrates and basic amino acids have often been used as adult diets. Teakle and Jensen (1985), however, reported a complex adult diet for *Heliothis punctiger*. Blanchard *et al.* (1942) showed that adults of the corn earworm, *Heliothis zea*, fed on a diet of beer were more fecund than those fed on honey solution (producing a mean of 275 compared to 140 eggs/female).

4.3 Life Cycle Studies

Detailed life cycle studies are of fundamental importance in the successful colonisation and rearing of an insect species. The description of an insect life cycle can be divided into several phases which correspond to distinct stages in insect ontogeny: embryonic development, the larval period, metamorphosis and imaginal life (Sehnal 1985). An understanding of these basic "facets" of an insect life form a foundational basis for colony maintenance and supply.

4.3.1 Temperature, Life Cycle Manipulation and "Storage"

Temperature is often ranked as one of the most important physical factors that regulate growth. After acclimatisation of the colony it is necessary to attempt to rear the insect at different temperatures with all factors remaining constant so as to determine the optimum and critical ranges (Ochieng'-Odero in prep). Manipulation of life cycle by temperature and its subsequent use in "storage" by use of "degree days" is a key to IRM.

It is now established that insects take a long time to develop through successive stages at low temperatures. As temperature increases, development time progressively decreases until the temperature becomes high enough to affect that growth and development negatively. In practice, information is obtained from laboratory experiments in which insects are reared at several different constant temperatures, and their development times recorded. Using the "linear approximation" method allows for the two important parameters of growth to be found — the lower development threshold and the upper temperature threshold. The heat accumulation for any one temperature equals the difference between the tempera-

ture and the lower threshold times the days to develop — commonly referred to as "degree -days" (°D).

$$°D = D\,(T\text{-}K)$$

where D is the duration of development, K is the lower developmental threshold or developmental zero and T is the constant rearing temperature (Guiterrez *et al.* 1981; Guppy 1981; Stevenson 1981; Wilson and Barnett 1983; McLeod *et al.* 1985). The same information can be used within a rearing system to allow for a greater control of the laboratory colony and also be able to arrange a manipulation and "storage" strategy for various stages of insects.

The importance of "storage" of insect stages cannot be over-emphasised. Several workers have developed "storage" techniques for different insects: e.g. Dutky et al. (1962) for the coccooned larvae of the greater wax moth (over a year at 15°C and 60% r.h.); Harvey (1958) for the "storage" of diapausing second instar larvae of spruce budworm (20 weeks at 21°C and 70% r.h.); Bouse and Morrison (1985) for "storage" of *Sitotroga cerealella* (Olivier) eggs parasitised by *Trichogramma pretiosum* (Riley); Bernado *et al.* (1986) for the "storage" of black flies' eggs (2–3°C for more than 2 weeks) and Richmond *et al.* (1971) evaluated the "storage" of pupae and adults of the pink bollworm (*Pectinophora gossypiella*) at 15.6 and 10°C.

4.3.2 Photoperiod

Photoperiod is an important factor in regulating many rhythms and is usually important in the entrainment of circadian rythmns (Bruce 1960; Beck 1968; Saunders and Lucuick 1976). Photoperiods can affect egg hatching (Lockwood and Story 1985); diapause (Ankersmit 1985); feeding (Bogus *et al.* 1987); calling of virgin females, pheromone release (Sower *et al.* 1970); and for mating and oviposition (Fatzinger 1973).

4.4 Containerisation

Smith (1966), Burton and Perkins (1984) reviewed the importance of containers in insect rearing systems. In each case the size and type of containers have developed as the technique of rearing and the production has demanded. Since containers should be utilised efficiently in a rearing programme, container type will be influenced by the size of the rearing operation; overall cost of production, labour,

versatility and durability of a given container, and the biology of the species being reared. Use of disposable containers is encouraged to reduce labour and risks of contamination.

5.0 Resources

Resources has been described as a complicated but important input into the rearing system. Time, space, personnel, materials and funds are major aspects of resource management but they ultimately depend on the project targets. The management of resources to achieve a balance that minimises waste in a temporal and spatial setting is probably the most challenging element in IRM to a rearing practitioner (see Singh and Ashby, 1985 for a discussion on resource management).

6.0 Quality Control

6.1 Quality and Performance

Monitoring the quality and quantity of insects produced should be a standard practice for rearing systems. Quality assessment is a gauge for the operation of the rearing system, measuring the interaction and impact of technology on the rearing operation (quality of the process) and on the product (the insect). Inputs of the rearing system are capable of a range of variability and therefore the selection of acceptable limits or standards is useful for production of insects targeted for optimal biological performance. Quality assessment highlights deficiencies in the rearing system that need immediate remedies. Quality control is effectively realised when the assessment (indicator) results in an appropriate adjustment or remedy (control).

A paper by Agee (1986) aptly illustrates the relevance of quality assessment in rearing systems. Agee found that the compound eyes of boll weevils (*Anthonomus grandis* Boheman) reared for many generations on artificial diets had reduced visual sensitivity when compared with wild weevils. These insects, however, were being used in large scale behavioural studies in the Sterile Insect Technique (SIT) programme in South Carolina. The validity and application of the results from the behavioural experiments could therefore be doubtful. Exhaustive reviews of quality in rearing systems are provided by Chambers (1975,1977,1980); Huettel (1976); Bush and Neck (1976); Boller and Chambers (1977a, b); Boller *et al.*

(1981); Fisher (1983); Chambers and Ashley (1984); Curtis (1985) and Moore *et al.* (1985).

The following are a series of standardised tests that a laboratory colony can undergo in each generation in order to maintain the level of quality and ascertain that insects produced gave reproducible biological performance. Size of the sample indicated refers to the numbers used in a colony strength of 600 pupae/ week.

6.1.1 Quality in Adults

(a) Fecundity and fertility of mated adult females is a reliable indicator of the adequacy of larval and adult diet, oviposition environment (e.g. humidity level) and genetic condition of the colony (n = 20).

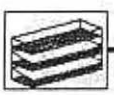
(b) Adult mortality/longevity served as an indicator of the general health of the colony. Diseased adults and inappropriate oviposition environment levels are detected by reduced longevity (n = 20).

(c) Adult malformation indicate nutritional inadequacy of the larval diet and problems in pupal management (n = 20).

(d) Mating success: Dissection of females to detect the presence or absence of spermatophores indicate the responsive behaviour of males to attractive females. Number of spermatophores (single or in multiples) indicate number of repeatedly successful matings (n = 20).

(e) Female attractancy is tested by tethering females overnight in field cages. A test of the cumulative attractive factors that make females functional in a semi-wild situation (n = 20).

(f) Pheromone production: Quantitative estimation in one attractive pheromone component of 2–5 day old virgin females by capillary GLC analysis using a 50m BP20 capillary column indicated changes that occur in the species mate recognition system (SMRS) over successive generations of rearing (n = 50).



6.1.2 *Quality in Eggs*

(a) Egg hatch: The percentage of the total eggs laid that hatched indicate success of mating, dietary adequacy, and the suitability of the egg incubation environment. Egg batches (ca. 30 eggs each) of each generation are monitored.

6.1.3 *Quality in Larvae*

Quality assessment of larvae is conducted on 100 larvae selected at random in each generation.

(a) Head capsule measurements is used to monitor larval size in each generation.

(b) Instar/stadia development is used to monitor the number of instars and their duration in each generation.

(c) Larval abnormalities: A record of the number of larval malformations obtained in each generation is kept.

(d) LD-50 susceptibility of neonates using *Bacillus thuringiensis* (*BT*) endotoxin is used to monitor low level trans-ovarial microbial infections. Infected neonates have higher susceptibility levels to *BT*.

(e) Smear tests are used to screen larvae suspected to have microsporidian infections.

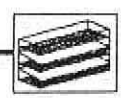
6.1.4 *Quality in Pupae*

The quality assessment of pupae is performed on 100 pupae of each generation.

(a) Pupal weight variation over each generation.

(b) Sex ratio for extreme deviation from a 1:1 ratio.

(c) Pupal abnormalities : Record of the number of deformed pupae in each generation.



(d) Percent non eclosion record of pupae that are unsuccessful in eclosion.

(e) Free amino acid (FAA) profiles of fresh pupae of both sexes using an HPLC amino acid analyser is used as a test for the stability of the internal biochemistry over successive generations.

6.1.5 Quality in the Rearing Process

(a) Inspection at strategic locations of the rearing flow process (e.g. diet dispenser, autoclave, temperature and light controls etc.).

(b) Quality of stored products (e.g. inspection of dietary ingredients and stored diet etc.).

The assessment tests listed above are adequate in regulating the general quality of the laboratory population. To ensure that overall quality is maintained in laboratory reared insects used in field release situations, several other tests need to be considered. Adult flight and orientation, adult sexual activity (mating propensity, frequency and compatibility), sexual physiology (sperm/egg production, sexual maturation) as well as the genetics of inbreeding.

It is recommended that within the laboratory, all production exercises be well documented. Colony establishment from inoculation records, pupal ratios and abnormalities from pupal recovery records can serve as relevant quality information. It is also recommended that a quality report form accompany each outgoing insect order.

Computerisation of all the quality information gathered over a period of time can aid in development of programmable models based on multivariate statistics of discriminant function and cluster analysis. Individual samples can then be entered and each "cleared" if conforming to acceptable variables. Data input and processing is important in keeping track of developments over time. Atkey *et al.* (1984) discussed steps in the formulation of good data documentation.

PROQREAR is a process and product quality control programme soon to be available for insect based rearing systems (Ochieng'- Odero *et al.* in prep.). The features of the programme include options for mean-range charts for indiscrete data, attribute charts for discrete data and the CUSUM charts for recording

changes over time. The programme will include a standards option for the standardisation of laboratory measurements.

6.1.6 Pathology

Nuclear polyhedrosis virus (NPV), granulosis virus (GV) and microsporidia have been reported as the main diseases of some laboratory reared Lepidoptera (Bathon *et al.* [in press]; P. Wigley [pers. comm.]).

7.0 Production

Production of high quality insects is the ultimate goal of rearing. The six input elements of IRM cumulatively lead to production. To initiate any production, an insect order (Singh and Ashby 1985) has to be formally submitted. It is a contract to supply specific insect stages, in specified quantities and at a given frequency of time. Singh and Ashby (1985) and Singh and Clare (in press.) described steps in the evaluation of the insect order and gave examples of how to plan for specific demands.

7.1 Production Systems

The insectary production of most insect species fall under two systems: maintenance and production. The maintenance system is designed primarily for perpetuation of the laboratory population at a level to enable build up and supply to insect orders. The production system is in response to specific insect orders and therefore stems out of the maintenance system. Both systems progressively examine production steps so as to increase efficiency.

7.2 Standard Operating Procedure

Standard Operating Procedures (SOP) or more specific, Standard Rearing Procedures (SRP) are important in the laboratory management of various insect species. These should be adhered to in order to ensure success of the rearing operation. They should include detailed instructions under the following sub-headings:

(a) **Adult Management** i.e. Oviposition systems;

(b) **Egg Management** i.e. egg collection, storage and hatching system;

(c) **Larval Management** i.e. larval rearing system; and

(d) **Pupal Management** i.e. pupal harvesting and storage procedures.

7.2.1 Colour Coding

In complex multiple-species rearing laboratories it is recommended to practice colour coding. Different insect species should be assigned a specific colour (with sub-colour for "strains"). All containers for handling specific insect species should have a colour sticker attached to the label (a larger sticker for the species and a smaller one for "strains". Colour codes of this type prevent confusion in species identification since at a glance information of the species contained is obtained.

8.0 Supply

In response to an insect order, the production system is set in motion. The final aspect of the production system is the supply of the order in scheduled time. There should be a model figure showing a hypothetical build-up process for one generation of an insect reared under optimum production conditions and on artifical diet. Such models can assist in evaluating insect orders and supply. All outgoing orders should be accompanied by the quality assessment form which is completed by user groups and returned to the rearing centre.

Sometimes the user groups are not located on the campus and in certain instances they are overseas. To meet their orders its often necessary to comply with the national and international cargo regulations for live animal specimens and quarantine formalities. Time required to ship insects from the rearing centre to its destination will dictate the stage at which they should be sent. The recommended practice is to ship all orders as early as possible in the week to avoid weekend delays. Toba and Lingren, (1984) review methods for handling mass-reared insects and shipping with emphasis on the cabbage looper (*Trichoplusia ni*); Miller and Cooper, (1980) describes a transportation system for giant silkworm moths; Baumhover *et al.* (1977) discuss a method of transporting the pupae of the tobacco hornworm. Other discussions on transport and shipping are by; Bouse and Morrison (1985) for *Trichogramma pretiosum*; Boldt and Drea (1960) for natural enemies and Graham (1970) for pink bollworm adults.

9.0 Discussion

9.1 Management and Rearing Systems

Management has been defined as an accomplishment of an end by judicious means. Rearing practitioners have not found it easy to achieve balanced efficient rearing practice due to the complex challenges and routine problems realised in production of living insects. The magnitude of the complexity can only be appreciated when considering the inherent variability introduced to the system by the insect and by the rearing or production process (Fisher 1984). Insect species undergo different developmental stages and need specific environmental inputs (both biotic and abiotic). They have particular nutritional needs for healthy development and propagation. As living organisms, they have a complex genetic build-up that is a function of the population and environment they represent and because of being in captivity they are more susceptible to diseases.

The background of the rearing system is further complicated with the introduction of variables due to the production process (the staff, the equipment, the space and other resources) and the specific constraints brought to bear by the insect order in terms of a target schedule to be met and optimal performance. Fisher (1984) noted that the rearing system is dynamic and that ideas, techniques and technology must therefore evolve flexibly to satisfy the almost fluid and changing system. Effective management defines and organises the variables that make up any functional system and then uses that system to maintain itself (Leppla 1984b). A suitable programme that would easily coordinate the various inputs of the rearing system is therefore necessary so as to constitute the production and work plan.

9.2 Insect Rearing Management (IRM)

Before Singh and Ashby (1985) formalised the management of insect rearing into the IRM concept, most rearing facilities would split the functional elements into small units for purposes of simple administration. Kavanau *et al.* (1971) criticised this approach in scientific management. Their recommended approach of operating whole systems as one unit is regarded of as a technological breakthrough, and IRM is proof of this. Unified systems of this type will almost run and regulate themselves as they react and adjust to routine challenges and problems.

The seven major elements of IRM are integral part of any rearing facility. The use of SOP is recommended to enhance repeatability in production and aid in "trouble-shooting" during process quality control. The most important element in the application of SOP is the skillfulness of the rearing practitioner (e.g. Insectary Manager, Fisher 1984). Insects will differ in their response to management. While others will successfully thrive on a pedantic use of proper materials and equipment, most however, use these resources to create an environment that would promote growth and development of the insect only when well managed. As insect rearing is a continuous learning process, IRM offers a feed-back loop system that allows the testing and application of new techniques. Whatever the programme and size of operation, the crucial assets are its personnel and their experience will be the yardstick of success, but due to the efficiency, economy, justifiable use of resources and the confidence IRM instills by orderly and reliable supply of high quality insects it should be a valuable asset to any rearing facility.

9.3 IRM and Multiple-species Rearing Centres

IRM is extremely useful in multiple-species rearing centres which either produce several different species to meet long term demand requiring various stages of different ages (for example 1000 ten day old pupae; 5000 "black head" stage eggs; 310 male adults of any age; 200 virgin and 70 three-day old females; all required of nine different Noctuids and three Gelichiids within one day); or rearing facilities that are constantly expected to rear many different species annually and at very short notice. The success of various laboratories in managing insects of such varied needs shows how well management techniques can cater for the rearing of multiple-species.

9.4 IRM as an Adaptable and Flexible Programme

The most attractive feature of IRM is its adaptability and flexibility. Contemporary rearing facilities differ considerably in design and production because of varied research needs and availability of resources (mainly fiscal, Leppla and Ashley 1978; Griffin 1984). IRM is designed to fit any rearing project simple or complex, so long as the seven constituent elements are identified. IRM has the ability to grow and expand with enlarging programmes due to the in-built flexibility of the system. In IRM, the insect decides, the resources decide and so does the rearing practitioner decide. Every constituent element is a crucial decision maker and effective insect management is developed through patient artistic, scientific and

business management practice taking account of each and every need of the constituent element of the system.

Ultimately, IRM is only a tool to help the rearing practitioner achieve their aims. To this end it is comparable to a fine instrument— well put together—but needing the skills of a rearing practitioner to make the harmony: efficiency and economy in resource utilisation and promote confidence and reliability in the production of high quality target insects, in scheduled time.

References

Agee, H. 1986. Laboratory-reared boll weevils (Coleoptera: Curculionidae) are visually impaired. *J. Econ. Entomol.* 79: 900–902.

Atkey, D. H., R. H. Jones and T. E. Walton. 1984. Systems analysis and automated data processing in insect rearing. A system for the biting gnat *Culicoides varipennis* and mosquitoes, pp. 269–280. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA ARS Publishers.

Ankersmit, G. W. 1985. *Adoxyphyes orana*, pp. 165–175. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing* Vol. II. Elsevier Science Publishers, Amsterdam.

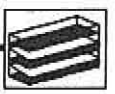
Baumhover, A. H., W. W. Cantelo, Hobgod, Jr., C. M. Knott and J. J. Lam, Jr. 1977. An improved method for mass rearing the tobacco hornworm. USDA/ARS-S-167, 13 pp.

Batiste, W. C. and W. H. Olson. 1973. Codling moth: Mass production in controlled-environment rearing units. *J. Econ. Entomol.* 66: 383–388.

Bartlett, A. C. 1985. Guidelines for genetic diversity in laboratory colony and establishment and maintenance, pp. 7–17. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing*, Vol. I. Elsevier Science Publishers, Amsterdam.

Beck, S. D. 1968. *Photoperiodism*. Academic Press, New York.

Bernado, E. W., E. W. Cupp and A. E. Kiszewski. 1986. Rearing black flies (Diptera: Simulidae) in the laboratory: colonization and life table statistics for *Simulium vittatum*. *Ann. Entomol. Soc. Am.* 79: 610–621.



Blanchard, R. A. , A. F. Satterthwaite and R. O. Snelling. 1942. Manual infestation of corn strains as a method of determining differential earworm damage. *J. Econ. Entomol.* 35: 508–511.

Bogus, M. I., J. R. Wisnieswski and B. Cymborowski. 1987. Effect of lighting conditions on endocrine events in *Galleria mellonella*. *J. Insect Physiol.* 33: 355–362.

Boldt, P. E. and J. J. Drea. 1960. Packaging and shipping beneficial insects for biological control. *FAO Plant Prot. Bull.* 28: 64–71.

Boller, E. F. and D. L. Chambers, (eds.) 1977a. Quality control: An idea book for fruit fly workers. *Bull. SROP*, WPRS, 162 pp.

Boller, E. F. and D. L. Chambers. 1977b. Quality aspects of mass-reared insects, pp. 219–235. In: R. L. Ridgeway and S.B. Vinson (eds.), *Biological Control by Augmentation of Natural Enemies.* Plenum Press, New York.

Boller, E. F., B. S. Katsoyannos, U. Remund and D.L. Chambers. 1981. Measuring, monitoring and improving the quality of mass-reared mediterranean fruit flies, *Ceratitis capitata* Wied.: 1. The RAPID quality control system for early warning. *Z. Angew. Entomol.* 92: 67–83.

Bouse, L. F. and R. K. Morrison. 1985. Transport, storage, and release of *Trichogramma pretiosum*. *Southwestern Entomologist Supplement*. no. 8: pp. 36–48.

Burton, R. L. and W. D. Perkins. 1984. Containerization for rearing insects, pp. 256–260. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA ARS Publ.

Bush, G. L. and R. W. Neck. 1976. Ecological genetics of the screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae), and its bearing on the quality control of mass-reared insects. *Environ.Entomol.* 5: 821–826.

Bruce, V. G. 1960. Environmental entrainment of circadian rhythms, pp. 29–48. In: *Biological Clocks*. Cold Spring Harbour Symposia on Quantitative Biology. Vol. xxv.

Chambers, D. L. 1975. Quality in mass-produced insects, pp. 19–32. In: *Controlling Fruit Flies—The Sterile Insect Technique*, IAEA, Vienna.

Chambers, D. L. 1977. Quality control in mass rearing. *Annu. Rev. Entomol.* 22: 289–308.

Chambers, D. L. 1980. Review: SIRM with special reference to quality control. Proceedings of Symposium on Fruit Fly Problems, *Natl. Inst. Agric. Sci.*, pp. 1–5.

Chambers, D. L. and T. R. Ashley. 1984. Putting the control in quality control in insect rearing, pp. 256–260. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA ARS Publ.

Curtis, C. F. 1985. Genetic control of insect pests: Growth industry or lead balloon? *Biol. J. Linn. Soc.* 26: 359–374.

Dadd, R.H. 1977. Qualitative requirements and utilization of nutrients: Insects, pp. 305–346. In: Rechcigl Jr. M. (ed.), *Handbook Series in Nutrition and Food. Section D. Nutritional Requirements*, Vol. 1. CRC Press, Cleveland, Ohio.

Davis, G.R.F. 1972. Refining diets for optimal performance, pp. 171–181. In: J.G. Rodriguez (ed.), *Insect and Mite Nutrition*. North-Holland / American Elsevier.

Dutky, S. R., J. V. Thompson and G. E. Cantwell. 1962. A technique for mass rearing the greater wax moth (Lepidoptera: Galleridae). *Proc. Entomol. Soc.* Washington 64: 56–58.

Etkind, P. H. 1976. The allergenic capacities of the gypsy moth *Lymantria dispar* L. Ph.D. thesis. Yale University, New Haven.

Fatzinger, C. W. 1973. Circadian rhythmicity of sex pheromone release by *Dioryctria abietella* (Lepidoptera : Pyralidae (Phycitinae)) and the effect of a diel light cycle on its precopulatory behaviour. *Ann. Entomol. Soc. Am.* 66: 1147–1153.

Fisher, W. R. 1983. Quality control of mass-reared Lepidoptera using the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), as a model. Ph.D. thesis, University of Florida. 170 pp.

Fisher, W. R. 1984. The insectary manager, pp. 295–299. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Graham, H. M. 1970. A method for shipping large numbers of pink bollworm adults. *J. Econ. Entomol.* 63: 2007–2008.

Griffin, J. G. 1984. General requirements for facilities that mass rear insects, pp. 70–73. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Goodenough, J. L. 1984. Materials handling in insect rearing, pp. 77–86. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Goodenough, J. L. and C. B. Parnell. 1985. Basic engineering design requirements for ventilation, heating, cooling, and humidification of insect rearing facilities, pp. 137–155. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing*. Vol. I. Elsevier Science Publishers, Amsterdam.

Guiterrez, A. O., J. U. Baumgaertner and K. S. Hagen. 1981. A conceptual model for growth, development and reproduction in the ladybird beetle, *Hippodamia convergens* (Coleoptera: Coccinellidae). *Can. Entomol.* 113: 21–33.

Guppy, J. C. 1981. Bionomics of the alfalfa blotch leafminer, *Agromyza frontella* (Diptera: Agromyzidae) in Eastern Ontario. *Can. Entomol.* 113: 493–500.

Harvey, G. T. 1958. A relationship between photoperiod and cold-storage treatment in the spruce budworm. *Science* 128: 205–206.

Harrell, E. A. and C. W. Gant. 1984. Automation in insect rearing, pp. 74–76. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Huettel, M. D. 1976. Monitoring the quality of laboratory-reared insects: A biological and behavioural perspective. *Environ. Entomol.* 5: 807–814.

Kavanau, L. L., C. J. Ancker, Jr. and N. F. Schneidedwind. 1971. Systems analysis,

design and operation procedures. In: H. B. Maynard (ed.), *Industrial Engineering Handbook*. MacGraw-Hill, New York. Chapter 8.

Knipling, E. G. 1966. Introduction, pp. 1– 12. In: C. N. Smith (ed.), *Insect Colonisation and Mass Production*. Academic Press, New York.

Knipling, E. F. 1984. What colonisation of insects means to research and pest management, pp. ix–xi. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Leppla, N. C. 1984a. Management of insect-rearing systems, 255 pp. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Leppla, N. C. 1984b. Systems management of insect-population-suppression programs based on mass production of biological-control-organisms, pp. 292–294. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

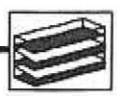
Leppla, N. C. and T. R. Ashley, (eds.) 1978. Facilities for insect research and production. *USDA Tech. Bull.* 1576. 86 pp.

Leppla, N. C., W. R. Fisher, J. R. Rye and C. W. Green. 1982. Lepidopteran mass rearing, an inside view, pp. 123–133. In: *Sterile Insect Technique and Radiation in Insect Control*. International Atomic Agency, IAEA-SM-255/14.

Lockwood, J. A. and R. N. Story. 1985. Photic, thermic, and sibling influences on the hatching rhythmn of the Southern green stink bug, *Nezara viridula*. *Environ. Entomol.* 14: 562–567.

MacLeod, D. G., R. Whistlecraft and C. R. Harris. 1985. An improved rearing procedure for the carrot rust fly (Diptera: Psilidae) with observations on life history and conditions controlling diapause induction and termination. *Can. Ent.* 117: 1017–1024.

Miller, T. A. and W. C. Cooper. 1980. A modular, transportable habitat system for colonization of giant silkworm moths (Saturnidae). *J. Lepidopterists' Soc.* 34: 51–60.



Moore, R.F. 1984. Message from the conference chairman, pp. vii–viii. In: E.G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Moore, R.F. 1985. Artificial diets: Development and improvement, pp. 67–83. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing* Vol. 1. Elsevier Science Publishers, Amsterdam.

Moore, R. F., T. M. Odell and C. O. Calkins. 1985. Quality assessment in laboratory-reared insects, pp. 107–135. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing*, Vol. I. Elsevier Science Publishers, Amsterdam.

Owens, C. D. 1984. Controlled environments for insects and personnel in insect-rearing facilities, pp. 58–63. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Richmond, C. A., H. M. Graham and C. T. Perez. 1971. Temperatures for storing pupae and adults of the pink bollworm. *J. Econ. Entomol.* 65: 435–439.

Saunders, C. J. and G. S. Lucuik. 1976. Factors affecting calling by the Eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Entomol.* 104: 1751–1762.

Sehnal, F. 1985. Growth and life cycles. pp. 1–86. In: G.A. Kerkut and L.I. Gilbert, (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. II. Pergamon Press.

Singh, P. 1977. *Artificial Diets for Insects, Mites and Spiders.* Plenum Press, New York.

Singh, P. 1983. A general purpose laboratory diet mixture for rearing insects. *Insect Sci. Applic.* 4: 357–362.

Singh, P. 1985. Multiple-species rearing diets, pp. 19–44. In: P. Singh and R.F. Moore (eds.), *Handbook of Insect Rearing*, Vol. I. Elsevier Science Publishers, Amsterdam.

Singh, P. and M.D. Ashby. 1985. Insect rearing management, pp. 185–215. In: P. Singh and R.F. Moore (eds.), *Handbook of Insect Rearing*, Vol. I. Elsevier Science Publishers, Amsterdam.

Singh, P. and G. K. Clare. 1989. Insect Rearing Management: An operating system for rearing facilities. In: T. E. Anderson and N. C. Leppla (eds.), Elsevier Science Publishers (in press).

Smith C. N. (ed.) 1966. *Insect Colonisation and Mass Production*. Academic Press, New York.

Sower, L. L., H. H. Shorey and L. K. Gaston. 1970. Sex pheromones of noctuid moths. xxi. Light:Dark cycle regulation and light inhibition of sex pheromone release by females of *Trichoplusia ni*. *Ann. Entomol. Soc. Am.* 63: 1090–1092.

Stevenson, A. B. 1981. Development of the carrot rust fly, *Psila rosae* (Diptera: Psilidae), relative to temperature in the laboratory. *Can. Entomol.* 113: 569–574.

Teakle, R. E. and J. M. Jensen. 1985. *Heliothis punctiger*, pp. 313–322. In: P. Singh and R. F. Moore (eds.), *Handbook of Insect Rearing*, Vol. II. Elsevier Science Publishers, Amsterdam.

Toba, H. H. and P. D. Lingren. 1984. Handling and shipping laboratory reared live specimens, pp. 121–125. In: P. D. Lingren and G. L. Green (eds.), *Suppression and Management of Cabbage Looper Populations. USDA, Tech. Bull.* 1684.

Wilson, T.L. and Barnett, W. 1983. Degree-days: an aid in crop and pest management. *California Agriculture*. Jan.–Feb., pp. 4–7.

Wolf, W. W. 1984. Controlling respiratory hazards in insectaries, pp. 64–69. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. Agricultural Research Service, USDA ARS Publishers.

Basic Principles of Diet Development, Establishment and Feeding of Phytophagous Insects on Artificial Diet

J. P. R. Ochieng'-Odero

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

1.0 Artificial Diets; Scope and Definition

1.1 Scope

With the demand for large numbers of insects required for fundamental research as well as insect control techniques, artificial diets have had an increase in use (Singh 1977, 1985). Programmes that require insect rearing on artificial diets include: development of insect-resistant strains of plants; to bioassay the effectiveness of insecticides; producing host larvae for entomophagous insects which have no adequate artificial diets; studying nutritional requirements; mass-rearing for sterilisation and subsequent release in pest control programmes; mass field releases of predators or parasites of pest insects; growing pathogens such as viruses; evaluating the nutritional quality of cereals inexpensively; an economical source of nutrients for animals used as human food such as fish, birds, and frogs; source of food for endangered insectivores in captivity; public education at museums, zoos and schools; uniform source of animals for bioassays; source for natural products such as endocrines or pheromones; continuous source of specimens for basic research in morphology, physiology, biochemistry etc. (Reinecke 1985).

1.2 Definition

Several definitions of artificial diets have been proposed (Ishii 1959; Vanderzant 1966; McKinley 1971; Dougherty 1959). Singh, in 1984 proposed an artificial diet to be an unfamiliar food which is formulated, synthesised, processed and/or concocted by man on which an insect in captivity can develop through all or part of its life cycle.

The diet should be inexpensive, easy to prepare, have good keeping quality, supply all nutrients needed to produce acceptable insects, allow for easy extraction or retrieval of insect stages, and should be aesthetically pleasing.

1.3 Classification

Dougherty (1959) classified diets into three categories: Holidic (chemically defined), Meridic (Holidic and unknown purity substance) and Oligidic (crude organic materials).

Despite the purity of holidic diets, they are expensive to prepare and need severe adherence to aseptic techniques in order to use them successfully (Singh 1977). For the normal laboratory culture, where cost is not as important as quality, then the Meridic Culture, where most of the nutrients are provided as pure or refined substances, are best suited. The Oligidic diet is of a greater interest to the Rearing Practitioner who has to produce insects in mass because of economics.

2.0 Advantages and Expectations of an Artificial Diet

The artificial diet allows for a more easier, simultaneous and economic rearing of insects. Insects can be reared throughout the year, giving uniform specimens of known age whose nutrition and metabolism can be regulated and manipulated.

Singh in 1977 reviewed four principal requirements in formulating a diet which are: it must be physically/chemically attractive so that it induces and stimulates insects to feed on the unfamiliar food; it must possess all essential nutrients in balanced proportions needed for normal growth, development and reproduction and it must be free from microbial contamination.

Qualitatively, nutritional requirements of most insects are remarkably similar and the choice of a particular food by a particular species is determined largely by more non-nutritional factors such as physical properties and phagostimulants. Singh (1977) therefore suggests that quantitative balance of the nutrients is the dominant factor in the success of a diet.

The success of a diet will depend on many aspects. The chemical and the physical feeding requirements must be more satisfied to allow for normal feeding behaviour. The successful diet should produce an average yield of at least 75% of initial egg numbers, and that the size and rate of development of the insects should be similar to those in nature and that the adults produced should continuously reproduce without loss of vigour or fecundity. The insects must be "normal" and of "acceptable" quality (Singh 1985).

3.0 Insect Nutrition

3.1 Insect Nutrition and Dietetics

Though nutrition and dietetics are closely related, Singh (1977) notes that the knowledge of nutrition is essential but not necessary in the development of a practical artificial diet. Insect nutrition is the study of the role and functions of individual chemically defined nutrients required by an insect in a diet. Insect dietetics is defined as the study of various proportions of optimal nutrients in a diet. Practical or applied nutrition according to Singh (1984) is dietetics.

3.2 Nutritional Components of Artificial Diet (Summary of Ideas From Reinecke's Review Paper (1985))

3.2.1 Carbohydrates

Most artificial diets for larvae contain carbohydrates as an energy source since natural food will contain some form of carbohydrates. Insects have been seen to generally survive better when supplied with carbohydrates even though lipids and proteins can be used as alternative energy sources. Non-nutritive carbohydrates are greatly used as bulking and gelling agents in insect diets (bran, pure cellulose, agar etc.).

3.2.2 *Lipids*

Certain lipids are dietary requirements for all insects, particularly the immature stages, and although many insects may use a wide variety of lipids as an alternative energy source to carbohydrates, very few are essential and only the sterols seem to be universally required.

3.2.3 *Proteins*

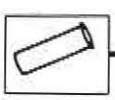
Except for a few, insects acquire amino acids from ingested proteins which are broken down by enzymes to amino acids and absorbed through the gut wall. There are a number of popular and often interchangeable protein sources used in insect diets. Ability to interchange is advantageous due to availability, cost reasons or new findings describing better colony growth. Casein is one of the more commonly used protein for insect diets. Wheatgerm, since its first use in the pink bollworm (Adkisson *et al.* 1960) is a popular addition to insect diets. Soybean and its products rival wheatgerm in frequency of use as a protein source. Other sources of crude protein for artificial insect diets include a number of legumes such as pinto, lima, and horse beans; carrots, cotton seed meat, peanut flour and walnut meat.

3.2.3 *Minerals*

Because insects seem able to thrive well on diets containing no other minerals other than those naturally present in crude additives or to perform well on diets containing standard salt mixtures originally designed for vertebrates, little interest has therefore been paid to minerals in artificial diets. The most commonly used is the commercially available Wesson's Salt Mixture.

3.2.4 *Vitamins*

Despite numerous reviews, there have been uncertainties in investigating the essentiality of some vitamin requirements of insects because the compounds are difficult to eliminate completely from the diet for critical tests. Some insects can also have enough reserves to survive and even pass adequate quantities to their progeny. Insects are now considered to have a general requirement for the following vitamins of B complex: Thiamine, riboflavin, niacin, pantothenic acid, pyridoxin, folic acid and biotin (House 1974; Dadd 1977). Choline is a frequent



additive in insect diets, though it can be spared or substituted for in some insect species. Other water soluble growth factors required only by restricted groups of insect species include carotene, nucleic acids and ascorbic acids. It has been difficult to establish a requirement for any fat-soluble vitamins. For a few insects (e.g. house cricket) growth has been enhanced by the presence of vitamin K in the diet. There are no known requirements for the D vitamins in insects. Because insects cannot synthesise carotenoids, they must obtain a dietary supply or derive them via symbionts. Though not found unequivocally to be essential, in some instances vitamin A has been shown to be a growth promoter.

3.2.5 Gels and Bulking Agents

When an artificial diet is to be moist and firm enough to support the feeding insect, a gel is used. The most popular is agar, but is a relatively costly component and can be a significant expense for rearing programmes. Agar substitutes therefore have been developed, e.g. carrageenan, Alginic acid, starches, gelatin, guar gum, locust bean gum, glutin, soya, lecithin and carboxymethylcellulose (CMC).

3.2.6 Phagostimulants

Because developing and supplying an insect with nutritionally adequate diet is useless if the insect will not feed on it, suitable chemical or physical agents that induce feeding are of prime importance. These could be amino acids, carbohydrates, sterols, vitamins etc. Physical phagostimulants include membranes (for sucking insects).

3.2.7 Antimicrobials, Antioxydants and Stabilisers

Insect diets need to be stabilised and protected from microbial contamination as this would otherwise lead to deterioration and spoilage too rapidly for effective use. Methods used include heating to deactivate enzymes, chilling for extended storage, addition of antioxidants to preserve unstable compounds, use of antimicrobial compounds and other methods such as autoclaving, UV and pH to retard microbial growth and employment of effective gelling agents to control water dispersement.

Singh and House (1970) reviewed literature on the effect of antimicrobial agents on insects. The more commonly used are formaldehyde, methyl p-

hydroxymethylbenzoate, potassium sorbate, sorbic acid, streptomycin, penicillin and aureomycin. However, before any can be used it is important to assess its level of toxicity and tolerance limits. Any method that decreases their use but withholds contamination, e.g. UV or irradiation will be of a greater value (Singh 1977, 1984).

4.0 Development of a Satisfactory Artificial Diet

Insects are among the most successful and the most adaptable of organisms. With comparative ease, many will adjust their metabolism so as to develop on sub-optimal diets or to use toxicants as metabolically useful compounds. Hence, other factors being equal insects develop normally within an adequate diet, regardless of the source of dietary components (Davis 1972).

Although basic nutritional needs of insects are understood in general, the problem of developing the most suitable diet for a specific insect can be very demanding. Ascertaining appropriate concentrations and ratios of required nutrients and non-nutritive components such as phagostimulants can be very difficult, particularly with fastidious feeders (Reinecke 1985).

In order to develop a satisfactory synthetic diet for insects, consideration should be given to the composition of the natural diet by means of proximate analysis. With such information diets may be prepared with proportions of protein, carbohydrates, lipids and minerals resembling the composition of the natural food. To such ingredients must be added those factors already proved essential for insects generally such as sterols and vitamins. This should form a basis of the initial diet.

4.1 Diets from Literature

Singh (1977) compiled a valuable source of information on artificial diets for the years 1908–1976. The well-organised volume contains abbreviated diet formulas, diet preparations, rearing procedures and insect development for over 700 species within 10 orders.

Within the volume is a list for lepidopteran diets which is overwhelming and which must have grown considerably since. Singh devotes 166 pages for the lepidopteran diets.

4.2 Multiple-species Rearing Diets

Singh (1985) describes a multiple-species diet as one which a number of species can be reared for one complete or several continuous generations. These diets have the advantage of rapidly facilitating the introduction of new species into the laboratory, for with slight modification one may satisfactorily rear the species. The General Purpose Diet (Singh 1983) is one such diet. Moore in 1985 discussed sequential steps to be followed in order to improve multiple-species rearing diets for any given species.

4.3 Animal Foodstuff Based Diets

Because man, herbivorous animals, birds and insects compete for the same food, naturally their nutritional requirements must be similar. It has been shown for example that the similarity between dietary requirements of various common laboratory animals and insects and within insect species are more striking than different (Clare et al. 1987; Singh 1984).

Due to this, the use of commercially prepared whole food for laboratory and farm animals (e.g."sheepnuts", and "rabbit pellets") as basic components of insect diets is of great interest because of the possibilities that this opens in terms of ease and economical cost. The use of animal foodstuff has been lucratively exploited in the rearing of the silkworm in Japan.

4.4 Host Plant and its Role in the Artificial Diet

The use of one or two host factors in the formulation of artificial diets is in no way new. On an overall overview, its advantages seem to outweigh the disadvantages (collection and processing costs). Due to soaring prices of refined substances and chemically defined compounds, coupled with a parallel cutback in funding for long term research in many insect rearing programmes, there has been an urgent need to develop simple, inexpensive, reliable and efficient diets using locally available ingredients. Ochieng and Bungu, (1983) gave a guiding principle that because insects survive in a simple and relatively hostile environment, to enable them to adapt to captivity, the change should never be drastic. The principle holds more true in insect diets than in any one given area of rearing.

Hence, in the colonisation phase, it is advisable to use diets with host factors. In a long-term mass-rearing, the plant factors should be phased out to allow for more readily available, easily standardised and sanitary constituents.

5.0 Principles of Establishment and Feeding of Phytophagous Insects on Artificial Diet

The principles and modes of establishment on artificial diets do not differ from feeding on the host food source. Artificial diets are meant to be a mimic or synthesised substitute for the host food source. Hence, factors that govern for example, host plant selection are similar to those that govern the selection of artificial diets. The establishment and feeding process is broadly divided into orientation and feeding steps (see Saxena, 1973). The orientation step involves mechanisms that determine attraction or avoidance responses. The feeding step involves tasting of the diet, continual feeding on the diet and the processes of digestion, assimilation and growth. Schoonhoven (1972) identified the feeding of insects to be due to four successive mechanisms: (a) a mechanism for attraction to the food source, (b) a mechanism for taste of the food source resulting in the initiation of feeding, (c) a mechanism for continual feeding, and (d) a mechanism involved in digestion and assimilation.

Attraction to an artificial diet is a complex process that involves visual, contact and chemical cues emanating from the diet. The interaction of an insect with these attracting cues results in the orientation to the diet (i.e. attraction or repulsion). Chemical cues can be important in cases of insects which are specialists in diet preference (mono- or oligophagous insects). Specialist insect species tend to "tune" to specific olfactory "token" chemical stimuli emanating from the host food source (Jermy 1966; Hsiao 1972; Schoonhoven 1968, 1972). The olfactory cues to a host food source may include the so called "green odours" (leaf alcohols etc. see Visser 1986; Jermy *et al.* 1988). The specificity of the host food source odour may also lie in a blend of the "green odour" compounds (Visser and Avè 1978). Such token stimuli are often very difficult to reproduce or emulate in artificial diets.

Tasting of the artificial diet is in most cases the final "arbiter" of a diet for an insect species. Mechanism that operate here involve phago-inhibitory chemical substances (referred to as feeding deterrents) as well as phago-stimulatory chemical substances (feeding attractants, see Saxena, 1973). Normally these

chemicals do not operate in isolation but at a highly complex synergistic interaction. It is now known that phago-stimulants are not only sugars (e.g. sucrose, glucose, fructose) but involve other nutrient stimulants like amino acids. Secondary plant chemicals (allelochemics) may influence feeding by:

(i) stimulating feeding response to specific chemicals restricted to certain food sources; or

(ii) inhibit feeding by chemicals distributed in certain food sources (Bernays and Chapman 1977).

Where allelochemics are involved, Jermy (1966) has suggested that insects select food in a negative manner. The rule of thumb is "eat everything not containing particular phago-inhibitors". Monophagous or oligophagous insects differ in that they tolerate fewer inhibitory allelochemics than do phytophagous insects. Consequently, the development of artificial diet for specialists is often a more difficult process.

Continued feeding depends largely on the outcome of the tasting process. Acceptable diets are usually fed upon. There is an hypothesis that certain "token" chemical stimuli actually enable insects to feed continuously. It is difficult to eliminate the effects of nutrition, digestion and assimilation in order to test the hypothesis. In all probability, substances that are readily digested and assimilated will support a process of continual feeding.

6.0 Principles of Development and Evaluation of Artificial Diets

An artificial diet has been defined as an unfamiliar food source formulated, synthesised, processed and/or concocted by man on which an insect can develop through all or part of its life cycle (Singh 1984).

The diet should be inexpensive, easy to prepare, have good keeping quality, supply all nutrients needed to produce quality insects, allow for easy extraction or retrieval of insect stages and be aesthetically pleasing. As insects are very adaptable, they can adjust their metabolism so as to develop on sub-optimal diets. Hence, other factors being optimum, insects will develop normally on artificial diets regardless of the source of nutrition (Davis 1972).

6.1 The Development of an Artificial Diet can be Accomplished by:

(1) Series of deletion sequences from a multiple-species diet to obtain a species-specific diet (Moore 1985; Dadd 1985);

(2) Series of "building block" sequences using information gathered from proximate analysis of host food source composition; and

(3) Series of inclusion sequences using a "carrier" diet incorporating aspects of a multiple-species diet to obtain a species specific diet (Ochieng'-Odero, unpublished).

The deletion and inclusion sequences are only possible when insect species can be reared fully or partially on a multiple-species rearing diet. "Building block" sequences need thorough knowledge of the chemical components of the host food source.

The classic approach to nutritional studies of insects is by the manipulation of specific constituents in an otherwise satisfactory artificial diet of sufficient definition and observing the consequences in terms of growth (increase in size and substance), development (change in form) and maturation and reproduction (Dadd 1985).

Use of a "carrier diet" is a more innovative method that follows the establishment of the reliance of the multiple-species rearing diet in giving satisfactory growth and reproduction and hence it forms a reference basis for all future evaluation work. The assumption is that it has at least all requirements needed for the development of the insect. However, it is too "rich" and has certain nutritives that are not really needed. It is possible to use the steps recommended by Moore (1985) to eliminate unnecessary substances. In these set of experiments, however, another diet which has nearly all the desired characteristics of the "ideal" diet (inexpensive, easy to prepare, good keeping qualities etc.) is identified (e.g. sheepnut-lucerne diet due to Thomas, 1968). The performance of this diet is such that it needs enriching and improvement. Using the "reference" diet as a basis of different substances to be selected for enriching, the diet is used as a "vehicle" or "carrier" in a series of inclusion experiments with nutritive and phagostimulant substances of similar concentration as in the reference diet. This allows then, the formulation of a species-specific diet that can be further improved in terms of

quantity ratios and relationship of substances due to "association" or synergistic effects with a number of stabilisers, antioxidants and antimicrobials to produce the species-specific diet.

The value of this method is basically that it measures both the nutritive and synergistic value of the nutrient and other substances in one step. Whatever evaluation process is followed, however, the development of the insect, reproduction, mortality, longevity and morphological deformities will form the criteria for the final selection.

7.0 The Diet-Rearing Practitioner Relationship

The importance of artificial diets in insect rearing cannot be overemphasized. Ochieng and Bungu, (1983) put the formulation of satisfactory artificial diets as the first step in the development of a practical rearing programme.

In many regards, it is the artificial diet that either makes or breaks the rearing practitioner. The central role that current integrated pest management has placed the rearing programme in means that the rearing practitioner has to now, more than ever before, be able to provide quality insects in expected time for research in such diverse fields as pheromone studies on one hand and fumigation on the other and in such numbers that rearing on host plant only becomes laborious, economically unviable and inefficient.

Artificial diets alone cannot rear insects. Many rearers have failed with the best possible balanced diets. There are numerous cases of insect diets performing excellently in one hand but failing dismally at the hands of another practitioner. Equally important is the right type of rearing container, the right conditions within a sound management procedure etc.

A successful artificial diet with a properly balanced IRM (Singh and Ashby 1985) solves these problems in a single stroke.

References

Adkisson, P.L., E.S. Vanderzant, D.L. Bull and W.E. Allison. 1960. A wheatgerm medium for rearing the pink bollworm. *J. Econ. Entomol.* 53: 759–762.

Bernays , E. A. and R. F. Chapman. 1977. Deterrent chemicals as a basis of oligophagy in *Locusta migratoria* (L.). *Ecol. Entomol.* 2: 1–18.

Clare, G. K., J . P. R. Ochieng'-Odero, S. J. Dalzell and P. Singh. 1987. A practical rearing method for leafrollers (Lepidoptera: Tortricidae). *NZ J. Zool.* 14: 597–601.

Dadd, R. H. 1977. Qualitative requirements and utilization of nutrients: Insects, pp. 305–346. In: M. Rechcigl, Jr. (ed.), *Handbook Series in Nutrition and Food. Section D. Nutritional Requirements*, Vol. 1. CRC Press, Cleveland, Ohio.

Dadd, R. H. 1985. Nutrition: Organisms, pp. 313–390. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 4. Regulation: Digestion, Nutrition, Excretion.* Pergamon Press.

Davis, G. R. F. 1972. Refining diets for optimal performance, pp. 171–181. In: J. G. Rodriguez (ed.), *Insect and Mite Nutrition.* North-Holland Publishing Company, Amsterdam.

Dougherty, E. C. 1959. Introduction to axenic cultures of invertebrate metazoa: A Goal. *N.Y. Acad. Sci.* 77: 27–54.

House, H.L. 1974. Nutrition, pp. 1–62. In: M. Rockstein (ed.), *The Physiology of Insecta*. Vol. 4. Academic Press, New York.

Hsiao, T. H. 1972. Chemical feeding requirements of oligophagous insects, pp. 225–240. In: J. G. Rodriguez (ed.), *Insect and Mite Nutrition.* North-Holland Publishing Company, Amsterdam.

Ishii, S. 1959. Methods of experiments with insects, pp. 126–143. *Konchu Jikkan Kaku* (in Japanese).

Jermy, T. 1966. Feeding inhibitors and food preferences in chewing phytophagous insects. *Entomol. Exp. Appl.* 9: 1–12.

Jermy, T., A. Szentesi and J. Horvàth. 1988. Host plant finding in phytophagous insects: the case of the Colorado potato beetle. *Entomol. Exp. Appl.* 49: 83–98.

McKinley, D.J. 1971. An introduction to the use and preparation of artificial diets with special emphasis on diets for phytophagous Lepidoptera. *Pans* 17: 421–424.

Moore, R.F. 1985. Artificial diets: Development and improvement, pp. 67–83. In: P. Singh and R.F. Moore (eds.), *Handbook of Insect Rearing*, Vol. 1. Elsevier, Amsterdam.

Ochieng, R.S. and M.D.O. Bungu. 1983. Studies in the legume pod-borer *Maruca testulalis* (Geyer) IV. A model for mass rearing: Rearing on artificial diet. *Insect Sci. Applic.* 4: 83–88.

Reinecke, J.P. 1985. Nutrition: Artificial diets, pp. 391–419. In: G.A. Kerkut and L.I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 4, Regulation: Digestion, Nutrition, Excretion.* Pergamon Press.

Saxena, K. N. 1973. Role of behaviour in host plant selection by insects, pp. 130–138. In: *Insect Physiology and Anatomy.* Div. of Entomol. IARI, New Delhi, India.

Schoonhoven, L. M. 1968. Chemosensory bases of host plant selection. *Annu. Rev. Entomol.* 13: 115–136.

Schoonhoven, L. M. 1972. Some aspects of host selection and feeding in phytophagous insects, pp. 558–566. In: J. G. Rodriguez (ed.), *Insect and Mite Nutrition.* North-Holland Publishing Company, Amsterdam.

Singh, P. 1977. *Artificial Diets for Insects, Mites and Spiders.* Plenum Press, New York.

Singh, P. 1983. A general purpose laboratory diet mixture for rearing insects. *Insect Sci. Applic.* 4: 357–362.

Singh, P. 1984. Insect diets. Historical developments, recent advances and future prospects in advances and challenges, pp. 32–44. In: E.G. King and N.C. Leppla (eds.), *Insect Rearing.* Agricultural Research Service, US Department of Agriculture.

Singh, P. 1985. Multiple-species rearing diets, pp. 19–44. In: P. Singh and R.F.

Moore (eds.), *Handbook of Insect Rearing*, Vol. 1. Elsevier, Amsterdam.

Singh, P. and House, H.L. 1970. Antimicrobial agents: Their detrimental effects on size of an insect; Agriaaffinis. *Can. Entomol.* 102: 1340–1344.

Thomas, W.P. 1968. An artificial diet for rearing the lightbrown apple moth *Epiphyas postvittana* (Walk.) (Lepidoptera: Tortricidae). *N.Z. Entomol.* 4: 31–32.

Vanderzant, E.S. 1966. Defined diets for phytophagous insects, pp. 273–303. In: C. N. Smith (ed.), *Insect Colonisation and Mass Production*. Academic Press, New York and London.

Visser, J. H. 1986. Host plant perception in phytophagous insects. *Annu. Rev. Entomol.* 31: 121–144.

Visser, J. H. and D. A. Avè. 1978. General leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. *Entomol. Exp. Appl.* 24: 738–749.

Ethological Aspects of Rearing Insects

Slawomir A. Lux

Biological and Chemical Ecology Research Unit (BCERU)
International Centre of Insect Physiology and Ecology (ICIPE),
P.O. Box 30772, Nairobi, Kenya

Introduction

Recently, many species of arthropods are being mass reared on a large scale, sometimes in an industrial-like process. Thus they are frequently called a "product" and are even commercially sold. Usually they are used for scientific investigation or applied for pest control (beneficial arthropods in Classical Biological Control, irradiated insects in Sterile Insect Techniques etc.). However, we have to keep in mind that this particular "product" is composed of living, individual creatures and thus suitability of the "product" to research or its effectiveness in the field depends on their behavioural properties. Nowadays it became obvious that many failures or difficulties of practical or research programmes based on such material should be attributed to some abnormalities or deficiencies in behaviour of the insects used (Calkins and Ashley 1989).

Insects produced in the process of mass rearing are particularly prone to many morphological, physiological and behavioural deviations due to highly unnatural factors associated with the rearing procedure (limited genetic base, artificial or factitious diet, high population density, lack of natural selection etc.). This could be minimised by proper correction of rearing procedure and by applying well designed quality control routines. However, in most cases quality control is not used at all or restricted only to elimination of morphological aberrants. We have to be aware that some differences in behavioural features between mass reared insects and natural ones are simply unavoidable and there is always a "trade off" relation between overall costs of rearing procedure and degree of such discrepancy. On the other hand, usually we can tolerate a certain degree of such

differences, at least in some aspects of behaviour of the material used. So, to keep the whole task feasible we shall fulfil the following conditions: (1) among many biological features of the reared insects select carefully those which are essential in a particular application and (2) set the minimum standards for the selected features (Bigler 1989; Laing and Bigler 1991; van Lenteren 1991; Lux 1991).

Various methods of insect rearing can be characterised not only by their scale of production and economical performance but also by specific risk of ethological abnormalities of the "product" associated with each method. The potential impact of factors which are the most frequently modified by the rearing procedures on ethological quality of the "product" is discussed in this paper.

General Methods and Scales of Rearing

Various methods of insect rearing can be roughly categorised into three classes: (1) multiplication of insects (usually low scale), (2) rearing insects for laboratory research (usually low or moderate scale) and (3) rearing insects for field research or IPM application (usually high or industrial scale). Each class has its own characteristic features, advantages and disadvantages and can be characterised by specific type of potential ethological distortion of the "product". There are also some special cases of rearing insects, which were not discussed in the paper. Rearing insects as a food for other organisms (eggs for egg-parasitoids, larvae for aquariums, insects for insectivora kept in zoos etc.) and small scale commercial rearing of insects as a bait for fishing (common in some countries e.g. in Florida, USA). In both cases no attention is paid to ethological properties or quality of insects since it is not essential for such applications. Also ethological problems associated with industrial rearing of insects for specific insect products (beekeeping, silk moth rearing etc.) are not covered because of their specificity. For the same reasons very interesting area of breeding insects for special behavioural features also remains uncovered (e.g. commercial breeding of honey bee queens, when females are artificially inseminated and sold with pedigree certificate).

1. Multiplication of Insects

Usually this is practised as the first step in the rearing of a new insect species. Insects are usually kept on natural diet in seminatural conditions (normal atmospheric conditions and natural seasonal regime). They are kept at moderate densities for limited number of generations (1–3) and then are renewed from

natural population. Usually distortion of biological characters is negligible, so the reared insects are resembling very much individuals from natural population, which is the main advantage of these methods. But these methods are very labour intensive since they frequently require parallel planting or rearing of natural host. They are also season restricted and there is a high risk of infestation by diseases, predators or parasitoids of the reared insects. Usually these methods are very difficult to standardise and relatively costly. So they are used during the first stages of research on new organisms of potential practical value, rearing of egzotic insects for museum or zoo-type exhibitions, rearing for highly demanding ethological research and also when no other method exists or using another method is non-economical due to very low scale of rearing planned. Good examples of such methods are given by Saxena (rearing of the brown plant hopper) and Bernon (rearing of parasitoids of the colorado potato beetle) in the respective chapters of this manual. In general, using this type of methods we shall not expect any considerable ethological distortions of the reared material and thus only superficial quality control shall be used, if any.

2. Rearing Insects for Research in Laboratory

This is the most diversified category of methods. Generally they may be characterised by highly artificial conditions, sometimes artificial or factitious diet, usually high number of generations (10–100) which are seldom renewed from natural population. Insects are kept at rather high densities and are frequently sexed at premature stages and then kept sexually isolated. In many cases insects are reared with shortened or broken diapause. The methodology is usually well standardised, not season restricted and seldom requires parallel planting or rearing of natural host. The risk of infestation by natural predators or parasitoids is relatively low but very high risk of infestation by diseases and some micropredators (e.g. predatory mites eating eggs). Due to low or medium scale and thus narrow initial genetic base as well as high number of generations kept without renewal inbred is very difficult to avoid and thus distortion of many biological characters is very likely. However, in some cases such distortions themselves are the objective of research and then are not considered as disadvantage. These methods are usually used in well established, long term research programmes, rearing insects for demonstrations, training or teaching, less demanding or larger scale ethological or physiological research, routine testing of pesticides or pheromones etc., genetic or "micro-ecological" research. The most commonly reared are stored product pests, haematophagous arthropods (mainly

ticks) and some phytophagous insects. Along with very likely distortion of many biological characters, considerable behavioural abnormalities shall be expected. The impact of this problem is very difficult to assess due to usually undefined limits of distortion of natural features which can be tolerated and their unclear relation to the subject of research. Moreover, to keep such distortion within acceptable limits the quantitative ethological standards for insects from wild population are necessary, which are almost always lacking. This results in frequent neglecting of quality control, although due to high risk of various distortions it shall be recommended. It is surprising how frequently these kind of problems are neglected even in reputed laboratories though it is known that it may lead to confusion or give results and conclusions non-applicable to natural population of studied insects. In the author's opinion, many research programmes failed to achieve practical results due to neglecting of this problem.

3. Rearing Insects for Field Research or IPM Application

The most apparent feature of these methods is their very high or even industrial scale. In consequence, usually highly controlled and reproducible artificial conditions are applied and very seldom natural diet is used, which is replaced by artificial or factitious one. Usually moderate, a controlled number of generations (3–20) is reared, periodically renewed from natural population. To economise the whole process diapause, if it exists in a given species, is much shortened or broken and population density is always high or extremely high. Rearing is usually not season restricted and parallel planting or rearing of natural host material is seldom practiced. Risk of infestation by predators and parasitoids is low and infestation by diseases and micropredators, although very likely, usually well controlled. Methodology is well standardised and due to industrial-like processing, cost per unit of "product" is moderate or relatively low. Since the "product" is frequently used for commercial pest control some warranty of efficiency is usually required. So limits of distortion which can be tolerated or some minimum standards of performance are usually defined and verified by routine quality control procedures incorporated into the rearing scheme (Leppla and Fisher 1989; Ravensberg 1991; Enkegaard and Reitzel 1991; Cerutti and Bigler 1991; Greenberg 1991; Frandon *et al.* 1991; Ochieng'-Odero 1991; van Lenteren and Steinberg 1991; Bigler *et al.* 1991). Although the routine quality control is sometimes costly and in some cases inadequately defined, it is obviously an indispensable component of a good rearing programme. So tendency to sacrifice quality for the sake of rearing efficiency or economy of the process shall be considered as short-sighted.

These methods are used in long term and large scale field research, large scale testing of pesticides and applied pest control (beneficial arthropods in Classical Biological Control, irradiated insects in Sterile Insect Techniques etc.). We shall be aware about possible behavioural deficiencies in the reared material, but they are usually predictable in sense of type and extent, provided well designed behavioural tests were included into quality control routines.

Factors Most Frequently Modified During the Rearing Process

As it was mentioned above there are many vital abiotic and biotic factors being manipulated and/or modified during the rearing procedure. This is done for the sake of simplicity, standardisation or cost reducing. Usually the limits of such manipulation are determined by ability of the reared species to maintain the basic biological characters e.g. fecundity, hatchability of eggs, emergence ratio, longevity and sex ratio within expected limits. These characters supplemented by survivorship during handling and transport determine the final number of living insects reaching the destination (e.g. field) which is usually specified in warranty certificates if any are provided. However, effectiveness of mass reared beneficial arthropods depends not only on the mere presence of living insects in the field but also on their behaviour. Among many aspects of behaviour, the most decisive factors seem to be: intensity and persistency of exploration and host searching, host acceptance and adaptability to diverse and changeable environmental conditions (Lux 1991; van Lenteren 1991). These important behavioural features may be distorted or impaired by factors modified in the rearing procedure. Among the most frequently manipulated factors are: (1) diet — usually factitious or artificial, (2) density of population, and (3) abiotic conditions which are frequently kept constant for the sake of standardisation and set within optimum range to maximise productivity. We have to be aware of the fact that the biological influence of such factors is usually far beyond their nutritional value (diet) or impact on survivorship (density, abiotic conditions).

1. The Impact of Diet Modification

(a) Diet as a Food

The nutritional importance of diet is obvious and usually sufficient attention is paid to this problem. However, this may give reasons to some difficulties at the

beginning of the rearing process, when the artificial or factitious diet is tested. Such a diet may be imbalanced or insufficient nutritionally or may not contain necessary phagostimulants. This may lead to malnutrition and alteration or retardation of the maturing process. Apart from easily detectable morphological changes (distortions or at least smaller size of emerging insects) this may result in lack, incomplete or distorted reproductive behaviour, impairment of mate or prey searching ability or persistency, response to specific cues (pheromones, kairomones), fecundity, sex ratio etc. (Rodriguez 1972; Scriber and Slansky 1981). However, in well established rearing processes nutritional problems are very seldom.

(b) The Role of Diet in Chemical Communication

(i) Diet Lacking Some Important Kairomones or Their Components

This deficiency may have no immediate, visible effects on insect development, so the effects may be easily left unnoticed. Insects may be mobile and "healthy" but their ability to locate, recognise or accept a natural host or prey may be considerably impaired, since in many cases this part of behaviour depends on their previous exposure to right stimuli (Papaj and Prokopy 1989; Vet *et al.* 1990). Sometimes behaviour of adults depends even on their indirect exposure to this kind of stimuli during previous developmental stages (as a larva). That is why the whole larval development may be successful (since it depends only on nutritional properties of the food), emerging adults will mate and lay eggs on the material offered, but will not behave normally in the field.

This may result in very serious problems in the case of IPM application or in misleading results of research based on such material. For example: parasitoids released into the field to control the pest may not be effective (will not recognise or locate the host in the field), phytophagous insects may show unnatural host plant preference (important during plant resistance study), insect larvae reared on such a diet as a food for parasitoids or predators may not be accepted due to lack of kairomones which should be "normally" acquired from the host plant (Chacko — personal communication) (this may lead to underestimating of potential value of parasitoids or predators during screening study).

(ii) Diet Containing Substances Utilised by Insects as "Misleading Kairomones"

Similarly like in the case described above this deficiency may also have no immediate, visible effects on insect development. During the rearing process insects are exposed to some stimuli from factitious or alternative host and then, due to conditioning or learning process are getting "tuned" to misleading signals (De Boer and Hanson 1984; Jaenike 1983; Papaj and Prokopy 1989; Prokopy *et al.* 1982a,b; Saxena and Schoonhoven 1982; Vet *et al.* 1990) (there are also documented cases of even more strange, unnatural preference induced to insects due to their exposure to some synthetic chemicals added to diet (Saxena and Schoonhoven 1978). Such insects display fully normal behaviour in "abnormal" situation; perfect ability to recognise and accept a factitious host, but no or little interest paid to their natural host.

So, parasitoids released may be mobile, may display perfect searching behaviour, may pass laboratory quality control tests and still may be ineffective in the field. Such material used for research may be even more "dangerous", since it may lead to seemingly appalling, but misleading results.

Recently the importance of this problem is being widely understood and usually some routines are incorporated into the rearing procedures (e.g. periodic passages through natural diet from the factitious one) and quality control routines (test for natural host acceptance) to keep possible distortions within acceptable limits.

(iii) Diet Lacking Some Substances Triggering Production of Pheromones

Also this diet deficiency may not give immediate effects, at least on larval development. Adults show normal feeding behaviour but their reproductive behaviour may be considerably impaired due to lack of pheromone emission and thus hindered communication between sexes and/or low female attractiveness. (Raina *et al.* 1992).

This may give very misleading results in pheromonal and ethological research (testing pheromonal blends, traps etc.). However, this effect may be reversible and such insects after release into the field and/or exposure to natural stimuli can regain their ability to produce a pheromone and then will behave normally.

(iv) Diet Lacking Some Non-nutritious "Secondary Substances" —Pheromone Precursors

Like in cases described above, also this deficiency may not lead to immediate, visible effects on insect development, but may alter or impair their reproduction. Insects display normal feeding behaviour, but reproductive behaviour may be lacking or may be distorted due to lack of pheromone emission and thus hindered communication between sexes and/or low attractiveness of females. However, in high density of population at laboratory conditions they may still reproduce if a short-distance communication mechanism is not dependent exclusively on the same pheromone.

This may also give very misleading results in pheromonal and ethological research (testing pheromonal blends, traps etc.). But contrary to the case described above, this effect may not be reversible, since such substances are frequently acquired and accumulated at a larval stage. So, even after release into the field and/or exposure to natural stimuli they may not regain their ability to produce a pheromone and thus may not behave normally.

(c) Condition of Diet as an Environmental Signal

Condition of the host plant is known to serve as an environmental signal for phytophagous insects. So, in the case of using the plant material in the mass rearing process a special attention shall be paid to standardise the physiological condition of the plants used. Host plants too old or stressed by deficiency of water, light or soil nutrients may change considerably behaviour of reared insects. Especially in diapausing species it may result in increasing their tendency to diapause, change their mobility, direction of geotaxis and/or higrotaxis, response to light, food-shelter preference, reduce the intensity of feeding and/or reproductive behaviour etc. In some species (aphids) it may also initiate metamorphosis of migrant stages and/or activate migration behaviour (Masaki 1980).

Such insect may not respond to pheromonal or kairomonal traps and to some other stimuli, which may result in accepting misleading research conclusions.

2. The Impact of High Density and Sexual Deprivation Due to Early Sexing and Isolation

Very frequently insects are kept under high densities and/or are sexed very early,

sometimes at premature stages to economise and simplify the rearing process. Such manipulations may expose insects to unnatural stress, very poorly tolerated by some species. In many cases high density as well as long sexual deprivation may induce serious changes in insect behaviour, most frequently manifested as increase in the tendency for migration or tendency to diapause. This may result in lack, incomplete or distorted ability to reproduce, reduced feeding behaviour, changed mobility, altered food-shelter preference, direction of geotaxis and/or higrotaxis, response to light etc. (Lux 1987; Lux and Dlugosz 1987).

Such insect may not respond to pheromonal or kairomonal traps and to some other stimuli, which may result in accepting misleading research conclusions.

3. The Impact of Constant and Optimum Rearing Conditions

Constant and optimum abiotic conditions (temperature, humidity, light intensity and photoperiod etc.) are usually maintained to facilitate standardisation and maximise production. Although this may produce no visible effects in laboratory, but due to lack of exposure to natural environmental stress may result in altered food-shelter preference, changed mobility and persistence of searching, increased tendency to hide and/or sit calm while in the field.

Thus parasitoids may be mobile and vigorous while in the laboratory but ineffective in the field except very narrow, well specified conditions resembling those used during rearing. Very uncertain results may also be obtained during field research; e.g. testing pheromones, artificial infestations, ecological or ethological experiments.

The importance of these factors is still waiting to be more widely recognised and some variability of rearing conditions (e.g. in the form of pre-programmed fluctuations) or at least acclimatisation procedure shall be included into the rearing scheme.

4. The Impact of Lack or Too Weak Genetic Verification — Natural Selection

The tendency to maintain optimum rearing conditions to maximise the number of insect produced impairs natural selection, which along with usually narrow genetic base of the stock colony may result in rapid accumulation of genetic aberrants. This is usually widely recognised and frequently some routines for

elimination of such aberrants are used. However, usually they are restricted only to eliminating the most apparent, morphological abnormalities. The fact that the vast majority of behavioural traits are inherited and therefore are also subject to the same mechanism of genetic deterioration is frequently neglected.

The Need and Prospects for Incorporating Control of Selected Behavioural Features Into Quality Control Routines

As it was discussed above, many factors manipulated during rearing procedure can result in very profound, unfavourable modifications of behaviour of the reared insects, not obviously associated with easily detectable morphological aberrations. Since value of the reared insects depends on their behavioural properties, including independent behavioural tests into the quality control procedures seems to be justified.

However, without special investigation it may be difficult to assess which behavioural properties of the reared insects are really crucial for a particular purpose and how to set quantitative standards for minimum acceptable performance. For example, in the case of mass reared *Trichogramma* spp. it is known that their field performance depends on intensity and persistence of foraging behaviour, ability to respond properly to semiochemicals and their adaptability to variable field conditions (Noldus 1989; Dicke *et al.* 1989, 1991). But relation between laboratory made quantification of these parameters and field performance still remains insufficiently clarified. On the other hand, direct, on-field measurement of these behavioural features is simply unfeasible. Moreover, behavioural tests are usually considered as very laborious, so difficult to include into the routine quality control, although this may be overcome by adaptation of already available automated systems for observation and analysis of insect behaviour (Noldus and Boccia 1989; Lux 1991). To combine sophistication of the ethological analysis with simplicity required for practical quality control methods, the four step procedure has been proposed (Lux 1991):

1. gaining detailed knowledge about complexity of insect behaviour through ethological field and laboratory research
2. selecting "computer digestible" attributes of behaviour, which could be easily monitored by automated devices
3. correlating the selected elements of behaviour with expected field performance of insects in the field

4. designing automated methods and equipment for behavioural diagnosis for routine quality control.

In spite of a very long and difficult research needed to complete these steps, the final product — equipment for automated diagnosis of selected behavioural features of mass reared insects — may be very simple and efficient in practical use. However, until such automated bioassay equipment will become easily available and well adapted, even very simple behavioural tests incorporated into control routines proved to be very useful in commercial rearing (Enkegaard and Reitzel 1991; Greenberg 1991).

References

Bigler, F. 1989. Quality assessment and control in entomophagous insects used for biological control. *J. Appl. Entomol.* 108: 390–400.

Bigler, F., F. Cerutti and J. Laing. 1991. First draft of criteria for quality control (product control) of *Trichogramma*, pp. 200–201. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Calkins, C. O. and T. R. Ashley. 1989. The impact of poor quality of mass-reared mediterranean fruit flies on the sterile insect technique used for eradication. *J. Appl. Entomol.* 108: 401–408.

Cerutti, F. and F. Bigler. 1991. Methods for the quality evaluation of *Trichogramma evanescens* Westw. used against the European corn borer, pp. 119–126. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

De Boer, G. and F. E. Hanson. 1984. Food-plant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm *Manduca sexta*. *Entomol. exp. appl.* 35: 177–193.

Dicke, M., M. De Jong, M. P. T. Alers, F. C. T. Stedler, R. Wunderink and J. Post. 1989. Quality control of mass reared arthropods: nutritional effects on perfor-

mance of predatory mites. *J. Appl. Entomol.* 108: 462–475.

Dicke, M., H. Dijkman and R. Wunderlink. 1991. Response to synomones as a parameter in quality control of mass reared arthropods, pp. 56–65. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Enkegaard, A. and J. Reitzel. 1991. A simple method for quality control of *Aphidoletes aphidomyza*, *Aphidius matricarinae* and *Encarsia formosa*, pp. 102–110. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Frandon, J., F. Kabiri, J. Pizzol and J. Daumal. 1991. Mass rearing of *Trichogramma brassicae* used against the European corn borer *Ostrinia nubilalis*, pp. 146–151. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Greenberg, S. M. 1991. Evaluation techniques for *Trichogramma* quality, pp. 138–145. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Jaenike, J. 1983. Induction of host preference in *Drosophila melanogaster*. *Oecologia* (Berlin) 58: 320–325.

Laing, J. E. and F. Bigler. 1991. Quality control of mass-produced *Trichogramma* species, pp. 111–118. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

van Lenteren, J. C. 1991. Quality control of natural enemies: hope or illusion? pp. 1–14. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

van Lenteren, J. C. and S. Steinberg.1991. A preliminary list of criteria for quality control of beneficial arthropods used commercially in greenhouse crops, pp. 195–199. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Leppla, N. C. and W. R. Fisher. 1989. Total quality control in insect mass production for insect pest management. *J. Appl. Entomol.* 108: 452–461.

Lux, S. A. 1987. The effect of sexual deprivation on behaviour of the colorado beetle. Abstracts from Symposium of the Polish Ethological Society, 24 – 26 November, Rogow, Poland.

Lux, S. A. 1991. Diagnosis of behaviour as a tool for quality control of mass reared arthropods, pp. 66–79. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Lux, S. A. and D. Dlugosz. 1987. The effect of population density on behaviour and development of the Khapra beetles, *Trogoderma granarium* Everts. Abstracts from Symposium of the Polish Ethological Society, pp. 24–26, Rogow, Poland.

Masaki, S. 1980. Summer diapause. *Annu. Rev. Entomol.* 25: 1–25.

Noldus, L. P. J. J. 1989. Semiochemicals, foraging behaviour and quality control of entomophagous insects for biological control. *J. Appl. Entomol.* 108: 425–451.

Noldus, L. P. J. J. and M. L. Boccia. 1989. Application of a computer system for observational studies of varying complexity. *Animal Behaviour* (in press).

Ochieng'-Odero, J. P. R. 1991. Pupal and adult indices of quality for Lepidoptera: the case of *Cnephasia jactatana* Walker (Lepidoptera: Tortricidae) and *Chilo partellus* (Lepidoptera: Pyralidae), pp. 161–173. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Papaj, D. R. and R. J. Prokopy. 1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Annu. Rev. Entomol.* 34: 315–350.

Prokopy, R. J., A. L. Averill, S. S. Cooley and C. A. Roitberg. 1982a. Associative learning in egglaying site selection by apple maggot flies. *Science* 218: 76–77.

Prokopy, R. J., A. L. Averill , S. S. Cooley, C. A. Roitberg and C. Kallet. 1982b. Variation in host acceptance pattern in apple maggot flies. Proc. 5th Symp. Insect-Plant Relationships, Wageningen, Pudoc pp. 123–129.

Raina, A. K., T. G. Kingan and A. K. Mattoo. 1992. Chemical signals from host plant and sexual behavior in a moth. *Science* 255: 592–594.

Ravensberg, W. J. 1991. A quality control test for *Encarsia formosa* (Hym., Aphelinidae) and the results over a ten year period. Quality control of natural enemies: hope or illusion? pp. 80–89. In: F. Bigler (ed.): Proceedings of the 5th workshop of the global IOBC working group "Quality Control of Mass Reared Arthropods". Wageningen, NL, March 25–28, 1991.

Rodriguez, J. G. (ed.) 1972. *Insect and Mite Nutrition; Significance and Implications in Ecology and Pest Management.* Amsterdam: Elsevier, North Holland. 702 pp.

Saxena, K. N. and L. M. Schoonhoven. 1978. Induction of orientational and feeding preferences in *Manduca sexta* larvae for an artificial diet containing citral. *Ent. exp. appl.* 23: 72–78.

Saxena, K. N. and L. M. Schoonhoven. 1982. Induction of orientational and feeding preferences in *Manduca sexta* larvae for different food sources. *Ent. exp. appl.* 32: 173–180.

Scriber, J. M. and F. Slansky. 1981. The nutritional ecology of immature insects. *Annu. Rev. ent.* 26: 183–211.

Vet, L. E. M., W. J. Lewis, D. R. Papaj and J. C. van Lenteren. 1990. A variable-response model for parasitoid foraging behaviour. *J. Insect Behav.* 3: 471–490.

D. Principles of Quality Assessment and Control

Quality Control in Insect Rearing Systems, *F. Bigler* 189

Nutrition and Quality Control in Mass Rearing of Phytophagous Insects, *G.L. Bernon and N.C. Leppla* 211

Microbial Contamination in Insectaries: Contamination, Prevention and Diseases Identification, *P.P. Sikorowski and A. M. Lawrence* 221

Identification of Insect Pathogens Common in the Insectary, *M. O. Odindo* 247

Animal Health Implications in the Laboratory Breeding of Small Mammals (With Particular Emphasis on Rabbits), *I.G. Onyango* 273

Insect Rearing and Inhalant Allergies , *R. Kfir* 277

Quality Control in Insect Rearing Systems

F. Bigler

Swiss Federal Research Station for Agronomy, 8046 Zurich, Switzerland

Abstract

Quality control is discussed under the aspect of diverse objectives in rearing insects. Basic procedures, independent of the purposes of insect production, are described and the relative importance of quality components are analysed. The usefulness of standardised tests and advantages of laboratory, semi-field and field methods are discussed. As an example, the quality control programme in the mass production of the egg-parasitoid* Trichogramma evanescens *is presented.

Introduction

By definition, quality has three components: skill, relativity and reference. That is, quality is the degree of excellence in some traits or skills relative to a reference. Thus, **skills** are the performance requirements for achieving an objective. **Relativity** has to do with ranking (comparing) the degree of excellence of the performance of skills. The **references** are the standards against which the skills are compared.

What is quality in insect rearing in the context of quality control? The definition of control includes the notion of checking, verification, comparison, regulation and periodicity. In the frame of quality there are also implications of input and feedback mechanisms, decision-making and the development of protocols for the imposition of control upon quality.

Procedures in Establishing Quality Control

There are several chronological steps that can be followed in any situation where quality control programmes have to be established in insect rearing systems (Fig. 1).

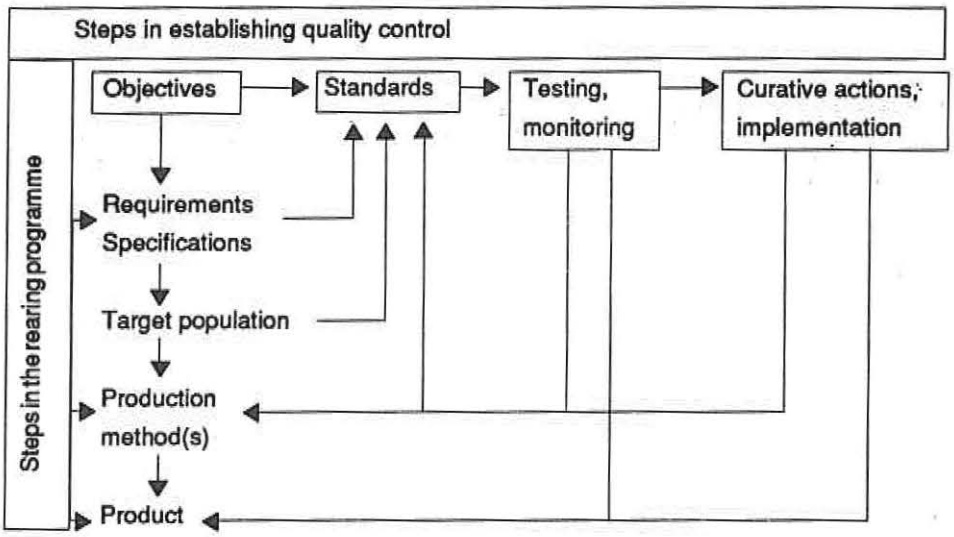


Fig. 1. Chronological procedures in insect rearing and quality control

- *Define the objectives.* Define for what purpose the insects are reared and identify the requirements.
- *Establish standards.* The required attributes of the insects produced have to be specified (specification).
- *Design and test the production methods* that satisfy the specifications.
- *Implement quality control* to ensure, within the confidence limits required, that the end product conforms to the specifications (via monitoring and corrective action).

Objectives

The purposes of rearing insects are manifold and range from the production of pathogens to the inundative release of the reared insects against a target population. The objectives to be achieved by the organisms will provide the general framework for the definition of those characteristics that must be present for performing the intended role. It is generally assumed that the strictest quality requirements must be anticipated in those cases where the mass-reared insects must have an immediate impact on the target population either as predators and parasites (in interspecific action) or as carriers of a genetic load (intraspecific action). The nature of the intended impact on the target is *the* important criterion for the definition of standards.

Standards

The standards provide the yardstick for measuring quality, and they may be considered as the very basis for the development and application of quality control procedures. It is therefore surprising that this crucial element of any comparative investigation of quality components is often obscure and vague. Standards should, by definition, be described as precisely as possible, including not only average performance levels, but also their tolerable band-widths, in order to indicate the acceptable plasticity or required rigidity of the trait. Vague definition of standards is partly caused by our lack of essential background information about the organism, especially with respect to its behaviour, ecology and genetics. This general lack of comprehensive knowledge suggests that the definition of standards in absolute terms (as is applied in industrial production) is not yet feasible and therefore its expectation may be unrealistic. One possible solution to this problem is the proper choice of a standard or reference strain against which our reared organisms are compared. It is generally agreed that the standard should be known to function well in its intended role to achieve the objectives.

In general the widely used concept of the *internal standard* can be applied wherever quality control is a matter of the detection of deleterious effects caused by intrinsic and extrinsic factors such as disease, diet, marking, chilling, sterilisation, handling etc. In these cases untreated samples of the laboratory strain are used as an internal standard. Quantification of standards becomes more complex as the objectives of the programme becomes more demanding.

Testing, Monitoring

Testing the performance of reared organisms prior to and sometimes after their use is the principal activity of a quality control programme that can provide input and feedback data for curative action. The routine monitoring of essential traits should provide an early warning that latent problems are at hand whenever significant deviations from the adopted standard are observed. These early indications of deteriorating quality components should set in motion more elaborate investigations aimed at a proper analysis and correction of the particular problem. Only the combination of insight gained by monitoring both performance (intrinsic quality) and production (output) will provide the necessary information needed to plan and carry out effective curative action.

Implementation

Figure 2 identifies steps that should be included in quality control systems and it indicates a probable order of implementation. However, the contingencies of individual programmes will dictate priorities.

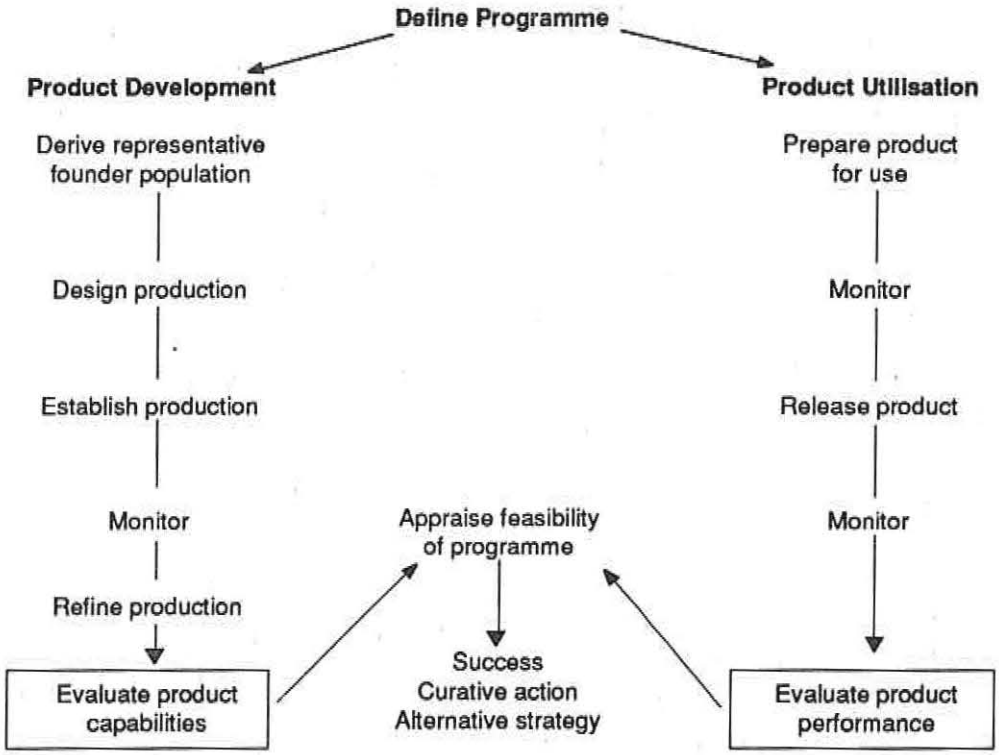


Fig. 2. Scheme for implementing quality control in operational insect rearing programmes

Relative Importance of Quality Components

Overall Quality

Overall quality of a laboratory population is measured in terms of how well it functions in its intended role. Sub-optimum success may be indicative of lowered insect quality, but it tells us nothing about the real causes of failure. It is certainly true that a successful programme is an indication of logistically adequate insect quality, but there is need to develop and to apply test procedures that assess quantitatively the overall quality at the very top of the hierarchy shown in Fig. 3. An assessment of integral performance may seem adequate as long as no major

problems are in sight. The limitations of this approach become evident as soon as problems start to appear. These problems might arise very rapidly because apparent success may mask concealed problems or gradual deterioration of quality. Then when unfavourable conditions cause a sudden breakdown of the performance of the insect and explanations must become available for the causes, it will become evident that descriptive overall quality measurement lacks the analytical power to uncover the causes of the failure. Thus, quality has to be divided into components amenable to analysis.

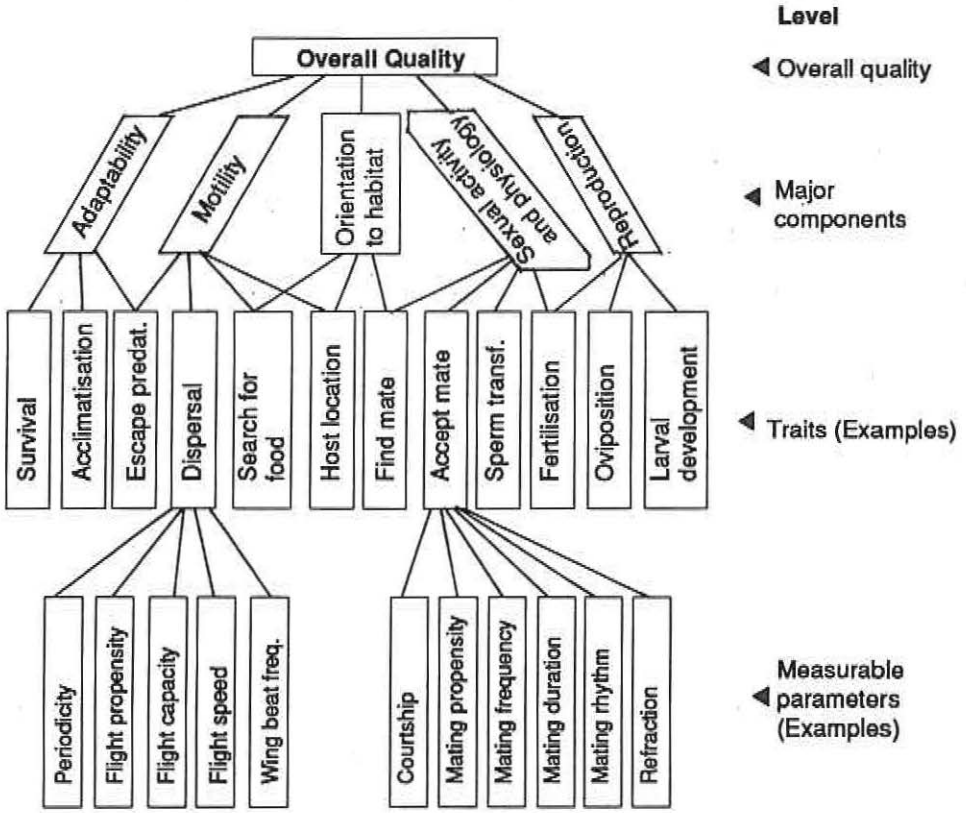


Fig. 3. Schematic presentation of a possible hierarchy of quality with examples of second and third order interrelationships (Boller and Chambers, 1977a)

One such division is shown in Fig. 3 where quality is divided into five *major components* covering the aspects of adaptability, motility, sexual activities (courtship and mating), reproduction, and colonisation. This division (or similar attempts to divide quality into major components) is necessary to sort out those components that might be of major importance for the observed failure or are known to be

especially sensitive to alteration. The selection of a few major quality components is a matter of personal judgement, but it is anticipated that a generally acceptable consensus may emerge which will be related to the objectives of the programme. The most difficult problem at this level is the determination of the relative importance of the individual major components for needed programme achievement. An example of such an attempt to weight the components in relation to the objective is given in Fig. 4. Placement of the priorities depends largely on the characteristics of the individual programme, and generalisations at this level will certainly have a limited value.

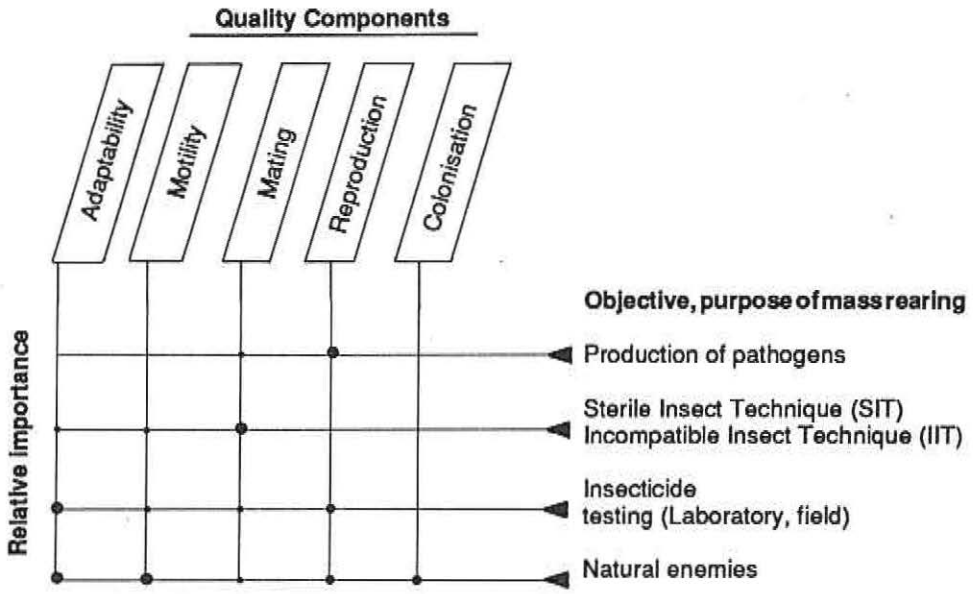


Fig. 4. Relative importance of major quality components according to the objectives of a rearing programme (after Boller and Chambers, 1977b, modified) (•, • , ● low to high relative importance)

These few major components are of no great help in the analysis of problems unless they are subdivided into *quality traits* containing measurable parameters. This level of the hierarchy shows by necessity a great variety of items, as many traits will reflect the peculiarities of the species under consideration. Certain traits will find broader application, and some of these are listed in Fig. 3. This checklist of quality traits should be made as complete as possible in the planning stage of quality control and then those singled qut that might have a major impact on overall performance.

The actual design of the quality tests will call for an even further division of the traits into *parameters* amenable to direct measurement. One example is the subdivision of the trait embracing the activities involved in the location of the mating site, the sexual partner, or the host. Because both physical and chemical stimuli might be involved in directing the insect to the proper site (such as colour, odour, shape, or sound) different techniques are required to measure and handle visual, olfactory and acoustical processes. This is the final level where a variety of techniques is to be developed for measuring and monitoring quality, where a wealth of data will be produced, and where it must be determined what techniques will produce information relevant for the events in the field.

The significance of measuring quality traits is best expressed in the following sentences: "... when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of science, whatever the matter may be". And, "... measurement is the comparison of an unknown with a standard" . And, "... quality in statistical quality control refers to some measurable property of the product that can somehow be translated into numbers".

The relatively complex structure of quality we have presented brings us back to the statement in the introduction that quality control procedures should be relatively simple in order to be applied widely and routinely. This breakdown of quality into innumerable parameters might indeed lead to the wrong conclusion, i.e., that quality control is so complex and sophisticated that it becomes the privilege (or pleasure) of a few specialists. This is not the case. Thanks to the accumulating experience, improved and simplified methods and devices, and not the least to the services provided by specialised facilities, entomologists should be able to analyse their problems and select or develop the proper key elements for biotests that monitor the quality of the insects they have to produce.

Methodology and Standardisation

Laboratory Versus Field Tests

The question arises whether laboratory tests for monitoring insect quality can adequately assess traits that have to function under field conditions. Criticism

focuses in particular on the validity of laboratory tests that measure behaviour. There is, however, a general consensus that both field and laboratory tests should be developed that complement each other because it has become obvious that both approaches have their definite advantages and shortcomings.

Advantages of the laboratory tests in general are: they are relatively cheap, quick, reproducible under standard conditions, independent of the season, comparable to a known standard and single parameters can be investigated.

What does a comparative laboratory test tell us? What does it mean when we observe that our laboratory strain shows significant deviations from the standard? First, we can state that the two strains produced significantly different results when tested under identical experimental conditions. Nothing can be said *a priori* about the relevance of the measured attributes and the significance of the observed differences to field performance. However, we have an indication that our lab strain has apparently undergone certain modifications during the rearing process that have altered its performance patterns or threshold values. Such data should serve as warning that changes are occurring. Then by comparing consecutive generations, one should be able to assess speed and intensity of this change. Secondly the warning signal should stimulate additional studies, especially in the field, to determine the relevance of the observed deviations to field performance. Thirdly, if the relationship between a single trait or a set of traits and field performance have been established previously, it may tell us what changes in field efficacy we have to expect.

Field tests or tests in larger field cages have the advantage that certain components such as dispersal, orientation mechanisms, survival rates and production of viable offspring can be studied under the direct influence of the complex environment of the target area. Unless carried out in a highly effective manner, field experiments often produce data that are influenced by a multitude of variables that cannot be identified precisely; hence, cause and effect are obscured and analysis is difficult. Therefore, these cost- and labour-intensive field tests are the logical followup to preliminary laboratory experiments that provide the first indications of when and in what direction research has to take in the field.

Standardisation of Test Conditions

Since we are dealing with phenotypes and phenotypic expressions of quality

traits, one realises that observed variation in data collected from a large number of individuals is the product of genotypic or inherited variation and environmental influences. If the performance of two strains has to be compared or the impact of certain treatments has to be assessed by comparison with an internal standard, it becomes a necessity to reduce the sources of variation to the greatest possible extent. Ideally, the comparison should be done with two identical strains that differ only in the characteristic under investigation. This can never be achieved because of the inherent variability of our test material. Therefore, in work with phenotypes exhibiting genetic and environmental variability, it is of prime interest to identify the environmental factors and those inherent to the insect population influencing our data, and to reduce the overall variation by standardisation or elimination of those influences that can be manipulated. Such factors are: temperature, light, humidity, sex, age, body size, mated or unmated, food (energy level), water, activity periods, crowding etc. After standardisation of these factors, variation of the data obtained of one parameter is generally reduced. As a result, the number of individuals tested will be lower with an acceptable accuracy. Variation still occurs and is caused to a large extent by the inherent variability of the genotype and by a residual environmental variation due to unidentified factors.

Therein lies one of the major advantages of laboratory tests: They can be carried out under defined experimental conditions that allow the detection of details that would be overlooked in the field.

Insect Rearing Programmes and Implementation of Quality Control

A rearing programme involving massive numbers of insects contains a number of major elements: These elements can be visualised as a series of stages and events, each of which affects the subsequent or preceding event by feedback. Figure 5 is a schematic diagram showing the principal elements of an insect mass-rearing programme and the implementation of quality control.

Founder Population

The selection of a target population depends basically on the objectives and requirements of the programme. Once the target is defined, it remains the delicate task to collect and choose the founder population. Both kind and range of

Programme Quality Control

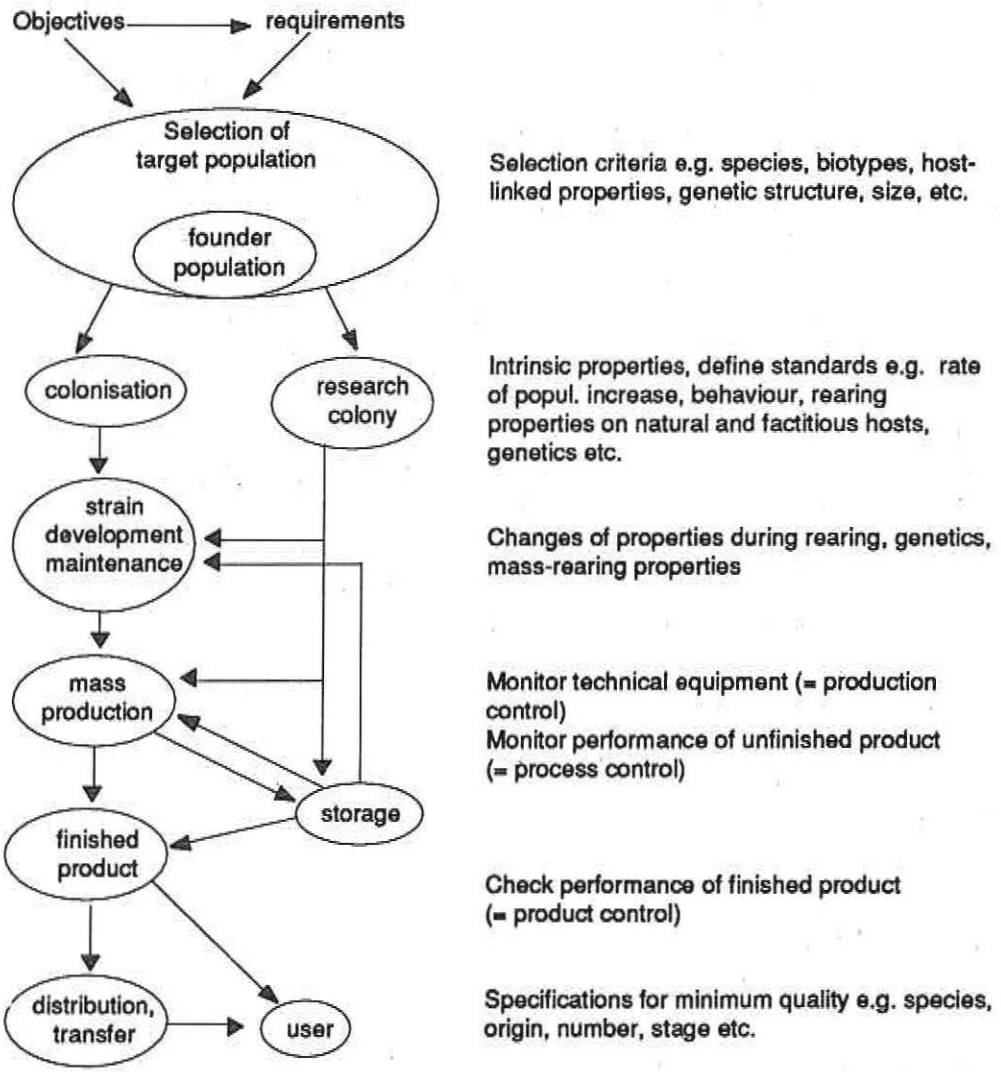


Fig. 5. Basic elements in insect mass rearing programmes and implementation of quality control

variability of performance traits must be retained if the insects are to be adequate. The difficulties in relating such factors as biotypes, genetic variability, host-linked characters, size, founder effects etc. to performance needs are presently, despite the spectacular advances in engineering, beyond the realm of possibility. Also selection and adaptation to the laboratory environment occur very early when the founder population is brought to the laboratory and forced to reproduce under artificial conditions.

Colonisation

Major "bottlenecks" occur during colonisation of the founder population.

There is no doubt that the removal of natural regulating factors is a benefit for the laboratory colony, but this very fact too often obscures the tremendous loss suffered at the very beginning of laboratory propagation. Sporadic introduction of wild material into existing colonies probably is of little value as such material stands a good chance of being eliminated by the same selective forces that affected the colony before. Such considerations might be helpful where mass rearing is still in the planning phase or has retained a certain flexibility.

Intrinsic properties and characters of the population as well as standards must be defined at the very beginning as well as in the course of the colonisation in order to follow changes in specific traits. Knowledge on speed and extent of selection and adaptation to the rearing environment is needed for the strain development and maintenance of the performance. At this stage of the programme, different rearing systems may be tested and developments aiming at the least modifications of the founder populations are made.

A method of monitoring production line continuity, the allozyme technique, has received increased attention. Loss of variation at specific genetic loci can be assessed by electrophoretic separation and identification of polymorphic enzymes of individual insects sampled from the test population. The advantages of such a technique include the objectivity of a chemical method, a relatively simple procedure that can be carried out at moderate cost.

Research Colony

Maintaining research colonies isolated from the mother-colony for many generations is often observed. This is in fact very dangerous because it is in general a small sample of the founder population, maintained under environments different from the mother-colony. Thus, the results obtained from the research colony may differ from the mother-colony and lead to wrong conclusions. Therefore it is recommended to work with research colonies taken regularly as samples from the mother-colony. This is not obvious because research colonies may be located in another place (research institute) than the mother-colony (producer).

Strain Development and Maintenance

The founding population from which production stocks are derived may be carefully selected, and the conditions of maintenance carefully managed. Nevertheless there are requirements for economy, scheduling, and productivity that restrict the design and conditions of rearing. These, plus a lack of recognition of all components of the natural habitat and a lack of capacity to incorporate them, lead to developing an insect strain in the factory that differs to some degree from the target or source population. The processes most contributory to genetic decay have been identified as the founder effect, inbreeding, genetic drift, and selection. In the large populations used as factory breeding stock, selection can be considered to have the greatest impact and the primary result is a quantitative rather than qualitative change in behaviour.

Situations exist where selection may be advantageous. For example, selected strains could provide improved performance of uniparental parasite strains, improved sexual performance of released sterile insects, engineered strains for genetic pest control, selected nondiapausing strains, colour and morphological markers, and other qualities such as suitability for production. Selection may increase homozygosity, but one may hope that serious disadvantage can be avoided. Therefore, known inbreeding or increased homozygosity should be warning signals rather than rejection criteria.

Mass Production

The quality of insects in mass production depends on the consistent performance of rearing operations at specified levels of excellence. **Production control** is the assurance that insect rearing and associated operations are performed (Fig. 6). Performance of rearing operations is controlled by directly monitoring procedures, equipment and environments. This involves the use of schedules, check sheets and other means of assuring the completion of each step in rearing and maintenance. **Process control** is the regulation of these rearing processes by monitoring unfinished products and comparing them with established specifications and standards.

Storage

Efficient storage of mass produced insects may determine the economic result and

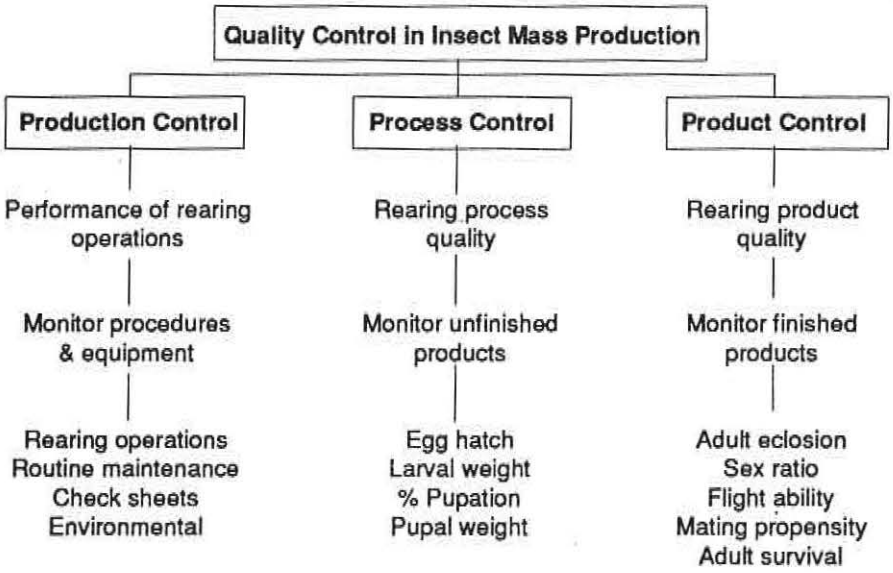


Fig. 6. The three primary subdivisions of quality control that occur in insect mass production. *Production control* assures the performance of rearing operations by monitoring related procedures and physical environments. *Process control* directly links every procedure and rearing environment with a specific stage of insect development. *Product control* evaluates the final stage that is produced and indirectly relates its quality to the rearing system (Leppla and Fisher, 1989)

the feasibility of the programme to a great extent. Storage capabilities are especially important if the product can be used only at certain periods in very large numbers (parasitoids, predators, sterile insects). Moreover, long term storage (months) of the stock colony with a high performance may be a tool to preserve the genetic variability in a culture. Stored individuals are a source for infusion of other genotypes or rejuvenation of the mass reared colony or they may be used in combination with other techniques of restoring the population.

Finished Product

Once the insect has reached the stage of a product, whatever purpose it has, a last quality control must be performed. **Product control** is the assurance that insects are appropriate for treatment, handling, and transfer to utilisation. In general, little time is left between the final preparations and the shipment of the product. Therefore, the tests have to be quick and simple but reliable. The quality control programme at this stage must be reduced to tests that assess attributes like emergence rates, sex ratios, size or weight, individuals per unit, fecundity and locomotory activity.

Distribution, Transfer

The production phase of the process terminates with transporting the insects to and using them in the target area. Techniques for handling, packaging, delivering, and releasing mass reared insects are tailored to each programme's needs; they vary from quick transport in simple containers to complex sorting, suppression of activity, controlled environment transport, and mechanical distribution. Release may involve either the active life stage or a preceding stage. In many cases, delivery is considered a part of treatment and monitoring is conducted on a subsample of the population delivered for use.

Quality Control in *Trichogramma evanescens*: A Case Study

Mass Production and Quality Control Procedures

Many species of the egg-parasitoid *Trichogramma* are mass produced worldwide and used in inundative releases against a variety of lepidopteran pests. All mass rearing systems have in common that the parasitoids are in general produced on eggs of an unnatural (factitious) host in very high numbers in crowded cages and often in an artificial environment. Too often, the number of individuals produced per time and space unit is the measure of rearing success. Thus, quality aspects are still neglected today.

In Switzerland, *Trichogramma evanescens* is mass produced since 1978 and released against the European corn borer, *Ostrinia nubilalis*, in maize. A significant loss of field efficacy was observed in 1980 (Fig. 7). By changing the mass production system and the colony maintenance, it was possible to improve the performance of the strain and keep a high quality for twelve years by now.

A thorough analysis of the production system and the performance requirements of *T. evanescens* under the maize growing conditions in Switzerland led to the discovery of important traits which are crucial for a high efficacy. Since the attributes like locomotory activity, host acceptance, host suitability and temperature tolerance were negatively affected by the former rearing system, we developed a new production unit. At the same time risk evaluations of other deteriorations in the strain were performed and methods for measuring single traits and the field performance were developed (Fig. 8).

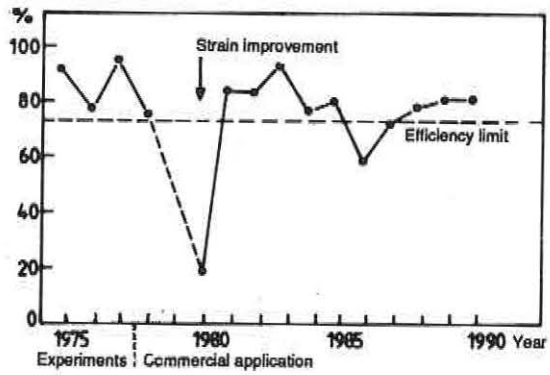


Fig. 7. Parasitism (%) of *Ostrinia nubilalis* eggs by *Trichogramma evanescens* in maize from 1976–90 in Switzerland (release of 3 x 50,000 parasitoids per hectare)

In recent years, the production system of *T. evanescens* has changed from a "high quantity/short period" to a "low quantity/long period" mass rearing system. Improvements of the long time storage of the parasitoids (diapause) has prolonged the mass production period from two to nine months per year. The main production steps are schematically presented in Figure 9. Table 1 lists the process and product control procedures in the mass rearing of the parasitoid.

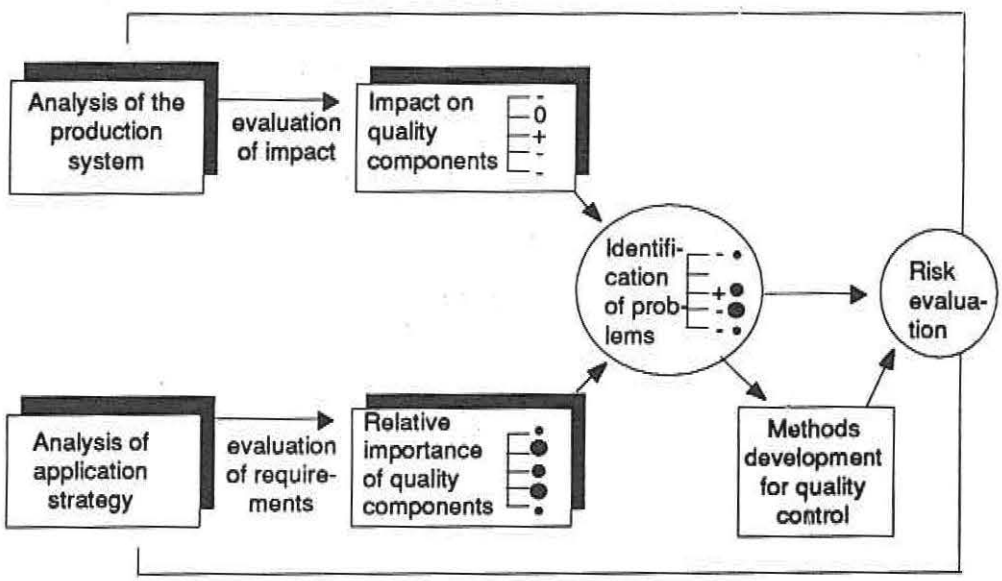


Fig. 8. Flow-chart showing the main steps in the development of quality control in the production of *Trichogramma evanescens*

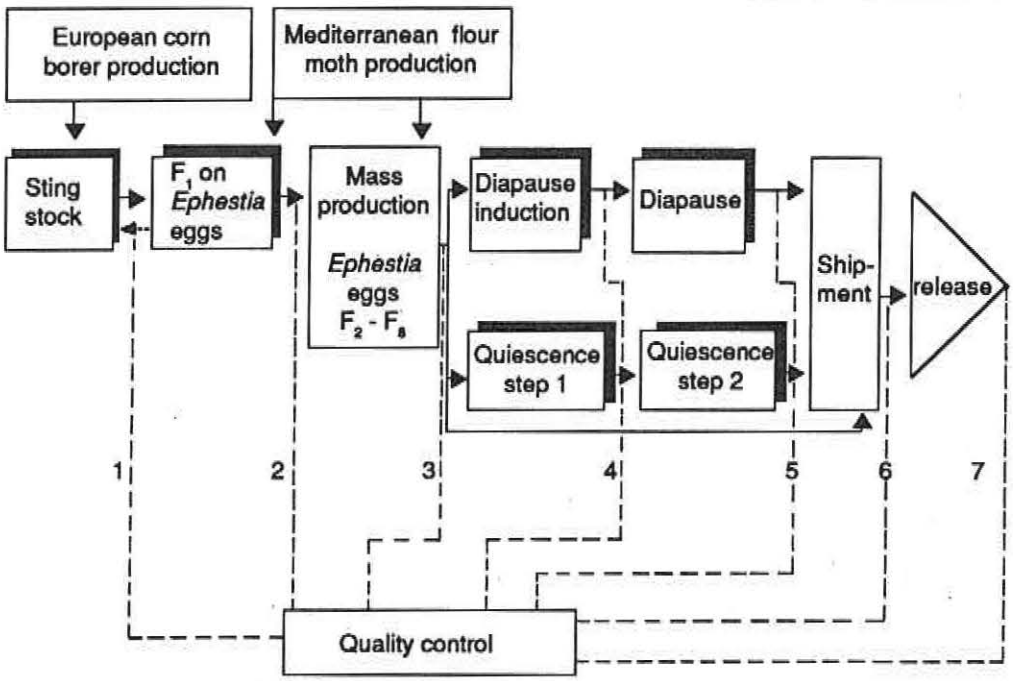


Fig. 9. The quality control system in the production of *Trichogramma evanescens* in Switzerland

Table 1. Process and product controls in the mass rearing of *Trichogramma evanescens* in Switzerland (numbers correspond to those on the quality control arrows in Fig. 2)

Flow-chart item	Quality control procedure
1. Sting stock,	Host acceptance Locomotion (walking, flight) Searching behaviour Fecundity Longevity
2. F_1	Host acceptance Host suitability Walking speed % females Fecundity Longevity
3. $F_2 - F_6$	% parasitism % females
4. Diapause ind. and quiesc. 1	% parasitism
5. Diapause and quiesc. 2	% emergence

Table 1. Continued

Flow-chart item	Quality control procedure
	Walking speed % females Fecundity Longevity % deformed females
6. Shipment	% parasitism % emergence % females % deformed females
7. Release	% parasitism In field (= efficacy)

Methods for Process and Product Controls

Laboratory Tests

Process control

Standardisation of the females for analysis

To avoid errors related to the manipulation of the wasps, great attention has to be paid to the standardisation of the rearing and handling procedures of the *Trichogramma* population which is tested. The wasps are prepared as follows: 0.1 g of parasitised eggs are placed in a small open plastic box (2 x 2 x 2 cm) which is put in a transparent plastic cylinder of 15 cm height and 10 cm diameter. The top of the cylinder is closed with a flexible plastic lid. The cylinder has 2 lateral holes of 3 cm diameter, covered with fine organdy, for ventilation. Tiny droplets of honey, about 2 per cm^2, are placed all over the lid as food. The parasitised eggs are reared at temperatures between 20 and 25°C, a relative humidity (r.h.) of 80% and 16 h light (L) and 8 h dark (D). At the first day of emergence, water is provided three times a day by spraying fine droplets of water in the cylinder through the lateral holes. The adults emerged during the first day have a higher fecundity and live longer than the ones emerged the subsequent days. For this reason only adults emerged during the first day are used. To avoid overcrowding, the small plastic box containing the parasitised eggs is removed during the first day of emergence when the population of emerged adults reaches a density of about 1000 individuals.

The next day the lid is removed and gently tapped on a white surface so that the adults get evenly scattered. They are caught individually by placing a glass tube (7.5 cm long, 1.3 cm in diameter) open end down. When the adults have walked into the vials, these are closed with a plastic plug with tiny holes for ventilation. The sex of each adult is then determined under the binocular lens. A droplet of water and a droplet of honey are then added directly to the wall of the vials containing a female.

Percentage of Deformed Females

During the sex determination at least 100 females are checked for deformed wings.

Walking Activity

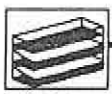
The walking activity is measured on 25 one-day old females (prepared as described in standardisation) with a computerised image analysis system. Each female is placed during 3 min. on a circular, flat Plexiglass arena of 10 cm diameter at a temperature of 19°C, r.h. 75% and a light intensity of 3600 lux (4 lamps JUST-NORMLICHT L15W-daylight 98 at 25 cm distance). Under these conditions the *Trichogramma* females show a pure walk-searching behaviour without flight induction. The position of the female is recorded each 0.48 sec. by the image analysis system (TE84-B405-SYSTEM, ELTEC EL. GmbH, D-6500 Mainz, camera CCD HR 450, HENZ AG, CH-5034 Suhr) which has a resolution of 47 x 23.5 pixels per cm in the xy-axes. The only parameter actually used to assess the walking activity is the distance covered.

Average Life-span

Survival is recorded daily on 25 females confined individually in glass tubes (see standardisation) at 25°C, 80% r.h. and 16 h L:8 h D.

Acceptance and Suitability of O. nubilalis *Eggs*

25 one-day old females confined individually in glass vials (see standardisation), receive 2 fresh egg-masses of *O. nubilalis* with at least 20 eggs each. After 24 hours at 25°C and 80% r.h., the egg-masses are removed and incubated for 3 days at the same climatic conditions. At this moment, i.e. just before the emergence of the *O. nubilalis* larvae, the number of parasitised (black) eggs is counted.



Fecundity

The fecundity is assessed on eggs of *E. kuehniella*. These eggs are collected daily and UV-sterilised. They are stored for max. 4 days at 3°C and 80–90% r.h.

25 pieces of cardboard (0.5 x 2 cm) with at least 250 eggs each (glued with water) are exposed individually for 3 days for parasitisation to 25 one-day old females (see standardisation) and kept at 25°C, 80% and 16 h L:8 h D. The pieces of cardboard with the eggs are then removed and incubated for 4 days at 25°C. The number of parasitised (black) eggs is then counted.

Emergence Rate

One ml of parasitised eggs is reared at 25°C. After the emergence of all adults, a sample of eggs is placed in a petri dish with alcohol (70%). The proportion of hatched eggs, out of 200 parasitised eggs, is determined under a binocular lens.

Sex Ratio

The sex ratio is assessed on 200 adults after their emergence. The wasps are placed in a petri dish with alcohol (70%) and examined under a binocular lens.

Product Control

Total Number of Parasitised Eggs per Release Unit

The average number of parasitised eggs per release unit (i.e. cardboard strips or cardboard shells) is assessed on six units counting all parasitised eggs of each unit.

Emergence Rate

Six release units are incubated at 25°C and 80% r.h. After the complete emergence of the adults, the rate is assessed on 200 parasitised eggs of each unit (totally: 1200 parasitised eggs).

Sex Ratio

The sex ratio is assessed on 6 release units, incubated at 25°C and 80% r.h., till the

end of the adults emergence. 100 adults per unit are checked (totally 600 adults).

Semi-field Tests

Laboratory cage experiments are carried out for assessments of host location, host acceptance and performance in varying environmental conditions. Potted maize plants of a standardised leaf area are confined in cages (50 x 50 x 80 cm) together with 40 females. Twenty corn borer egg-masses are attached to the leaves and observed and the number of females on the egg-masses is registered in short time intervals. After an exposure period to the parasitoids of 4 hours, the egg-masses are retrieved and incubated for 5 days for determination of parasitism. Environmental conditions (temperature, humidity, light) are strictly standardised.

Similar to the laboratory, cage experiments are performed in large field insectaries (4 x 2 x 2 m) where additional information on flight capacity can be obtained and where the parasitoids are exposed to natural environmental conditions.

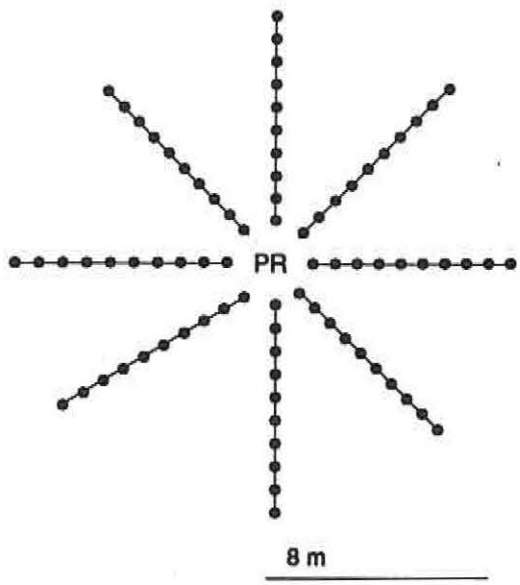


Fig.10. Experimental design for investigating the overall quality of *Trichogramma evanescens* in maize

Field Tests

The field performance is routinely checked by using basically two methods 1. Sentinel egg-masses of the European corn borer are attached to leaves of maize plants in commercial maize fields. Each dot in Figure 10 represents one egg-mass. Adults of *T. evanescens* are released in the centre (PR). The egg-masses are retrieved shortly before the corn borer larvae hatch from the unparasitised eggs. Then the egg-masses are incubated in the laboratory until parasitism can be determined. This method is used for comparisons of the overall performance of different species or strains and to assess the impact of treatments. At least three repetitions are performed per strain.

The overall performance of the released strains is measured, in commercial maize fields by assessing the parasitism on naturally oviposited egg-masses. A number of plants are checked once a week.

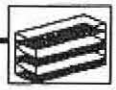
Conclusions

Quality control has made substantial progress within the last few years. Thanks to fruitful collaborations between laboratories that devote considerable time and effort to research aimed at a better understanding of the basic and applied aspects of insect quality, we have reached the point where a few applicable principles and techniques are at hand. It can be anticipated that such mutual efforts will continue to increase and to produce results.

Quality control research combines the interests of specialists involved in basic research at the academic level with those of applied field workers that know the problems at the farm and laboratory levels. There is excellent opportunity for a multidisciplinary approach involving all levels of research and application. One step in this direction could be workshops on quality control problems at the location of major rearing plants in operation. These could provide the necessary direct contact between individuals involved in basic and applied research, including those that have to carry the burden and responsibility for front line action.

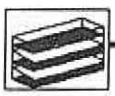
References

Boller E.F. and D.L. Chambers. 1977a. Quality Control. In: Boller and Chambers, (eds.), *An Ideabook for Fruit Fly Workers. Bulletin IOLB/SROP*, 5.



Boller E.F. and D.L. Chambers. 197 b. Quality aspects of mass-reared insects. In Ridgway and Vinson (eds.), *Biological Control by Augmentation of Natural Enemies*. Plenum Press.

Leppla N.C. and W. R. Fisher 1989. Total quality control in insect mass production for insect pest management. *J. Appl. Ent.* 108: 452–461.



Nutrition and Quality Control in Mass Rearing of Phytophagous Insects

G. L. Bernon[1] and N. C. Leppla[2]

United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Methods Development

[1]Otis Methods Development Center
Otis Air Base, Building 1398
Massachusetts 02542 USA

[2]Federal Building, Room 540
6505 Belcrest Road
Hyattsville, MD 20782 USA

Introduction

Nutrition was the primary consideration in mass rearing phytophagous insects until it was determined that their requirements are similar to those of humans (Rodriguez 1973; Vanderzant 1974). Consequently, diets are easily formulated to incorporate a balance of proteins (essential amino acids), carbohydrates (complex and simple sugars), fats (essential fatty acids), vitamins, and minerals. Secondary plant chemicals and special nutrients may be added to stimulate feeding and promote growth. The ingredients are suspended in a hydrated medium, such as agar, and the diets are adjusted for optimum pH and protected with food preservatives. The goal is to produce reliable diets and reduce costs by improving food utilisation (dietetics). As a result, standard or generic diets (Singh 1983) have been developed and marketed as industrial products. Nutrition and diet preparation therefore become major concerns only if insect growth becomes asynchronous and survival declines (Reinecke 1985).

Quality control theory has advanced to the point of implementation in insect mass production (Leppla 1989; Leppla and Ashley 1989). For individual colonised species, it is essential to assure competitiveness, avoid genetic deterioration, enforce production control (monitor all rearing operations in terms of personnel, materials, equipment, schedules, environments, etc.), establish process control (sampling immature insect stages to predict quality and determine sources of variability, such as nutrition), perform product quality control at the rearing facility and at critical points to field application, measure field performance, and provide feedback to optimise production and field delivery. Total quality control incorporates all factors that affect quality and distributes responsibility for quality among every aspect of the production, utilisation, and optimisation system.

Nutrition and Mass Rearing

Insect Diets and Private Industry

Artificial insect diets have become an industrial product in the United States. Formulations are obtained from the literature (Singh 1977; Singh and Moore 1985), and marketed without specific regulation. For example, gypsy moth diets are available from two companies, both relying on similar but not identical formulations. Researchers with limited needs for gypsy moth, *Lymantria dispar* (Linnaeus), purchase commercial diets because either it is not economical to buy ingredients separately or the suppliers of the ingredients may require a minimum purchase that greatly exceeds needs.

The gypsy moth egg masses used for laboratory research are not available commercially. The cost of mass rearing gypsy moths prohibits the private sector from producing this resource. Therefore, egg masses are often obtained from the Animal and Plant Health Inspection Service (APHIS), an agency of the United States Department of Agriculture (USDA). This facility, the Otis Methods Development Center, has been able to mass rear gypsy moths with good yields. Various rearing problems occur when the USDA strain is reared on commercial diets at other facilities. To evaluate this situation, a test was conducted on the commercial diets, using the USDA strain and diet formulation as a control. Four commercial diets were tested by rearing APHIS gypsy moth strain and monitoring larval growth. One commercial diet was completely contaminated by mould, with all larvae failing to reach the pupal stage. It was confirmed that methyl

paraben had been omitted from the diet. Obviously this formulation was not acceptable and should not be marketed without a mould inhibitor. The other diets yielded pupae with differences as shown in Fig. 1. Long term comparisons would require evaluating results after several generations.

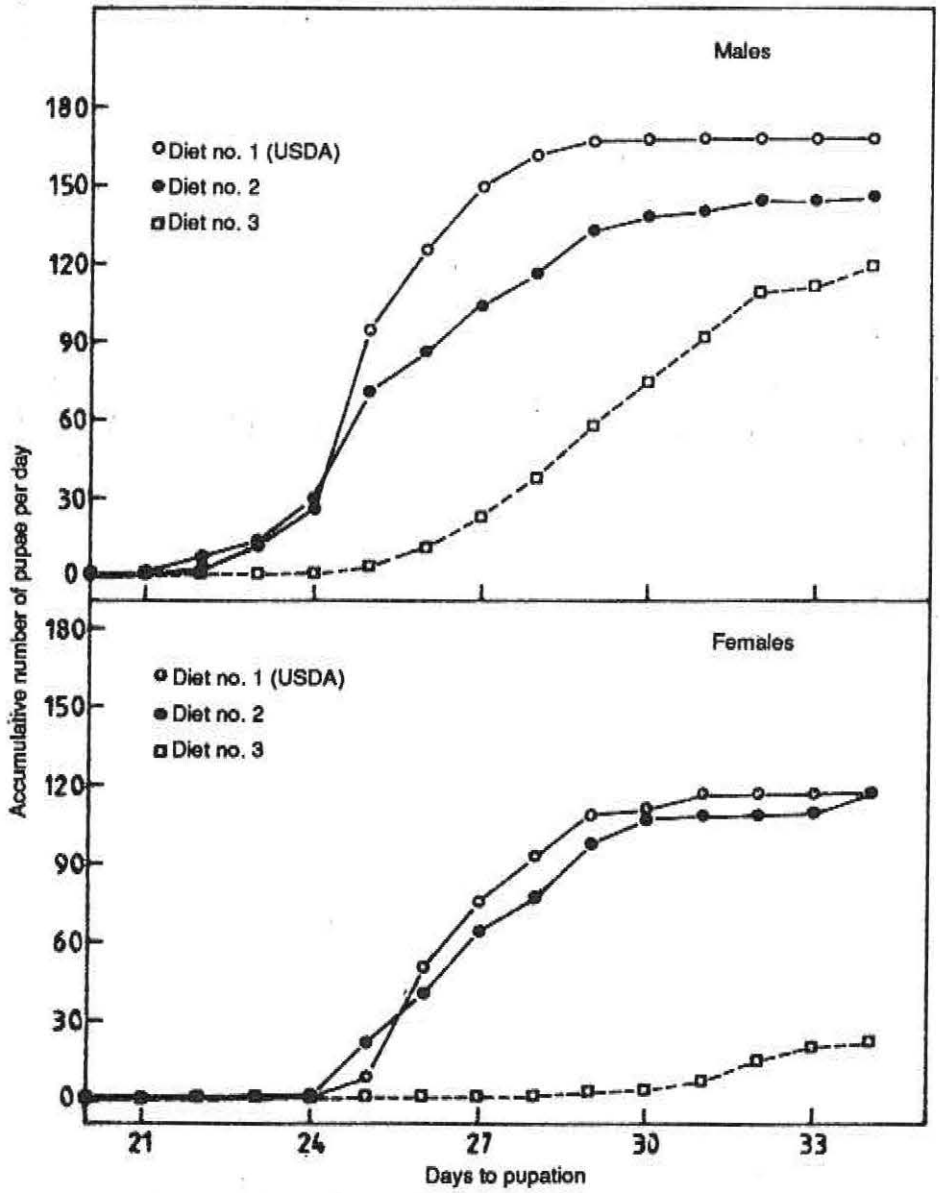


Fig. 1. Rate of gypsy moth pupation on three artificial diets

Selection versus Nutrition

A key component to a successful mass rearing programme is synchronised development. Synchrony of larval development permits a single, high yield collection of pupae. Laboratory colonies will naturally tend to become more synchronised over time, often as a result of accidental selection pressure. For example, when mass rearing the gypsy moth, slowly developing sixth instar female larvae are discarded when pupae are collected. As a result, sixth instar larvae are less common in laboratory colonies than in field populations or newly colonised strains (Fig. 2). Both strains were reared on the same diet, and in this

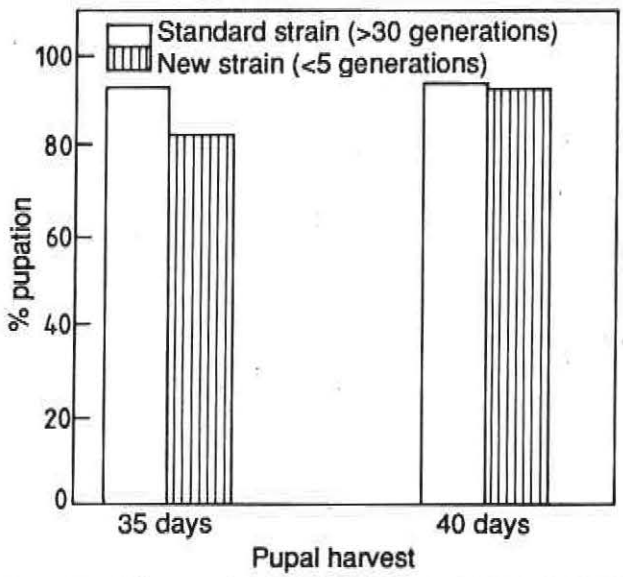


Fig. 2. Comparative time to pupation for two laboratory reared gypsy moth strains

example, resulted in laboratory adaptation. However, if the pupal collection is delayed until day 40, the yields from the two strains are more similar. Unfortunately, this is not feasible for routine rearing because adults begin to eclose after 35 days. Altering length of development also might affect competitiveness in the field.

Nutrition and Asynchronised Growth

Nutrition apparently can cause abnormal development in the larval stage, a major concern for insect mass rearing. This phenomenon was first noted in the gypsy moth when a laboratory colony was reared on an unnatural diet (Forbush and Fernald 1896). Larvae from the egg mass were reared individually on diet under

standard conditions. The rates of larval development were extremely varied, even when similar amounts of diet were eaten. This early report may have involved insufficient nutrients. However, even when an optimal diet is provided, the same pattern can occur.

In 1988, research programmes that depended on mass reared gypsy moths, including the sterile male technique and virus production, were almost stopped due to the reappearance of abnormal development (Fig. 3. modified from Tanner, unpublished data). This asynchronised larval development, referred to as "straggling" larvae (Tanner and Weeks 1980), may be part of an overall growth abnormality referred to as "Abnormal Performance Syndrome", or APS (ODell 1992). The problem can make the cost of mass rearing gypsy moths prohibitive. As a result, a massive research project was initiated in 1989 to identify the cause of APS.

Although straggling was eliminated in 1989 from the USDA, APHIS Otis

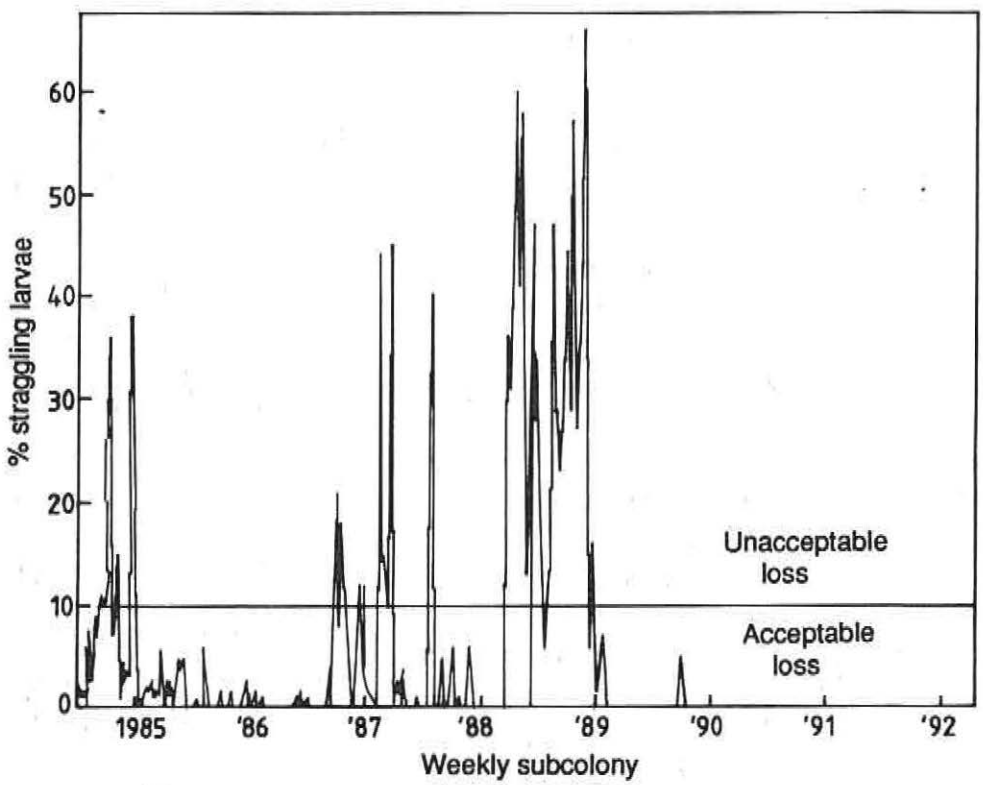


Fig. 3. Occurrence of delayed larval growth in mass reared gypsy moths

Methods Development Center, that produces most of mass reared gypsy moths in the United States (Fig. 3), the specific cause remains unknown. However, the problem continued to plague another gypsy moth rearing facility even though the same diet and strain were used. Some straggling is a natural occurrence, and a subjective threshold level of 10% is considered acceptable. This type of occurrence is not unusual in insect mass rearing; it is unpredictable, appearing and disappearing for no apparent reason.

Preliminary results of APS research indicates that nutrition may actually be involved in causing the problem, even when the same diet formulations are used to produce normal and abnormal colonies. The answer to this riddle may involve the quality of diet ingredients. Two of the facilities use different sources to obtain salt mix, one of the ingredients in the standard gypsy moth diet (Bell *et al.*, 1981). Apparently, one formulation of the salt mix is suboptimal, although the exact cause is still unknown. The concern does however again indicate a need to stress quality, and not necessarily quantity of insect nutrients.

Quality Control and Mass Rearing

Total quality control provides a means of optimising mass rearing systems and assuring that there is a dependable supply of competitive insects (Leppla and Fisher 1989, Fig. 4).

Figure 4 is a new organisational framework divided into eight generic elements: Management, Methods Development, Material, Production, Research, Utilisation, Personnel, and Quality Control. The elements and sub-elements are described as follows: **Management** determines policy, performs planning and administration and controls programme design as it evolves. **Research** is the source of new technologies and **Methods Development** creates new operational procedures. **Material** encompasses purchasing, establishing specifications and standards, assigning responsibility for quality materials, verifying compliance, and storage. The components of **Production** are facilities, equipment, operations, and production control. **Utilisation** is the treatment, handling and distribution of finished insect products. Management delegates responsibility for the selection, training, motivation and health and safety of employees to **Personnel**. **Quality Control** impinges on all programme elements with primary emphasis on process and product control. This element provides information management must have to optimise the entire pest management programme.

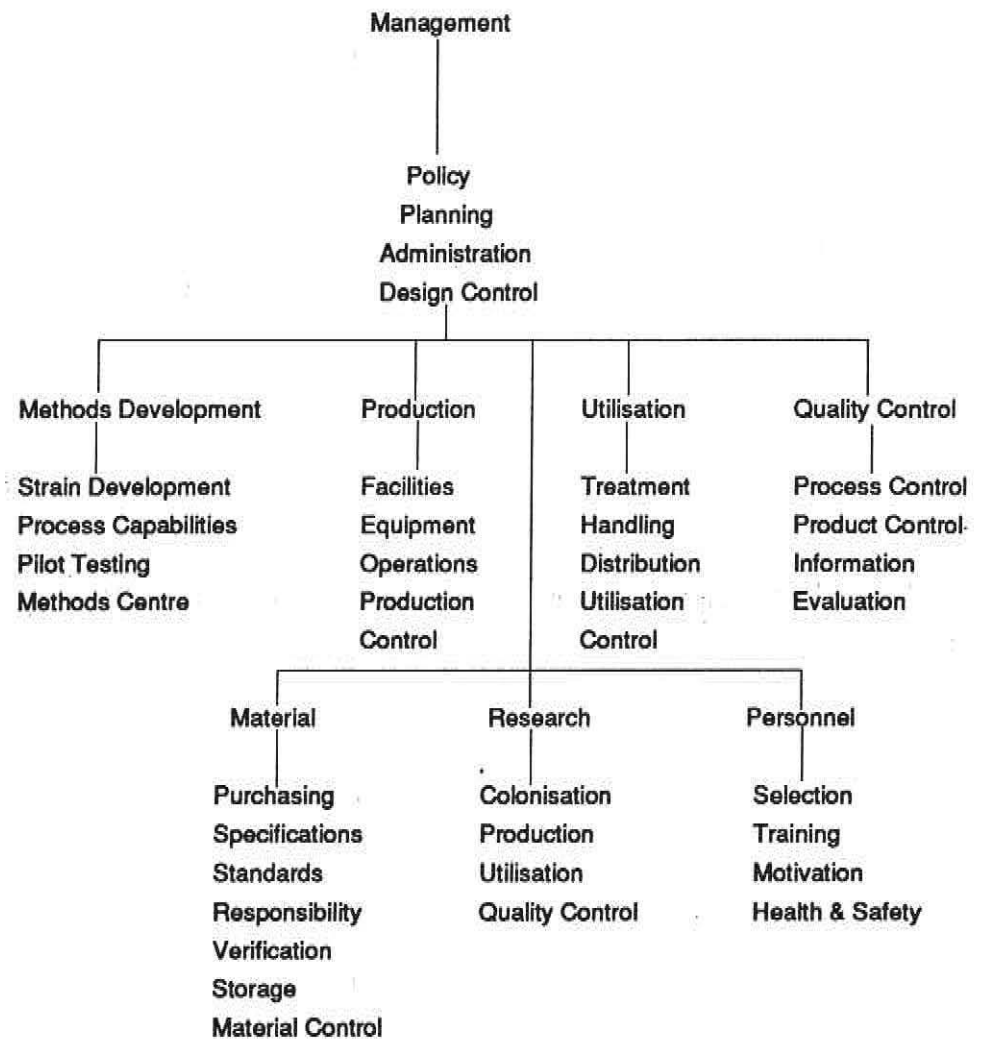


Fig. 4. Total Quality Control

It is essential that insects be mass reared according to their intended use and that they be collected and reared in a way that preserves their essential behavioural traits (Huettel 1976). These traits must operate in both the mass rearing facility and in the field because the insects must survive and perform in both environments. The insects must tolerate crowding and associated epizootics, and environmental variability caused by changing materials, equipment and techniques. Performance in the field depends on the ability to survive through delivery and release, recover and seek suitable habitats, and find and interact with wild-type populations.

A total quality control system extends from planning through production and field delivery to field evaluation that provides for quantitative assessment and optimisation of the associated insect suppression programme (Fig. 5).

Quality control procedures must be implemented and periodic on-site reviews performed to assure consistency. A quality control review team can perform the assessments and provide specific recommendations to improve support for insect control operations. They can also develop standardised tests, establish data acquisition and analysis capabilities, provide training, specify uniform protocols for preconditioning and bioassaying the insects, recommend the best available rearing and delivery procedures, improve field evaluation techniques and assure the use of quality control data in a decision support system.

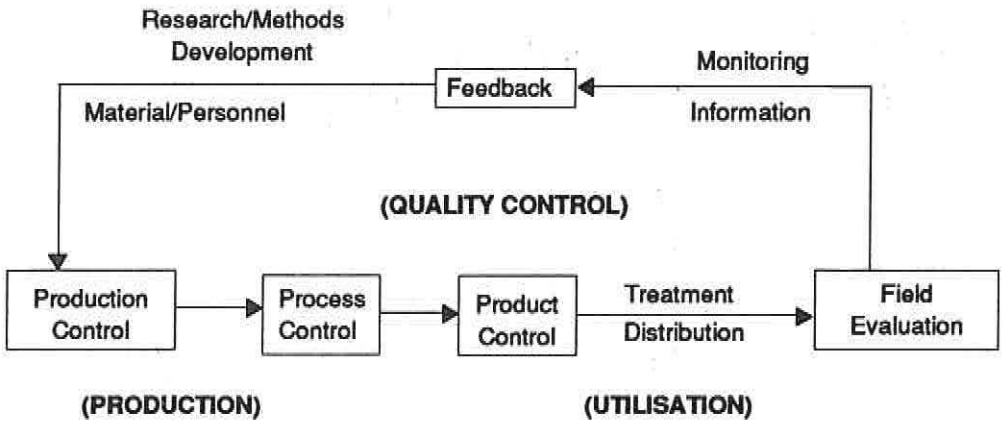


Fig. 5. Total quality control in an insect pest suppression system

An operational quality control manual, such as the one used by the USDA, APHIS (Brazzel *et al.* 1986), is required to assure continuity among the programme elements. The APHIS manual is designed to provide standard procedures for required quality control, packing, irradiation and shipping as well as specifications for Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), to be used for sterile insect release. The required tests are pupal size, percent emergence, flight ability, mating propensity, stress and sex ratio. Data are plotted on mean and range charts, and used to prepare a capability analysis for each of the quality control tests. A protocol should be added to operational manuals that specifies an efficient feedback system extending from field evaluation to the management factors that affect production and delivery of the flies (Webb 1984).

Conclusion

Virtually every nutritional problem encountered in the mass rearing of phytophagous insects has been caused by environmental rather than genetic changes. Dietary ingredients have oxidised during storage (fatty acids in wheatgerm and vitamins) and become contaminated with pesticides (wheat and corn). Improper preparation and formulation (vitamins, minerals, acid and water) are also common. These changes are difficult to avoid and may accumulate during many generations. However, total quality control and standardisation, as in industrial processes, can help assure that environmental conditions are maintained and competitive insects are produced. As insect mass rearing becomes more pervasive, the need for dependable production at the lowest possible cost will increase, and total quality control will be implemented.

References

Bell, R. A., C. D. Owens, M. Shapiro, and J. R. Tardif. 1981. Mass rearing and virus production, pp. 599–655. In: C. C. Doane (ed.), *The Gypsy Moth: Research Towards Integrated Pest Management. USDA Bull.* 1584. 757 pp.

Brazzel, J. R., C. O. Calkins, D. L. Chambers and D. B. Gates. 1986. Required quality control tests, specifications, and shipping procedures for laboratory produced mediterranean fruit flies for sterile insect control programs. U.S. Dept. Agric., Animal and Plant Health Inspection Service. APHIS 81–51. 28 pp.

Forbush, E.H. and C. H. Fernald. 1896. *The Gypsy Moth.* Wright Co. Boston. 95 pp.

Huettel, M. D. 1976. Monitoring the quality of laboratory-reared insects: A biological and behavioral perspective. *Environ. Entomol.* 5: 807–814.

Leppla, N. C. 1989. Laboratory colonization of fruit flies, pp. 91–103. In: A. S. Robinson and G. H. S. Hooper (eds.), *Fruitflies, Their Biology, Natural Enemies and Control.* Elsevier, Amsterdam. 447 pp.

Leppla, N. C. and T. R. Ashley. 1989. Quality control in insect mass production: A review and model. *Bull. Entomol. Soc. Amer.* Winter. pp. 33–44.

Leppla, N.C. and W. R. Fisher. 1989. Total quality control in insect mass production for insect pest management. *J. Appl. Entomol.* 108: 452–461.

ODell, T.M. 1992. Straggling in gypsy moth production strains: A problem analysis for developing research priorities. In: T. E. Anderson and N. C. Leppla (eds.), *Advances in Insect Rearing for Research and Pest Management*. Westview, Boulder, Colorado. (in press).

Reinecke, J. P. 1985. Nutrition: Artificial diets, pp. 391–419. In: G. A. Kerkut and L. I. Gilbert (eds.), *Comprehensive Insect Physiology. Vol. 4, Regulation: Digestion, Nutrition, Excretion.* Pergamon Press. 639 pp.

Rodriguez, J. G. 1973. *Insect and Mite Nutrition.* American Elsevier, New York. 717 pp.

Singh, P. 1977. *Artificial Diets for Insects, Mites, and Spiders.* Plenum Press, New York. 606 pp.

Singh, P. 1983. A general purpose laboratory diet mixture for rearing insects. *Insect Sci. Applic.* 4: 357–362.

Singh P. and R. F. Moore (eds.). 1985. *Handbook of Insect Rearing*, Vol. I & II. Elsevier, Amsterdam. pp. 488 and 514.

Tanner, J. A. and B. P. Weeks. 1980. Establishing Standards. USDA, APHIS Otis Methods Development Center, Annual Laboratory Report (unpublished).

Vanderzant, E. S. 1974. Development, significance, and application of artificial diets for insects. *Annu. Rev. Entomol.* 19: 139–160.

Webb, J.C. 1984. The closed-loop system of quality control in insect rearing, pp. 87–89. In: E. G. King and N. C. Leppla, (eds.) *Advances and Challenges in Insect Rearing.* Agric. Res. Serv., USDA, US Government Printing Office, Washington, DC. 306 pp.

Microbial Contamination in Insectaries: Contamination, Prevention and Diseases Identification

P.P. Sikorowski and A. M. Lawrence

Department of Entomology, Mississippi State University, Mississippi State, MS 39762 USA

Abstract

This chapter reports how microorganisms affect the rearing of insects and what measures are required to prevent it. One of the major problems encountered in rearing of insects is microbial contamination. The major sources of contamination in the rearing facilities are workers, unsterilised or partially (surface) sterilised eggs, and partially sterilised diet. The biochemical changes, produced by microbes alter the nutritional value of the diet. Microbial degradation of the diet usually reduces both the number of insects per unit diet and the quality of insects produced. Four ways of controlling microbial contamination are: (1) enforcement of sanitation programme; (2) egg surface sterilisation; (3) diet sterilisation and dispensing; and (4) air filtration. The most critical areas in rearing facilities are: (1) diet preparation and dispensing and (2) egg and larval planting room(s). Insect pathogens are common in newly colonised insects and must be eliminated from initial stock.

Introduction

Microbial contamination is harbouring of, or having contact with, microorganisms without a symbiotic or pathogenic relationship. Microbial contaminants are usually composed of seemingly innocuous microbes (Steinhaus and Martignoni 1970). Opportunistic bacteria are saprobes which are able to multiply rapidly in a diet or in a stressed insect and cause often severe or fatal diseases. Studies in

animal pathology have shown that contaminants or opportunistic microorganisms previously considered innocuous may multiply extensively in the tissues of weakened hosts and cause severe diseases (Neter 1974).

Out of over 1 million insect species, about 1,400 have been reared in the laboratory, and of these about 3 dozen are reared continuously (Singh and Moore 1985). The majority of insectary-reared phytophagous insects are reared on synthetic or semisynthetic insect diets. Such diets are usually subject to spoilage by many species of bacteria and fungi. Spoilage is the result of metabolic activity associated with microbial growth which causes diet degradation and the release of products of microbial digestion. The biochemical changes produced by microbes alter the nutritional value of the diets and consequently the insects being reared. In addition many bacteria and fungi produce toxins harmful to insects.

Humans may be affected by microbial contamination. Several airborne microorganisms such as *Aspergillus, Pseudomonas,* and *Streptococcus* spp., which grow on almost any organic matter, are also human pathogens and present some hazard to personnel in insect-rearing programmes (Sikorowski 1984).

The cost of rearing insects can be greatly reduced by establishing an environmental sanitation programme. Much information pertinent to laboratory rearing of insects has been obtained only recently, and most subjects covered in this chapter merit further study. However, research results to date suggest that sanitation will play an important part in rearing of most insects, and that more attention will have to be given to enforcement of basic sanitary measures to assure volume production of healthy insects.

Microorganisms of Healthy Insects

It is known that insects reared on artificial diets have a different microbial flora from those reared on plant or animal diets. It is also known that many other insects have no internal flora while in still others, the flora may change with the life stage or even the host.

Understanding the microflora of insectary-reared insects is based on a knowledge of the normal flora of feral insects. The most commonly occurring, internally harboured microorganisms (symbionts) in insects are bacteria or bacteria-like forms that are found in Blattodea, Isoptera, Homoptera, Hemiptera,

Phthiraptera, Coleoptera, Hymenoptera, and Diptera. Flagellates are found in wood-feeding insects and yeast and yeast-like organisms in Homoptera and Coleoptera (Steinhaus 1949 and Chapman 1971). Steinhaus (1941) studied the bacterial flora of 30 species of insects and isolated 83 strains of bacteria, 2 strains of yeasts, and 2 fungi from the insects. In most cases, the bacterial species found in specimens of any given insect species were surprisingly constant. A study of the internal microbial flora of 2,016 unfed specimens of the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, showed that only 1.6% of the adults harboured bacteria (Steinhaus 1942). Over 75% of the field-collected adult boll weevils, *Anthonomus grandis grandis* Boheman, of various ages examined by Sikorowski (1984) had 100 or fewer bacteria per insect. Antibacterial constituents found in cotton plants (such as gossypol, caryophyllene, gallic acid, and tannins) are responsible for the low bacterial content of wild weevils (Hedin *et al.* 1978). Greenberg (1962) reported that 73% of house flies, *Musca domestica* L., and 54% of stable flies, *Stomoxys calcitrans* (L.), are germ free at emergence from the puparium. He concluded that metamorphosis tends to sterilise the digestive tract.

Insects that feed on diets deficient in certain elements (for example, wood; deficient in nitrogen and vitamins: and wool, feathers, hair deficient in vitamins) usually have microorganisms associated with them. This suggests that the microorganisms supplement the diet deficiencies. But, in many cases, the precise nature of this association is not known (Chapman 1971; for reviews of the literature about microorganisms of healthy insects, see Brooks 1963).

Microbial Contamination and Rearing Environment

The level of sanitation maintained in the insectary has a direct influence on diet contamination and its resulting acceptability as a source of nutrients. Sanitation is very important where diets with a low level of antimicrobials are used.

A. Sources of Contamination

1. **Diets.** Shapiro (1984) and others isolated microbial contaminants from wheat germ, casein, torula yeast, and tap water. It is well known that various components of artificial diets contain numerous bacteria and fungi, and that these diets provide the nutrients essential for growth not only for insects, but also various microorganisms, including some human pathogens (McLaughlin and Sikorowski 1978).

2. **Eggs.** Insect eggs are another source of microbial contamination which may affect all insect diets. Egg surfaces must be decontaminated or the neonates will contaminate larval diet.

3. **Air.** Laboratory air serves as a vehicle for many species of bacteria, fungi, viruses, protozoa, and ricketsia. The fact that microorganisms are ever present is of particular concern in insect rearing, particularly if insect diets are not prepared under laminar airflow cabinets or in a laminar airflow room.

4. **Personnel.** Humans are also a very important source of microbial contamination, and the level of contamination relates directly to activity and density of personnel (Favero *et al.* 1966, 1968; Runkle and Phillips 1969). Even the healthy human body harbours millions of microorganisms on the skin, in the mouth, respiratory tract, genitourinary tract, and intestines (Fig. 1). Jawetz et al. (1978) arranged the normal microbial flora of the human body into two groups: (1) the resident flora consisting of fixed types of microorganisms regularly found in a given area and on persons of a given age; and (2) the transient flora consisting of microorganisms that inhabit the skin and mucous membranes for hours, days or weeks. The latter are environmentally derived, do not produce disease, and do not establish themselves permanently on the surface. Jawetz *et al.* (1978) reported that constant exposure to, and contact with the environment results in skin contamination with transient microorganisms.

Smith and Bruch (1969) monitored healthy individuals who exercised naked for 30 minutes and found that each dispersed 2–6 million viable microorganisms into the air. Sikorowski (1975) showed that freshly laundered and sterilised cotton uniforms do not provide an efficient barrier for movement of bacteria through the cloth into the environment (Fig. 2). The hair of 50 individuals (1 hair per person) possessed from several to many bacteria per hair (Fig. 3). Cultures from hands almost always produced bacterial colonies even shortly after normal washing (Fig. 4). The presence of bacteria in the breath is illustrated in Fig. 5.

In conclusion: The number of workers should be kept at a minimum in critical areas (diet dispensing and egg or larval planting rooms). Properly cared for equipment, instruments, walls, floors, etc. are minor sources of microbial

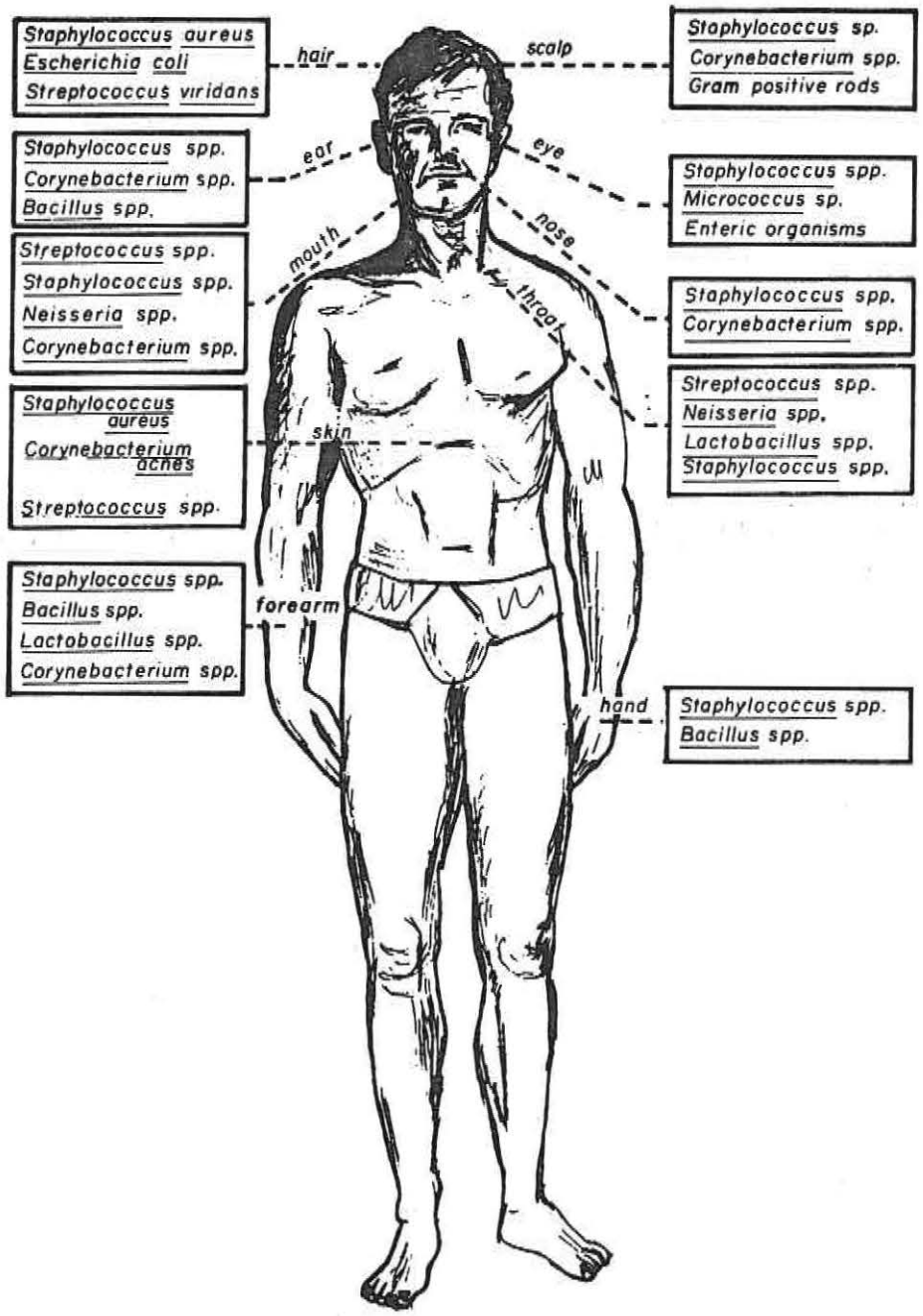


Fig. 1. Prevalent microbial flora of man (based on National Aeronautics and Space Administration 1969)

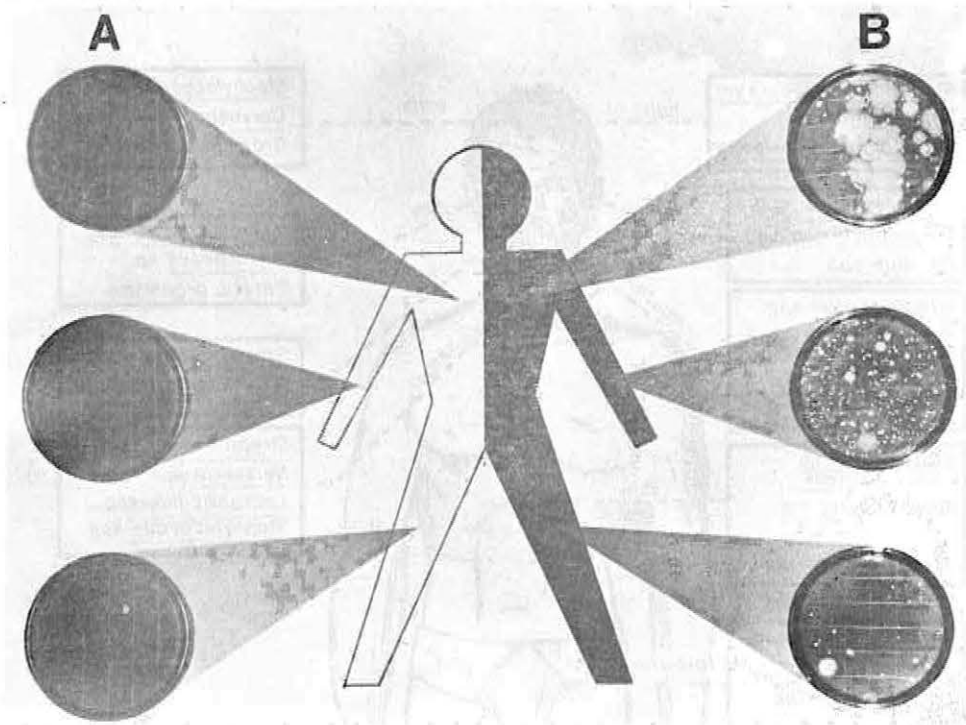


Fig. 2. Penetration of bacteria through cloth into the environment. (A) freshly laundered uniform; (B) uniform worn for 4 hours. (From Sikorowski 1984)

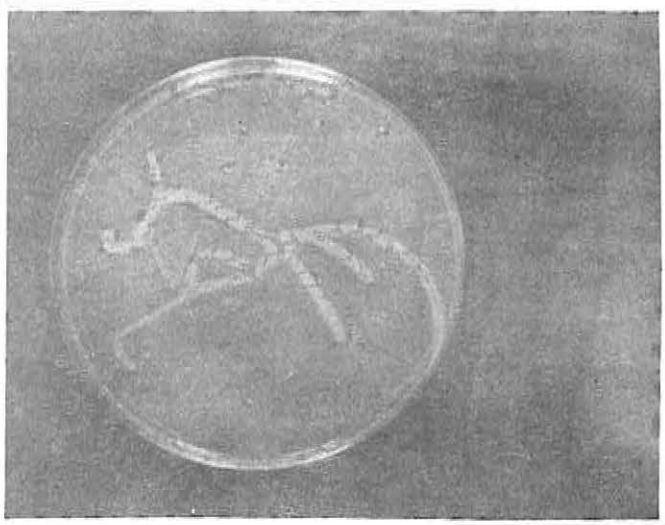


Fig. 3. Human hair (unsterilised) bacterial colonies on Trypticase soy agar plates incubated for 48 hours at 36 ± 1°C

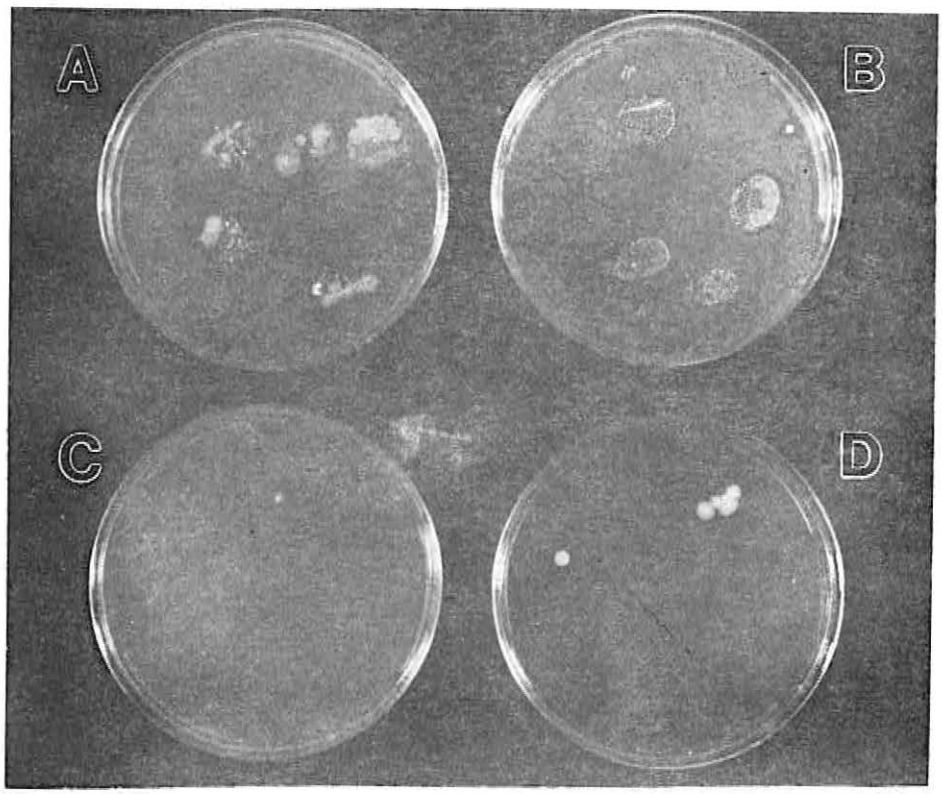


Fig. 4. Microbes on hands. (A) bacterial colonies from finger prints of unwashed hands; (B) washed with soap; (C) washed with 0.5% sodium hypochlorite; (D) washed with 5% Mikro-quat^{R} (quaternary ammonium compound)

contamination. The microbial content of the air in a room usually reflects the total microbial contamination of this space (Loughhead and Moffett 1971).

B. Effects of Microbial Contamination

The more lethal microbial contaminants are relatively easy to determine and eliminate from insectaries. However the enzootic or debilitating diseases are normally more of a problem since they often escape discovery (Goodwin 1984).

1. **Viruses.** Oral infection is the most common mode of virus acquisition by insects. Viruses are frequently introduced to the laboratory insects when undiagnosed field-collected insects are introduced into an established

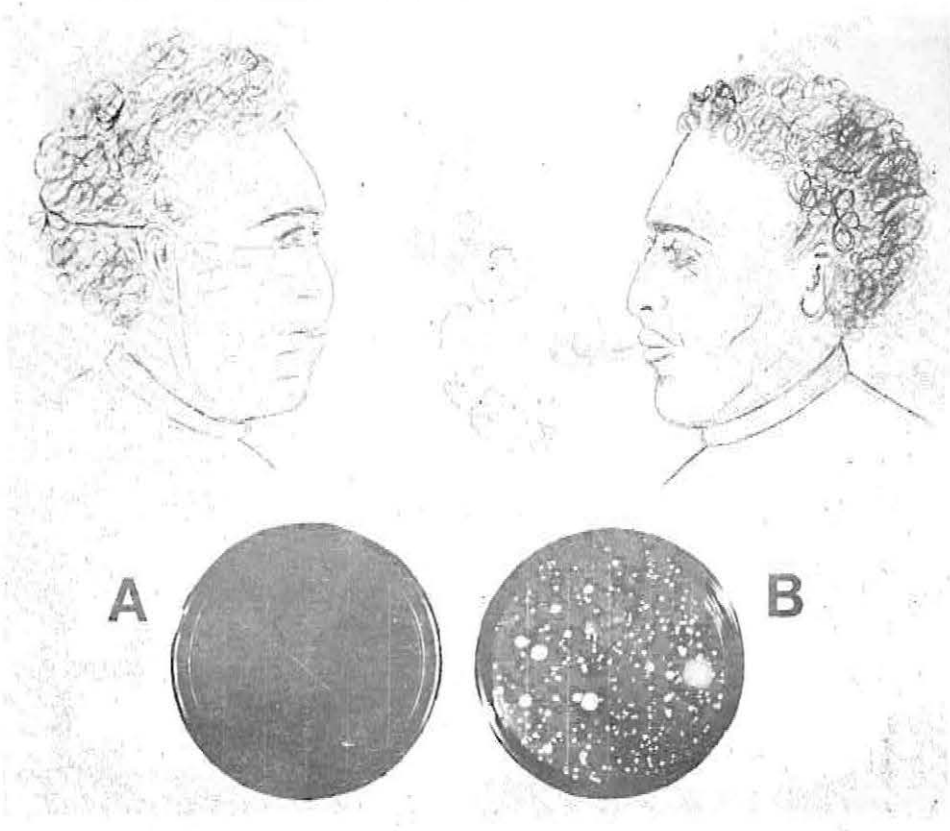


Fig. 5. The effects of a face mask on spread of oral microorganisms. (A) bacterial counts with masks; (B) without masks

healthy colony. Many insect viruses are highly infectious and drastic sanitary measures (such as disinfecting surfaces with antiviral compounds, (for example; 5% sodium hypochlorite) may be required to eliminate them. There are four major types of insect viral diseases encountered in rearing insects (3 inclusion-type and less common non-inclusion-type diseases). Inclusion-type diseases are caused by cytoplasmic polyhedrosis viruses, nuclear polyhedrosis viruses and entomopox viruses.

Cytoplasmic polyhedrosis viruses (CPVs) produce chronic diseases which cause structural damage to the midgut epithelium. Effects of CPVs on insects have been investigated by numerous workers and their findings can be summarised as follows: low larval mortality, larval stage infection producing smaller than normal pupae and moths, reduction in pupation rate and adult emergence,

frequent pupal and moth deformities, and impaired female insect reproduction. CPVs are highly infectious and can persist undetected in insect populations. Egg surface contamination is a major means of transmission of CPVs to the next generation.

Nuclear polyhedrosis viruses (NPVs) in insect cultures are manifested by high larval mortality in the insect. There are over 300 recorded isolates of NPV from Lepidoptera, Hymenoptera, Diptera, Coleoptera, Crustacea and Arachnida. Insect cultures with high percentages of virus infection can be decimated.

Entomopoxviruses (EPVs) cause high mortality and prolonged developmental times in insect cultures. In lepidopterans, death is often preceded by abdominal paralysis and regurgitation or defecation of fluid containing inclusion bodies. In the Lepidoptera and Orthoptera, virus replication usually takes 10–30 days. In Scarabaeidae (Coleoptera), where the host life cycle may take 1–2 years, virus replication may take 5–10 months or longer (Goodwin 1984).

In non-inclusion type diseases inclusions are absent and the virus particles occur freely in the tissues. This group includes iridescent viruses, parvoviruses, picornaviruses, and several viruses affecting the honey bee and fruit fly (*Drosophila*) species. This group of viruses are not discussed in this chapter since they require the use of electron microscopy for detection and are not frequently found in insectaries.

2. **Protozoa.** The protozoa are a diverse and heterogenous group of one-celled organisms. Their relationships with insects range from commensalistic to pathogenic. Most protozoans associated with insects produce chronic infections characterised by general host debilitation and nonspecific signs and symptoms. Microsporidia are perhaps the best known group of protozoa associated with almost all species of colonised insects. Most members of this group produce chronic infections characterised by delayed larval development, body colour changes, incomplete metamorphosis, deformed pupae and adults, and lowered adult fecundity and longevity.

3. **Bacteria.** The impact of bacteria on rearing of insects is the most common and perhaps most difficult contamination to cope with. Effects of bacterial contamination on boll weevil development have been reviewed by

Sikorowski (1984) and are summarised as follows: bacterially contaminated boll weevils differed from noncontaminated individuals in amino acid and fatty acid content, pheromone production, digestive system histopathology, haemolymph chemistry, and insecticide susceptibility. The numerous effects of contamination were reflected in the increased time required for adult emergence and the fewer adults produced per petri dish of diet.

Boll weevils and their diet are modified by excretory products (including toxins) of the bacteria (Thompson and Sikorowski 1978; Wyigul and Sikorowski 1986; and Thompson and Sikorowski 1988). Modification of the diet by bacteria may vary not only with bacterial species, but also between strains of the same species. The impact of a bacterially modified diet on the larvae varies also with the age of larvae, since young larvae are normally more susceptible than older larvae to bacterial contaminants. Bacterial contamination of the host's parasites also has a devastating effect on parasite production (Sikorowski *et al.* 1992).

4. **Fungi.** Many fungi associated with insect rearing are frequently parasitic or saphrophytic opportunists, rather than primary pathogens. Members of the genus *Aspergillus* and yeasts frequently contaminate insect diets. Effects of *Aspergillus*-contaminated media vary with the developmental stage of the insect. Early contamination of diet with *Aspergillus* may result in few or no adults. Late contaminations have little, if any, effect on numbers of insects produced. Yeasts may occasionally infect breeding colonies, causing reduction in fecundity and high mortality.

Prevention of Microbial Contamination

Rearing facility contamination may have a devastating influence on the entire rearing programme. Contamination can increase cost of rearing insects, cause high mortality of all stages, increase work load, lower the quality of insects, and cause loss of confidence in work. Although use of sanitation measures may be expensive, the lack of sanitation can be even more expensive. Employees must be trained properly in use of equipment, sanitary procedures, and personal hygiene. Many people without basic training or experience in sanitation microbiology have difficulty realising that microbes can be present on apparently clean hands, garments, or equipment.

A. Training of Insectary Personnel

Personnel must understand rearing procedures and be aware that their own potential for contaminating the area is high. The training should include a demonstration of the presence of microbes on unsanitised floors (Fig. 6), clothing, shoes, hair, hands, and in the breath (Figs. 2, 3, 4, and 5). Monitoring techniques should be used to show that a chosen sanitary measure can reduce microbial contamination to safe levels. A series of exercises designed to show the presence of microbes on clothing, shoes, hair etc. used in the Robert T. Gast Rearing Laboratory are given by Sikorowski (1984).

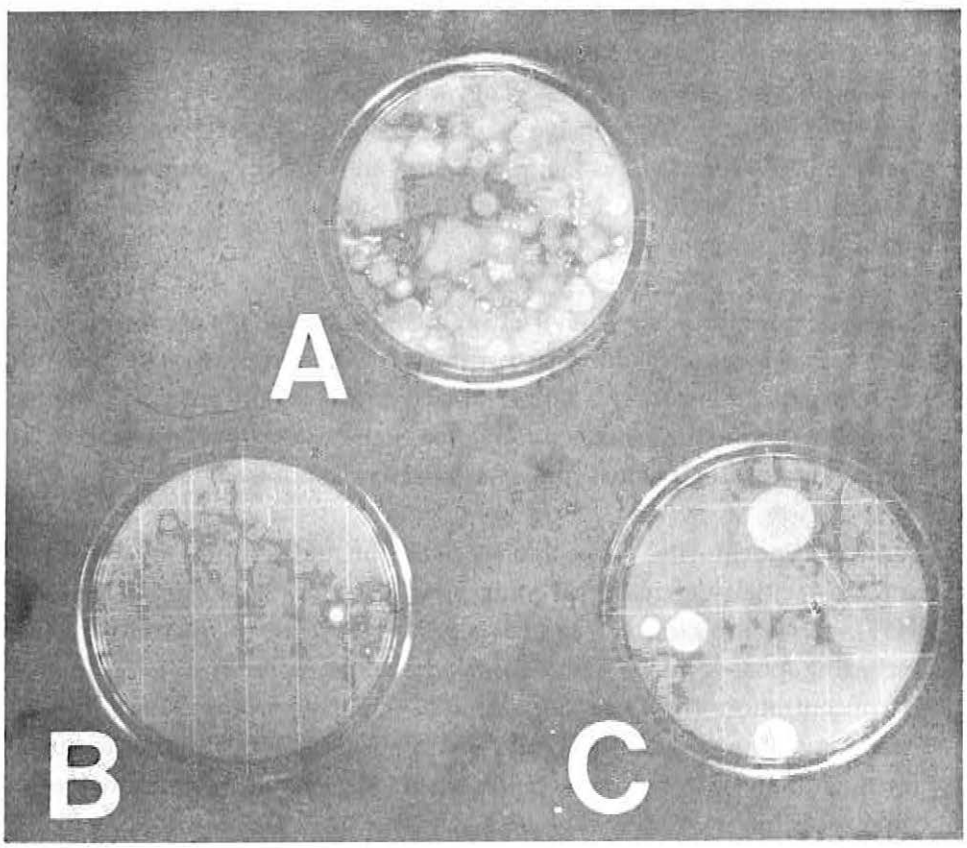


Fig. 6. Microbes on floor. (A) unsanitised; (B) sanitised with 0.5% sodium hypochlorite; (C) sanitised with Mikro-quat® (quaternary ammonium compound)

B. Antimicrobial Compounds

1. **Iodophors.** Iodophors are formulations consisting of iodine and a carrier. Their extended activity is based on the slow release of iodine. Iodine is effective against a wide variety of microorganisms; it is lethal to viruses, bacteria (including spore-formers), fungi, protozoa, and algae. Trueman (1971) reported that iodine is less sporicidal than hypochlorite.

2. **Quaternary ammonium compounds.** The bactericidal quality of the quaternaries is high against Gram-positive bacteria, but they are less effective against Gram-negative bacteria. They are not sporicidal, but they also have bacteriostatic characteristics far beyond their bactericidal concentration. They are also fungicidal for common insectary fungi. Other features include low mammalian toxicity, low corrosiveness, and combined germicidal and detergent properties. They are stable, odourless, nonstaining, water soluble, and inexpensive in comparison to other chemicals. (See Petrocci 1977 for reviews of quaternary ammonium compounds and Martignoni and Milstead 1960 for discussion of their use in surface sterilisation of insect eggs.)

3. **Phenolic compounds.** Phenol in high concentrations is a protoplasmic poison that penetrates and disrupts the cell wall and precipitates the cell proteins. At lower concentrations, it inactivates essential enzyme systems. Phenolic compounds are effective against vegetative cells of bacteria and, at high temperatures, against fungal and bacterial spores.

4. **Sodium hypochlorite.** Hypochlorites (calcium and sodium) are the oldest and most widely used chlorine compounds used for disinfection (Dychdala 1977). They are effective against a wide spectrum of microorganisms, including spore-formers. The sodium hypochlorite solutions are formulated in concentrations from 1 to 15% with 1–5% chlorine products used domestically and stronger solutions industrially. Today, hypochlorites are used for microbial control in households, hospitals, schools, restaurants, food-processing plants, dairies, canneries, wineries, and beverage bottling plants (Dychdala 1977). Hypochlorites are also used extensively as disinfectants in rearing facilities. Sodium hypochlorite solution is available in most grocery stores as laundry bleach (in the USA, Clorox and Purex).

5. **Formalin.** Formalin is commonly a 37–40% solution of formaldehyde in water. This antimicrobial compound is sporicidal, bactericidal, and, at lower concentrations, bacteriostatic. Formalin contains certain stabilisers, 8–15% methanol being the one most commonly used, to prevent the formation of solid polymers if the solution is chilled or is kept for a long time (Phillips 1977). Formalin is most effective at high relative humidity. It penetrates very slowly; therefore, its application should be limited to surface sterilisation. Although formalin has been widely used for at least 80 years, it should be used only when no other method is available. (For more information about formalin, see Hoffman 1971 and Trujillo and David 1972.) A New York University study provided decisive confirmation of industry findings that formaldehyde is an animal carcinogen (Sun 1981).

6. **Soaps.** Many soaps have bacteriostatic or bactericidal properties. Germicidal soaps can be ordinary toilet soaps with various antimicrobial agents added to a maximum concentration of 2%. Phenol derivatives, especially creosols and bi-phenols are often mixed with soaps. (For more information on soaps, see Frobisher *et al.* 1969 and Hamilton 1971).

C. Insectary Sanitation

The key purpose of an insectary sanitation programme is to eliminate contamination that interferes with the rearing of healthy insects. The degree of cleanliness required for each room in the rearing facility should be established so that the needed technologies can be determined (even better before the insectary is built). Most of the sanitary regimens published for various insects (Steinhaus 1953; Sikorowski 1975; Davis 1976; and Martignoni and Iwai 1977) include personal hygiene, maintenance of a clean and sanitary environment, and various methods of sterilising and sanitising insectary equipment and environment.

1. **Personal hygiene.** Many skin microorganisms cannot be satisfactorily washed off the skin surface since they replicate in subsurface areas. The number of microorganisms present on, and under the skin of individuals varies widely, but in general, people with high bacterial skin contents can be expected to shed microorganisms into the environment. The average individual with a normal skin flora will shed approximately 10,000 viable particles (mainly Gram-positive cocci and diptheroids) each minute (Phillips

and Runkle 1973). Microorganisms are shed with the human skin scales to which they are attached. Showering creates about a five-fold increase in the rate of shedding of microorganisms, thus decreasing microbes on the skin surface. However, the normal flora returns in one or two hours after showering.

Special clean room clothing is now available and may be used in critical areas. The major purpose of such uniforms is to limit the passage of skin microbes into the environment (Fig. 2).

In conclusion: The personal hygiene for insectaries should include:

1. Wearing of protective outer clothing.
2. Wearing of hair caps and face masks by workers handling diet and antimicrobial agents.
3. Take a shower in the insectary if possible.

2. **Laboratory cleaning.** Litsky and Litsky (1968) and many other workers in the field of microbial contamination have demonstrated that flooding and wet-vacuuming is the most effective method for cleaning and disinfecting floors. Portable vacuum cleaners may be used only if the vacuum cleaner exhaust is filtered to the same extent that the air is filtered through the air-filtration system in the room. Because sweeping and dry-mopping stir up microorganisms, they should be avoided (Nagasawa *et al.* 1970). Runkle and Phillips (1969) recommended elimination of sweeping through the use of dry or wet pickup vacuum cleaners with high-efficiency exhaust air filters. General lab cleaning routines are given by Sikorowski (1975), Davis (1976) and Martignoni and Iwai (1977).

   In conclusion: Wet-mopping with a good antimicrobial compound at least once a day (evening) or twice a day (evening and morning) is recommended.

3. **Sterilisation and sanitisation of equipment.** Sterilisation means the complete destruction of microorganisms. Wet steam under pressure (121°C at 103.5 kPa) or dry heat (360–380°C) are the most reliable and universally acceptable methods of sterilisation and should be the method of choice wherever possible. All living microorganisms can be rapidly destroyed in the presence of steam under pressure.

The most effective antibacterial wavelength of ultraviolet light is between 240 nm and 280 nm; the optimum is about 260 nm. Ultraviolet light kills bacteria because it is absorbed by them and damages their DNA (Willett 1976). However, ultraviolet light has poor penetrability; it does not penetrate solids, and it penetrates liquids only slightly. Microorganisms are protected by dust in the air and by dust on ultraviolet light bulbs, thus clumping of microbial cells to dust allows those in the central part of the clump to escape the effect of ultraviolet light.

Large equipment that is permanently attached to walls is sanitised usually with chlorinated cleaners, iodophors and quaternary ammonium compounds. Small instruments that cannot be autoclaved may be sanitised by immersion in sanitising solution.

In conclusion: Wherever possible, steam under pressure is recommended for sterilisation, while for large equipment, chlorine compounds or quaternary ammonium compounds are widely used.

4. **Air disinfection and air filtration.** Air filtration is one of the most important tools to control microbial contaminations. Filtration is usually recommended when maximum removal of bacterial particles from the air is required. Particles carrying bacteria are usually greater than 4 μm in diameter, with a median of 10–20 μm. Single bacterial spores free of dust carriers are uncommon. HEPA (high-efficiency particulate air) filters remove 99.97% of particles that are 0.3 μm in diameter and larger. Thus laminar airflow prevents microbial accumulation in a room atmosphere because the air content in the room is usually changed several times in an hour. Laminar-air flow (LAF) devices can be classified as follows: horizontal, vertical, and curvilinear-airflow units. In the R.T. Gast Rearing Facility, air in the egg-implanting area has been filtered with considerable success through HEPA filters having a 0.3 μm pore size (Sikorowski 1975).

In conclusion: After several years' experience in the use of laminated airflow, we find that this method is one of the most useful tools in controlling microbial contaminants. In our opinion it is required only in diet dispensing and larval planting or egg-implanting rooms. J. Roberson (Entomologist in charge of one of the largest mass rearing facilities in the USA) is of the opinion that LAF is the most efficient and "in the long run" least expensive method for controlling microbial contamination.

D. Egg Surface Sterilisation

Many contaminants and pathogens are transmitted from one generation to the next on the egg surface. For this reason surface sterilisation of insect eggs is implemented routinely in most insectaries. While many different germicides have been used for this purpose formaldehyde and sodium hypochlorite are the agents most often used.

1. **Formaldehyde.** Formaldehyde is a carcinogen and must be used only under a fume hood. At high concentrations it destroys all microorganisms including spore-formers and viruses. Different workers use different formaldehyde concentrations in sterilising solutions, but 8–10% formaldehyde has been used most frequently. We recommend 10% freshly prepared formaldehyde solution for sterilisation of boll weevil eggs (Sikorowski *et al.* 1977). The eggs should be sterilised in this solution for 20 minutes, and agitated during this time on a shaker. After sterilisation the eggs must be rinsed several times (10 minutes each) with sterile water.

2. **Sodium hypochlorite.** This compound is a strong oxidizing agent and will inactivate protein; thus it is lethal to all microorganisms including spore-formers. Concentrations of sodium hypochlorite used for egg surface sterilisation vary greatly for different insects thus the optimum concentration of sodium hypochlorite must be determined experimentally for the eggs of each newly colonised insect species.

In conclusion: Both methods produce good results when used properly. High concentrations of sodium hypochlorite will destroy the insect's egg shell — the thickness of the egg shell varies with the insect species.

E. Diet Sterilisation

Diet sterilisation is particularly important for diets with low levels of chemical antimicrobials. The most popular method of sterilisation for a small to medium size insectary is a combination of heat and chemical sterilisation; for a large insectary flash sterilisation.

The combination of heat (about 80°C) and antimicrobials (sorbic acid and methyl p-hydroxybenzoate) in *Helicoverpa* diet is widely used, but produces diet

only partially free of contaminants. The diet is heated to about 80°C for 5–10 minutes to reduce the number of microbes present in unsterilised diet. Antimicrobials such as parabens, sorbates, chlorotetracycline etc., are added before or after heating of the diet.

Flash sterilisation is the best and most expensive method of diet sterilisation. Flash sterilisers operate on the principle that a thin layer of medium moves rapidly through a heat exchanger maintained at a high (145–160°C) temperature. This method is used in large insectaries.

In conclusion: Flash-sterilisation of the diet is the best method for production of microbial-free diet, but original investment may be costly. Diets in the rearing containers are frequently overlaid with sterile corn cob grit mixed with 0.04% (w/w) Phaltan^{R} —and 0.03% (w/w) tetracycline hydrochloride to shield it from microbial contamination.

5. Monitoring Contamination

The monitoring programme evaluates the effectiveness of the contamination control programme. Such programmes usually include microbiological sampling of air, diets, eggs, equipment, walls, floors, surfaces, and the adult insects (the final product).

A. Microbial Content of Air

Air microbial content can be determined by various methods ranging from sedimentation to computerised multichannel particle counters. Air sampling by sedimentation allows the suspended particles to settle on plain surfaces or on nutrient agar surfaces. This permits determination of the number of viable particles or organisms and the size of all particles that settle in a given time. Advantages of sedimentation samplers include: low cost, simplicity, and collection of particles in their original state. Disadvantages include: inability to give quantitative counts unless the aerosol sample settles in a closed container, a long waiting period for settling of smaller particles, and some viability loss in the settling process. Petri dishes containing trypticase soy agar or brain heart infusion agar are placed in predetermined locations. Covers from the petri dishes are removed for desired periods of time, (usually 2–12 hours), replaced, and half the dishes are incubated for 24–48 hours at 36 ± 1°C

for bacteria. The remaining dishes are left at room temperature for 7 days for fungi. All dishes are examined and the number of colonies counted with a stereomicroscope (100x magnification).

In conclusion: A sedimentation method is perhaps sensitive enough for monitoring microbial content in a small to medium size rearing facility.

B. Sterility of Diet

Samples of about 15–20 ml of diet are placed aseptically in sterile empty petri dishes at predetermined time intervals during diet dispensing into rearing containers. The dishes are incubated and examined as with the sedimentation tests.

In conclusion: A dish with one or more colonies of bacteria or fungi is considered an indication of significant diet contamination.

C. Sterility of Eggs

Samples of eggs are implanted in petri dishes with agar and incubated and examined as with the sedimentation test.

In conclusion: One or more colonies of bacteria or fungi per dish is considered an indication of egg contamination.

D. Microorganisms on Work Surfaces

The methods used for detection of bacteria and fungi on surfaces are the swab-rinse method and the agar contact method. A sterile cotton swab is rubbed over the surface of the object to be sampled. The tip of the swab is broken into a tube of sterile dilutant and shaken, and the rinse fluid is plated on appropriate culture medium. Care is taken to avoid contaminating the swab. The major disadvantages of this method are that there often is poor recovery of bacteria or fungi from the surfaces sampled, that results are not reproducible between different workers or laboratories, and that cotton retains microorganisms (Patterson 1971).

The agar contact method employs the Rodac plate, a modified petri dish that contains a raised nutrient agar bed of trypticase soy agar with lecithin and polysorbate 80 medium. The agar is placed in contact with the tested surface, and samples are incubated as given for the sedimentation tests.

In conclusion: Rodac plate method produces more reproducible results than swab method.

6. Disease Identification and Insect Examination

The information given in this section is very fragmentary and final determination of the cause of a disease should be made by a person trained in insect pathology. It is important to remember that the presence of a bacterium or fungus in a dead insect does not necessarily mean that it was the cause of mortality. Saprophytic bacteria and fungi are fast multipliers and quickly colonise insect cadavers. Bacteria, fungi and protozoa are most frequently recorded as causative agents of insectary diseases.

A. Bacteria

In the insectary environment where artifical diets are made, many bacteria may colonise the diet, and subsequently the insect's alimentary canal, resulting in continuous insect losses. Some bacteria are frequently present in small numbers of many healthy insects, but they are able to multiply and cause disease when an insect's vigour is greatly reduced.

Diagnostic characteristics of insects infected with bacteria vary greatly. For example, insects infected with red strains of *Serratia marcescens* are usually pinkish-red, but white-gray when infected with a nonpigmented strain. Infections by *Pseudomonas aeruginosa* turns the insect body black at the time of death but, insects infected with *P. flourescens* become gray and remain gray for several days.

In conclusion: Definitive symptoms associated with bacterial infections rarely occur. The number of contaminant cells per insect, rather than its identity may be more important and this can be determined in the following way:

A sample of adults is frozen (-20°C) for 15–20 minutes, surface-sterilised for 5 minutes with 0.5% sodium hypochlorite solution, and washed in 2 changes of sterile water (5 minutes each). Each adult insect is blended in 40 ml sterile water for 30 seconds. Forty ml of the homogenate is mixed with 30 ml of melted double strength agar (45°C) and poured into sterile petri dishes. After incubation, the colonies are counted. This method was used to evaluate the contamination of mass reared boll weevils (Sikorowski 1984) and adult *Microplitis croceipes* (Sikorowski *et al.* 1992).

B. Protozoa (Microsporidia)

Most protozoan infections produce no characteristic symptoms. Insects that are transparent (for example mosquito larvae) are often wholly or partly milky white because of spore masses packed into the infected tissues such as hypodermis, fat body etc. Infected insects may manifest symptoms such as sluggishness, have uncoordinated movement, morphological deformities, difficulty in moulting, loss of balance, appear leathery, and have white fecal exudate. However, most are general symptoms which also occur in insects infected with a variety of insect pathogens. For detection of microsporidia in adult weevils we use the following method:

A sample of 25 adult weevils is blended in 25 ml of water. The suspension is then filtered through cheesecloth and centrifuged at 3,000 RPM for 10 minutes. The resulting pellet is resuspended in a small quantity of water, smeared on slides, and examined under a phase-contrast microscope. (For more information about diagnostic techniques used in identification of insect pathogenic protozoa see Brooks 1985).

C. Viruses

According to Sikorowski (1984), if a virus disease is suspected, the host tissue is examined with a light or electron microscope as required. Insect viruses can be divided into two broad groups: occluded viruses (nuclear and cytoplasmic polyhedroses and the insect pox diseases); and free viruses, which are not embedded in a crystalline matrix. Inclusion bodies of the occluded viruses can be easily detected with light or phase-contrast microscopes. For detection of nonoccluded viruses, an electron microscope must be used. However, an

infectivity test would be a more general and probably less expensive method for detection of nonoccluded viruses.

D. Fungi

If a fungus contamination is suspected, the methods given by Poinar and Thomas (1984) or Goodwin (1984) will be helpful to place it in a proper group.

7. Conclusions

The contaminants encountered in insectaries are usually microbes that are normally innocuous. However, it is well-documented in the literature that innocuous microorganisms can multiply extensively in the tissues of weakend hosts or on favourable diets and so cause disease that may be severe or even fatal. Insect diets are frequently complex and subject to spoilage by many species of bacteria and fungi. The biochemical changes produced by microbes alter the nutritional value of the diet. Microbial contaminants may have multiple effects on insects, depending on species of insect, vigour, age, composition of diet, and above all, species of bacterium. Some microbial products such as bacterial toxins and mycotoxins may greatly reduce the quality of insects produced. Among the more common problems associated with microbial contamination are high mortality of young insects, prolonged developmental time, diminutive pupae and adults, reduced pheromone production, reduced amino and fatty acid synthesis, wide fluctuations in the quality of insects and direct pathological effects. For the above reasons an environmental sanitation programme should be employed to eliminate contamination that can interfere with the rearing of healthy insects.

The most critical areas in the insectary from the microbial point of view are:

1. diet preparation and dispensing room
2. egg and larval planting room.

The most critical procedures are:

1. egg surface sterilisation
2. diet sterilisation and dispensing.

Acknowledgement

We are very grateful to the following persons for critically reviewing the manuscript: C.H. Collison, L.D. Corpus, F.M. Davis and P.A. Hedin.

References

Brooks, M. A. 1963. The microorganisms of healthy insects, pp. 215–243. In: E. A. Steinhaus (ed.), *Insect Pathology*, Vol. 1. Academic Press, New York.

Brooks, W. M. 1985. Entomogenous protozoa. In: C. M. Ignoffo (ed.), *Natural Pesticides. Vol. V. Entomogenous Protozoa and Fungi*. CRC Press, Inc. Boca Raton, Florida.

Chapman, R. F. 1971. *The Insects: Structure and Function*. Elsevier, New York. 819 pp.

Davis, F. M. 1976. Production and handling of eggs of southwestern corn borer for host plant resistance studies. *Miss. Agric. For. Exp. Stn. Tech. Bull.* 74, 11 pp.

Dychdala, G. R. 1977. Chlorine and chlorine compounds, pp. 167–195. In: S. S. Block (ed.), *Disinfection, Sterilization and Preservation*. Lea and Febiger, Philadelphia.

Favero, M. S., J. R. Puleo, J. H. Marshall and G. S. Oxborrow. 1966. Comparative levels and types of microbial contamination detected in industrial clean rooms. *Appl. Microbiol.* 14: 539–551.

Favero, M. S., J. R. Puleo, J. H. Marshall and G. S. Oxborrow. 1968. Comparison of microbial contamination levels among hospital operating rooms and industrial clean rooms. *Appl. Microbiol.* 16: 480–486.

Frobisher, M., L. Sommermeyer and R. Fuerst. 1969. *Microbiology in Health and Disease*. W. B. Saunders Co., Philadelphia. 549 pp.

Goodwin, R. H. 1984. Recognition and diagnosis of diseases in insectaries and the effects of disease agents on insect biology, pp. 96–117. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA.

Greenberg, B. 1962. Host-contaminant biology of muscoid flies. II. Bacterial survival in the stable fly, false stable fly, and the little house fly. *J. Insect Pathol.* 4: 216–223.

Hamilton, W. A. 1971. Membrane-active antibacterial compounds, pp. 77–93. In: W. B. Hugo (ed.), *Inhibition and Destruction of the Microbial Cell.* Academic Press, London.

Hedin, P. A., O. H. Lindig, P. P. Sikorowski and M. Wyatt. 1978. Suppressants of gut bacteria in the boll weevil from the cotton plant. *J. Econ. Entomol.* 71: 394–396.

Hoffman, R. K. 1971. Toxic gases, pp. 225–258. In: W. B. Hugo (ed.), *Inhibition and Destruction of the Microbial Cell.* Academic Press, London.

Jawetz, E., J. L. Melnick and E. A. Adelberg. 1978. *Review of Medical Microbiology.* Lange Medical Publications, Los Altos, Calif. 550 pp.

Litsky, B. Y. and W. Litsky. 1968. Investigations on decontamination of hospital surfaces by the use of disinfectant-detergents. *Am. J. Public Health* 58: 534–543.

Loughhead, H. O. and J. A. Moffett. 1971. Air sampling techniques for monitoring microbiological contamination. *Bull. Parenter. Drug Assoc.* 25: 261–265.

McLaughlin, R. E. and P. P. Sikorowski. 1978. Observations of boll weevil midgut when fed natural food or on bacterially contaminated artificial diet. *J. Invertebr. Pathol.* 32: 64–70.

Martignoni, M. E. and J. E. Milstead. 1960. Quaternary ammonium compounds for the surface sterilization of insects. *J. Insect Pathol.* 2: 124–133.

Martignoni, M. E. and P. J. Iwai. 1977. Sanitation program for diet preparation. Standard Operating Procedures, RWU-2203, Forestry Sciences Laboratory. 9 pp. US Forest Service, Corvallis, Oreg.

Nagasawa, S., T. Tsuchiya and H. Nishimura. 1970. Hospital disinfection, pp. 275–289. In: M. A. Benarde (ed.), *Disinfection.* Marcel Dekker, New York.

NASA SP-5076. 1969. *Contamination Control Handbook.* Office of Technology Utilization, National Aeronautic and Space Administration, Washington, DC.

Neter, E. 1974. Opportunistic pathogens: immunological aspects, pp. 37–48. In: J. E. Prier and H. Friedman (eds.), *Opportunistic Pathogens.* University Park Press, Baltimore, Md.

Patterson, J. T. 1971. Microbiological assessment of surfaces. *J. Food Technol.* 6: 63–72.

Petrocci, A. N. 1977. Quaternary ammonium compounds, pp. 325–347. In: S. S. Block (ed.), *Disinfection, Sterilization, and Preservation.* Lea and Febiger, Philadelphia.

Phillips, C. R. 1977. Gaseous sterilization, pp. 592–610. In: S. S. Block (ed.), *Disinfection, Sterilization and Preservation.* Lea and Febiger, Philadelphia.

Phillips, G. B. and R. S. Runkle. 1973. *Biomedical Applications of Laminar Airflow.* 180 pp. CRC Press, Cleveland, Ohio.

Poinar, G. O. and G. M. Thomas. 1984. *Laboratory Guide to Insect Pathogens and Parasites,* pp. 105–170. Plenum Press, New York.

Runkle, R. S. and G. B. Phillips. 1969. *Microbial Contamination Control Facilities.* Van Nostrand Reinhold Co., New York. 198 pp.

Shapiro, M. 1984. Microorganisms as contaminants or pathogens, pp. 130–142. In: E. G. King and N. C. Leppla (eds.), *Advances and Challenges in Insect Rearing,* USDA.

Sikorowski, P. P. 1975. Microbiological monitoring in the boll weevil rearing facility. *Miss. Agric. For. Exp. Stn. Tech. Bull.* 71, 21 pp.

Sikorowski, P. P. 1984. Pathogens and microbiological contaminants: their occurrence and control, pp. 115–168. In: P. P. Sikorowski , J. G. Griffin, J. Roberson and O. H. Lindig (eds.), *Boll Weevil Mass Rearing Technology.* University Press of Mississippi, Jackson.

Sikorowski, P. P., J. M. Wyatt and O. H. Lindig. 1977. Method for surface-sterilization of boll weevil eggs and the determination of microbial contamination of adults. *Southwest. Entomol.* 2: 32–36.

Sikorowski, P.P., J.E. Powell and A.M. Lawrence. 1992. Effects of bacterial contamination on development of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). *Entomophaga* (in press).

Singh, P. and R. F. Moore. 1985. *Handbook of Insect Rearing*, Vol. I. Elsevier, New York. 488 pp.

Smith, F. W. and M. Bruch, 1969. Reduction of microbiological shedding in clean room. *Dev. Ind. Microbiol.* 10: 290.

Steinhaus, E. A. 1941. A study of the bacteria associated with thirty species of insects. *J. Bacteriol.* 42: 757–790.

Steinhaus, E. A. 1942. The microbial flora of the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles. *J. Bacteriol.* 44: 397–404.

Steinhaus, E. A. 1949. *Principles of Insect Pathology*. McGraw-Hill Book Co., New York. 757 pp.

Steinhaus, E. A. 1953. Diseases of insects reared in the laboratory or insectary. *Calif. Agric. Exp. Stn. Ext. Serv. Leafl.* 9, 26 pp.

Steinhaus, E. A. and M. E. Martignoni. 1970. An abridged glossary of terms used in invertebrate pathology, 38 pp. Pacific Northwest Forest and Range Experiment Station, US Forest Service, Corvallis, Oreg.

Sun, M. 1981. Study shows formaldehyde is carcinogenic. *Science* 213: 1232.

Thompson, A. C. and P. P. Sikorowski. 1978. The effect of bacterial load on amino acids in the boll weevil, *Anthonomus grandis*. *J. Invertebr. Pathol.* 32: 388–389.

Thompson, A. C. and P. P. Sikorowski. 1988. Effect of antibiotics on the physical and biochemical properties of irradiated boll weevil adults (*Anthonomus grandis* Boh.). *Comp. Biochem. Physiol.* 90: 161–167.

Trueman, J. R. 1971. The halogens, pp. 137–183. In: W. B. Hugo (ed.), *Inhibition and Destruction in the Microbial Cell.* Academic Press, London.

Trujillo, R. and T. J. David. 1972. Sporostatic and sporocidal properties of aqueous formaldehyde. *Appl. Microbiol.* 23: 618–622.

Willett, H. P. 1976. Sterilization and disinfection, pp. 226–241. In: W. K. Joklik and H. P. Willett (eds.), *Zinsser Microbiology.* Appleton-Century-Crofs, New York.

Wyigul, G., and P. P. Sikorowski. 1986. The effect of staphylococcal enterotoxin B on pheromone production in fat bodies isolated from male boll weevils. *J. Invertebr. Pathol.* 47: 116–119.

Identification of Insect Pathogens Common in the Insectary

M. O. Odindo

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

Insect pathogens occur in all the major groups of bacteria, viruses, protozoa, nematodes and fungi. Many of these insect (and mite) pathogens have been, or are being studied, for the control of pests of crops or vectors of diseases, and are therefore highly beneficial. However, a great many also cause diseases in insect rearing facilities or in domesticated insects such as honey bees and silkworms. Indeed the earliest records of animal diseases were reported in these two groups of domesticated insects by such early philosophers as the Chinese, Guan (?– 645 BC), the Greek, Aristotle (384–322 BC) and the Roman, Virgil (70–19 BC).

To date, losses caused by insect pathogens in insectaries and other insect producing facilities worldwide amount to many millions of dollars annually.

In order to avoid diseases in the insectary, care must be taken in the maintenance of the insectary. The breeding or founder stock must be healthy and disease-free. Subsequently, they must be reared in clean conditions, with minimum distress and contamination. Further, the diet on which they are reared must be clean, well balanced and nutritious. Insects hate frequent handling, and therefore disturbance should be reduced to the minimum. Any new insect introduction into the colony should be held under quarantine before they are introduced into the main insectary.

If through some accident, and in spite of the care in production, diseases still occur in the colony, then, depending on the nature of the disease and the history of the colony, all the contaminated insects must be removed and eliminated from the insectary. The rearing facilities must also be decontaminated.

Insect Pathogens

A. Viruses

Insect viruses may be identified through the symptoms of the infected insects, or staining and examination of sections and smears of body tissues in a compound microscope.

Steps	Key points
1. Identification through symptoms of infected insects	• Lepidopteran larvae may show retarded growth. • Insect reared on natural diets are usually prone to viral infections (Fig. 1). • Larvae climb to the top of the rearing container. • In late stages of infection, the midgut may be visible through the integument as a whitish region.

Fig. 1. Rearing the African armyworm *Spodoptera exempta* on maize leaves

Steps	Key points
	• The integument becomes extremely fragile and disintegrates at the slightest touch. • The body becomes liquified, releasing many polyhedral inclusions in the rearing container and the diet. • In some virus infections, the host integument may not disintegrate, but the colour changes to white or pale. • The iridoviruses cause specific colour changes of iridescence, green, brown, blue or opalescence to the infected organs. • In *Drosophila* sp., infection by the non-included sigma viruses causes the insects to be highly sensitive to carbon dioxide. • In the honey bees, acute or chronic paralysis viruses shows peculiar symptoms of trembling and loss of coordination. • Also in the honey bee, the sacbrood virus causes the dead insects to extend lengthwise on the floor of the cell of the hive, and head becomes darker than the rest of the body. • In mosquitoes, virus infections in larvae, appear as milky white in the fat body and midgut due to cytoplasmic virus infection. • DNA virus infection in tsetse flies causes extension and hypertrophy of salivary glands (Fig. 2) which may be visible through the integument in teneral flies.
2. Procedure for examination	• If a virus infection is likely, examine the insects in a dissecting microscope and look for symptoms as outlined above.

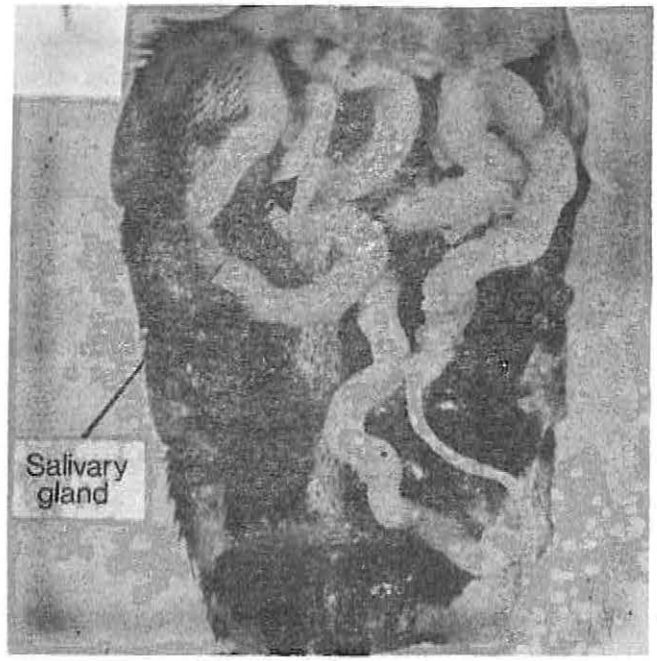


Fig. 2. Grossly enlarged salivary glands of the tsetse fly *Glossina pallidipes* due to infection with the tsetse DNA virus

Steps	Key points
	• Dissect two or three insects and place portions of the fat body, integument and midgut on glass slides.
	• Macerate the tissues well using a pair of forceps or other suitable instrument.
	• Add a drop of clean sterile distilled water and mix thoroughly.
	• Cover with glass cover slip and examine on a compound microscope.
	• Use phase contrast or dark field if your microscope has these facilities.
	• In oil immersion, polyhedral inclusion bodies (PIBs) appear as large, irregularly-shaped granules (Fig. 3).

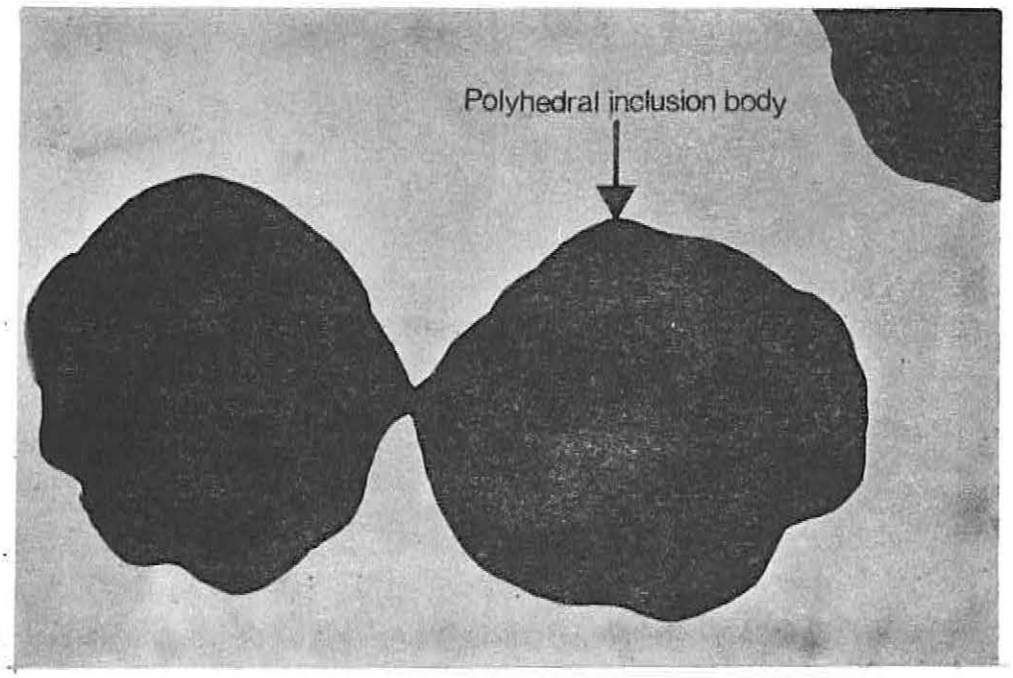


Fig. 3. Outline of the polyhedral inclusion bodies of the *Spodoptera exempta* nuclear polyhedrosis virus

Steps	Key points
3. Methods for isolation of insect viruses	• Inclusion body viruses may be isolated simply by placing diseased larvae on top of a gauze wire in a test tube filled with water.
	• Place the tubes in an undisturbed position for 2–3 days. PIBs accumulate at the bottom of the tube as a white layer.
	• Carefully decant off the top layer of insect tissue bacteria and other products of decomposition.
	• Wash the PIBs by centrifugation.
	• Inclusion body viruses may also be isolated by direct maceration of infected insects and filtration.

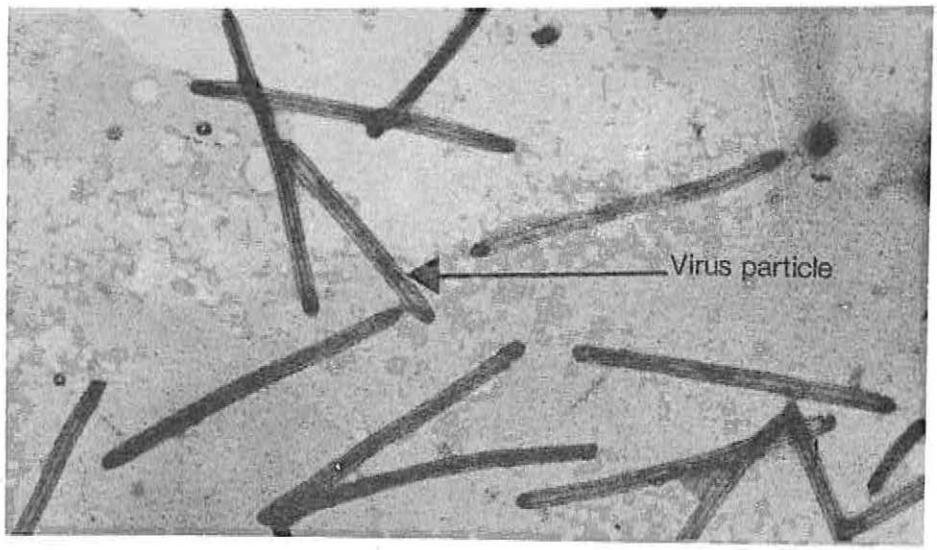


Fig. 4. Tsetse DNA virus particles

Steps	Key points
	• Alternatively, macerate several insects in test tube and filter through several layers of mosquito netting.
	• Non-inclusion body viruses have to be isolated through high speed centrifugation.
	• In an electron micrograph, the tsetse DNA virus particles appear as long rods (Fig. 4).
4. Staining techniques	• Prepare a smear on a clean glass slide and allow to air dry.
	• To differentiate fat body cells from PIBs fix the smears in ethanol and stain in aqueous Sudan III for 10 –15 min.
	• Virus inclusion bodies may be stained with Giemsa with acid hydrolysis.

Steps	Key points
	• Fix the smear in Bouin-Dubosq-Brasil for 10 min. • Wash the smear in 70% ethanol for three changes, one per hour. • Rinse in distilled water (5 min). • Rinse in Giemsa buffer (185.0 ml distilled water, 0.5M KH_2PO_4, and 0.5M Na_2HPO_4, pH 6.8) overnight. • Rinse in distilled water. • Differentiate in 80% ethanol with 5% glacial acetic acid. • Drain off excess ethanol and dip in 1:1 xylene/acetone mixture. • Immerse in xylene (2 changes). • Mount in DPX.
5. Inferences	• Sudan III stains fat droplets red while PIBs do not stain. • In Giemsa, PIBs are stained purple and virus rods will be red. • In histological sections, nuclear polyhedrosis viruses are easily recognisable within the nuclei of infected cells (Fig. 5).

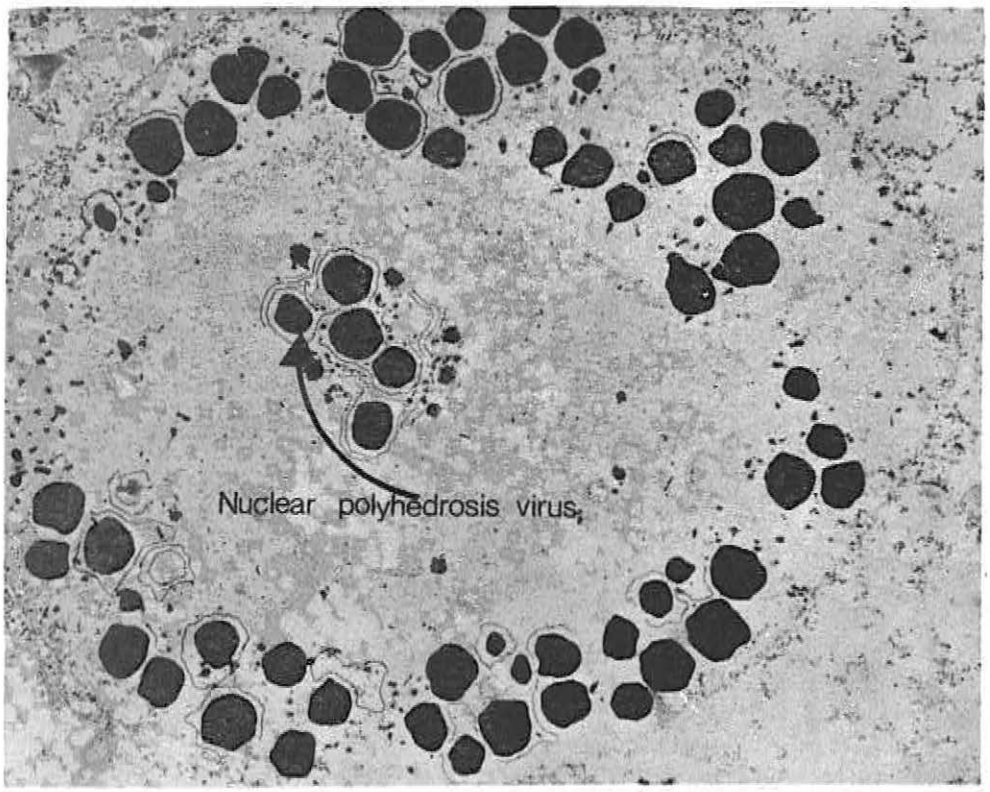


Fig. 5. Polyhedrosis viruses in the nuclei of infected cells

B. Bacteria

Bacteria are the most abundant of all microorganisms and occur in all insectaries at various levels. By their ubiquitous nature they are found on the diet, on the surface of the insect body, within the gut, in water, or on the rearing container. Fortunately, a great majority of them do not cause disease or affect the insect life in any way.

However there are important entomopathogens from among the bacteria, and care must be taken so that they are not introduced into the insectary.

For practical purposes straightforward techniques may be used for bacterial identification. However specialised laboratories must be contacted for the more complicated and final techniques of genetic analysis, serology, bacteriophage typing, and enzymology.

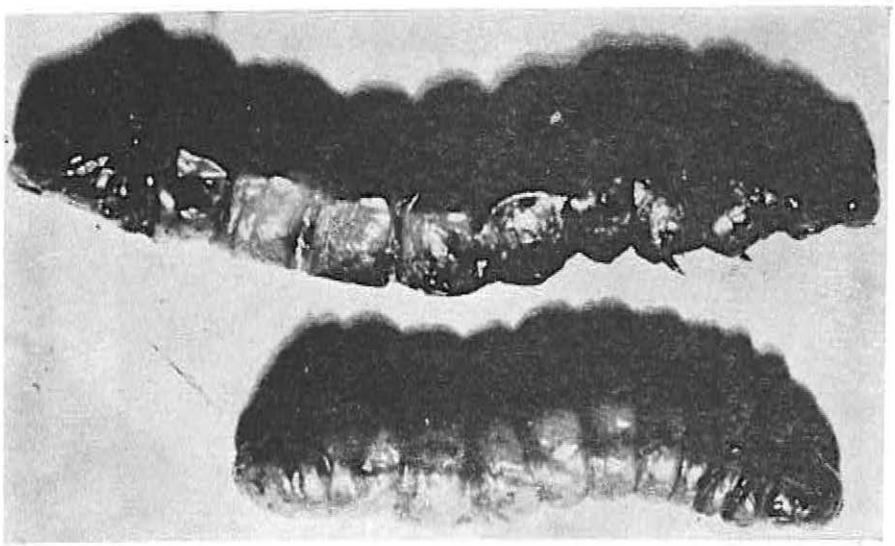


Fig. 6. Bacterial infection in the cereal stemborer *Busseola fusca*

Steps	Key points
1. Identification through disease syrup forms	• Infected insects have loss of appetite, stop feeding, have diarrhoea, show gut paralysis, and regurgitate ingested food. • Insects show uncoordinated movement, are sluggish, and may show paralysis. • Dead insects turn black, accompanied by an offensive smell due to putrefaction (Fig. 6).
2. Examination of the haemolymph	• Bacterial infection may be recorded by examining a drop of haemolymph in a compound microscope. • Bacterial rods (bacilli) Fig. 7 or round cells (cocci) may be seen using phase contrast.
3. Isolation	• If bacterial infections are suspected, then steps must be taken to isolate the causal organism under sterile conditions.

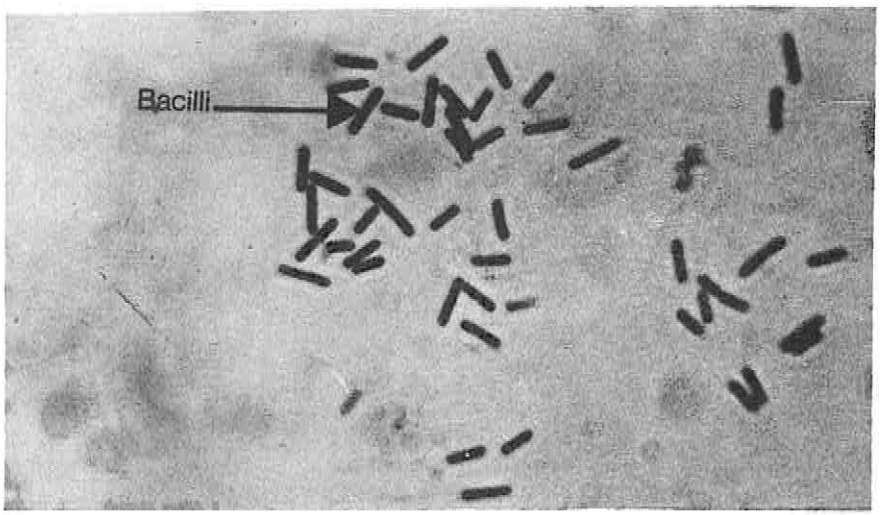


Fig. 7. Bacterial cells from the haemolymph of infected spotted stemborer *Chilo partellus*

Steps	Key points
	• Ligature the anterior and posterior openings of the insect.
	• Dip the insect into 70% alcohol for 2 seconds.
	• Dip into 5% sodium hypochlorite for 3–5 min to sterilise the insect from surface contaminants.
	• Dip into 10% sodium thiosulphate to remove free chlorine.
	• Rinse in several changes of sterile distilled water.
	• Dissect insect on a sterile petri dish using sterile forceps.
	• Place portions of body fluids and tissue into sterile Ringer's solution.
	• Using streak method place suspension onto Nutrient Agar or Brain-Heart Infusion Agar.

Steps	Key points
	• Incubate plates in an inverted position at 30°C overnight.
	• The pure bacterial isolates can now be examined for colony growth characteristics.
4. Characteristics	• Using a dissecting microscope examine the form of the colony.
	The following forms may be observed:
	• Circular: round colony with smooth edges >1 mm diameter.
	• Irregular: periphery non-uniform.
	• Punctiform: small colonies just visible to the naked eye < 1 mm diameter.
	• Filamentous : Irregular, threadlike colonies.
	• Rhizoid : Root-like spreading colony.
	• Spindle: Occur within the agar colonies larger at either end than in middle.
	Elevation of colony may be:
	• Raised: Hill-like elevation above the media surface.
	• Flat: Thin plateau above the media.
	• Umbonate: Only centre of colony is raised.
	• Convex: Curved colony.
	• Pulvinate: Colony is cushion-shaped.

Steps	Key points
	The surface of the colony may show the following characteristics:
	• Concentrically ringed: Colony in form of rings one within another.
	• Contoured: Colony in form of contours more like a relief map.
	• Smooth: Even surface.
	• Radiately ridged: Colony in form of ridges which radiate from the centre.
	• Rugose : Surface with a wrinkled appearance.
	Colony margin may be:
	• Erose: Irregularly notched.
	• Undulate: Having a wavy margin.
	• Entire: Margin smooth.
	• Lobate: Having rounded projections or lobes.
	• Filamentous: Long threads branching out from colony.
	• Curled: Parallel cilia-like chains of threads radiating from the colony.
	Density of the colony may be:
	• Translucent: transmits light through the colony.
	• Opaque: Light cannot pass through.

Steps	Key points
	• Colony may produce certain characteristic colours (chromogenesis).
5. Steps in Gram staining	• Staining: Gram-staining divides the bacteria into two major groups which are important for identification, Gram-positive and Gram-negative. Use young cultures (8–16 hr) for Gram staining. • Prepare smear on a glass slide and fix by passing it quickly through bunsen flame several times. • Stain with ammonium oxalate-crystal violet (1 min). • Rinse in running tap water (5 sec). • Rinse in Gram's iodine then stain in the same solution (1 min). • Rinse in running tap water (5 sec). • Pass through propyl alcohol (3 changes, 1 min each). • Rinse in running tap water (5 sec). • Rinse in sufranin counter stain, then immerse in stain and leave for 1 min. • Rinse in running tap water (5 sec). • Air dry and examine under oil immersion.
6. Results and inferences	• Gram-positive organisms are blue-violet. • Gram-negative bacteria are red. • Examination of bacteria under oil immersion may show

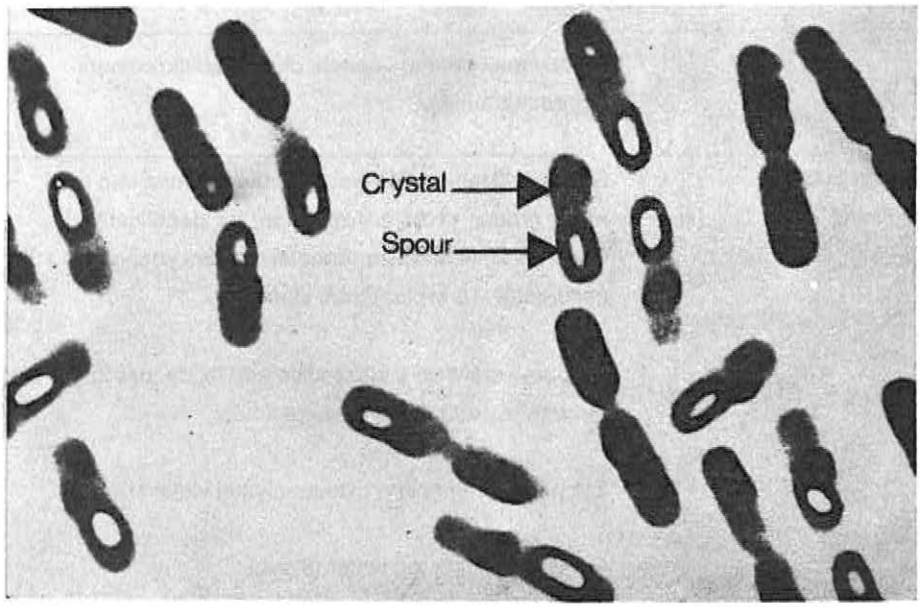


Fig. 8. Cells, spores and parasporal crystalline bodies of *Bacillus thuringiensis*

Steps	Key points
	spores and parasporal bodies.
	• Specialised media may be used to identify specific bacteria e.g. *Streptoccocus faecalis*. Differential medium for isolating *S. faecalis*, or Tergitol-7 FTTC Agar (T7 + TTC) for isolating Gram-negative coliform bacteria such as *Escherichia* sp. and *Xenorhabdus nematophilus*, and *Pseudomonas* spp.
	• Chromogenesis results may indicate certain bacteria e.g. *Pseudomonas aeruginosa* produces greenish pigments on media, while red and orange-red pigments are produced by *Serratia marcescens* due to presence of the parasporal crystals and spores when examined in a phase contrast microscope (Fig. 8). Heat fixed smears stained with strong Giemsa or crystal violet show the crystals deeply stained with the oval unstained spores in outline.

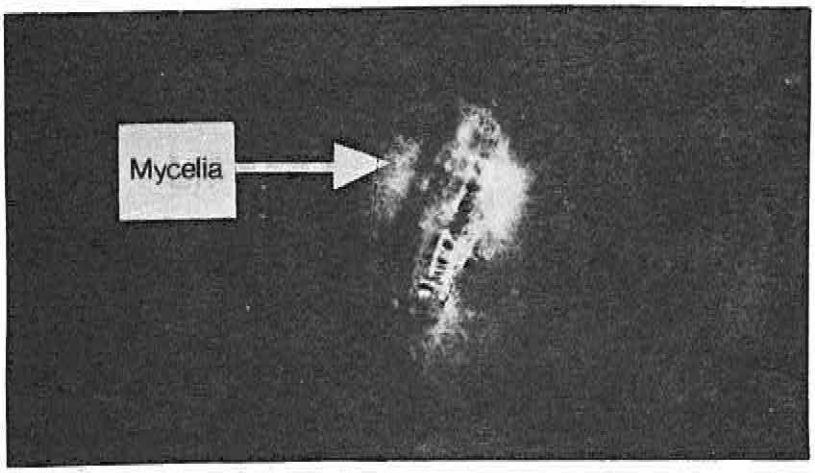


Fig. 9. Mycelial growth on a 3rd instar *Chilo partellus*

C. Fungi

Fungal infections in insects are probably the easiest ones to recognise in an insectary. This is due to the easily noticeable abundant growth of mycelia and conidia on the insect integument. They are abundantly found in both terrestrial as well as aquatic insects and attack all stages of the insect. They often have a broad host spectrum, and the same fungal isolate may infect a wide range of different insect groups.

Steps	Key points
1. Symptoms of infection	• Fungal infections are easily diagnosed where mycelia occur on the surface of the infected insect (Fig. 9.) Mycelia may also be observed within the insect tissues.
	• Fungi are the most common causes of diet spoilage (Fig.10).
	• Initially infected insects may show cessation of feeding, uncoordinated movement, and weakness.

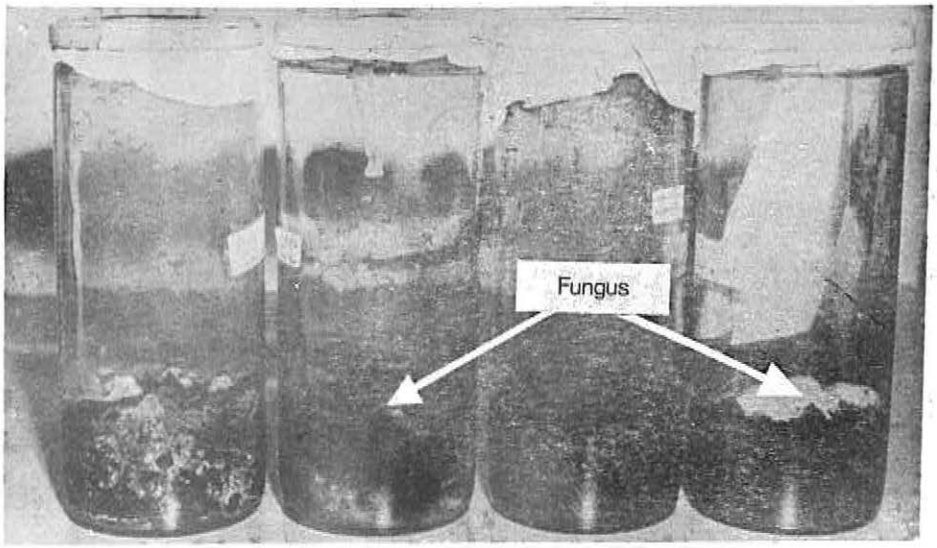


Fig. 10. Fungal growth on artificial diet for rearing stem borers

Steps	Key points
	• The integument may change colour and become spotted or white.
	• Dead insects become waxy or hard and brittle.
	• Colony of mycelia on the insect may give its identity.
2. Isolation of insect fungi	• Fungi may be isolated from the spores or mycelia • Sterilise the surface of the insect by immersing it in 5% sodium hypochlorite.
	• Rinse thoroughly in sterile distilled water.
	• Dissect the specimen in a sterile glass slide, and transfer a portion of tissue onto Sabouraud dextrose agar (SDA) plates. Mycelia and spores may also be placed onto the plates directly.

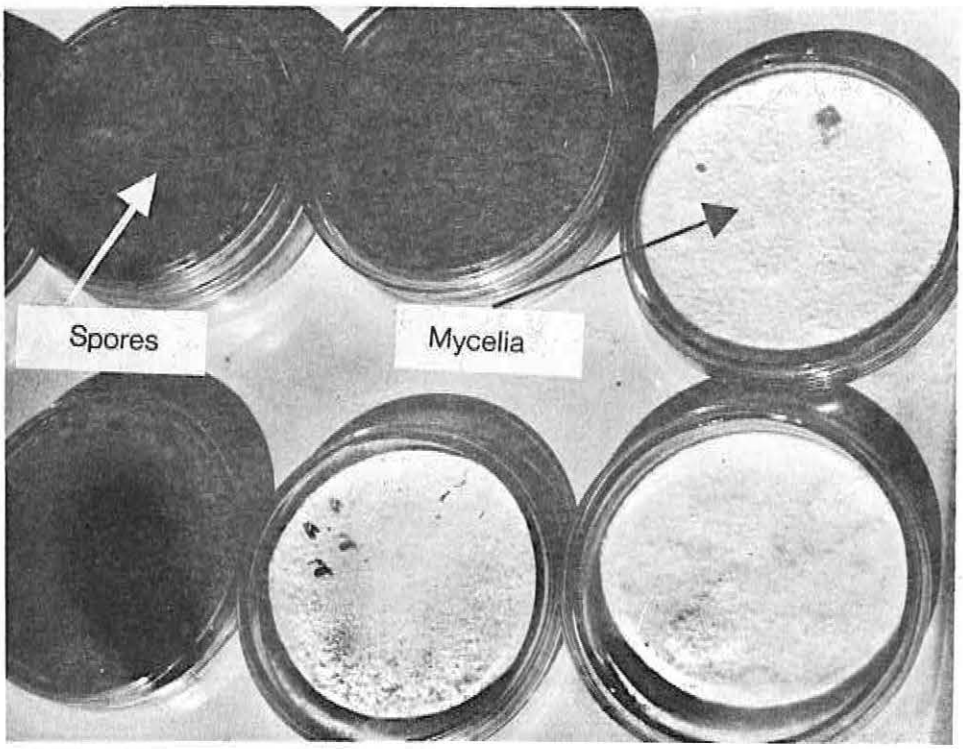


Fig. 11. Mycelia (right) and spores (left) of *Hirsutella thompsonii* on a growth medium

Steps	Key points
	• Incubate at 30°C for 5 to 10 days by which time mycelia growth and sporulation should be evident. (Fig. 11) Take portions of the mycelia and fruiting bodies, and examine in a compound microscope.
3. Important characteristics in fungi	• Certain key points are important in fungus identification. These characteristics include: • Size and shape of fungus spores. • Position of attachment of spores onto the hyphae. • Nature and type of the fruiting body. • Nature of spores (septate, motile, occurring in slime drops). For example in *Hirsutella thompsonii* the conidia are borne on conoid or ellipsoid phialides (Fig. 12.).

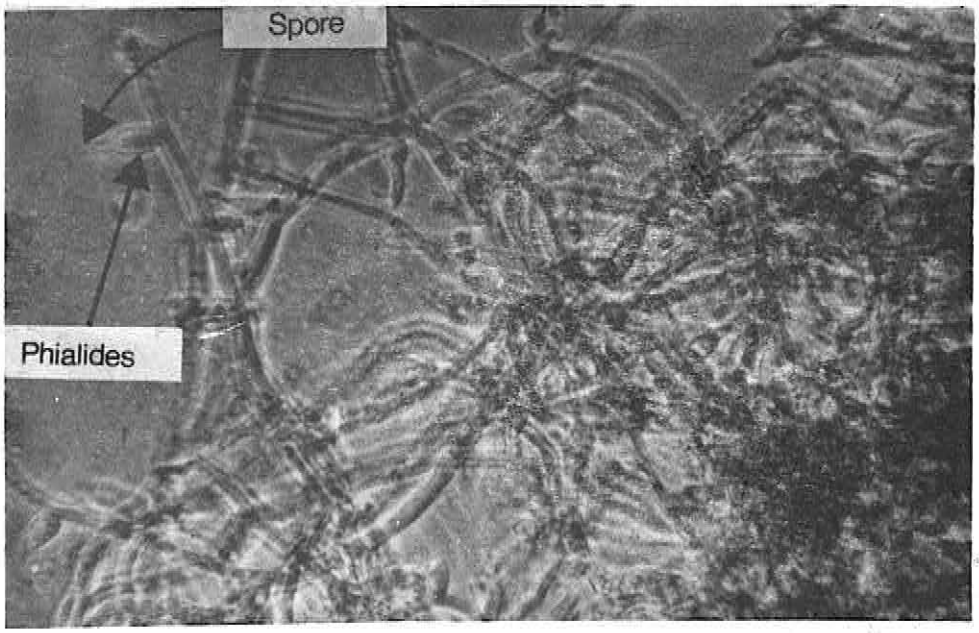


Fig. 12. Conidia and phialides of *Hirsutella thompsonii*

Steps	Key points
4. Inferences	• Certain characters of colour and form may indicate the identity of the fungus. • *Beauveria bassiana* infection appears as white powdery spores covering the dead insect. • *Metarhizium anisopliae* spores are green. • *Aspergillus flavus* spores are greenish-yellow. • *Paecilomyces farinosus* infection are found as yellowish spores. • Insects dead from *Entomophthora* and *Neozygites* spp., especially locusts, grasshoppers, and flies die attached to the subtratum with a white halo of spores scattered around the cadaver.

D. Nematodes

Nematode parasitism is rarely a problem in insect rearing facilities. Most of these would have been introduced into the colony within field-collected insects, and are likely to be mermithids and steinernematids.

Insects reared on natural substrates are more likely to be exposed to parasitism by nematodes. Wet soil and sand usually harbour nematodes (along with other insect pathogens) and care must be taken when they have to be used in an insectary, for example in rearing mosquito larvae or other aquatic insects.

Steps	Key points
1. Dissection and isolation	• Mermithids may sometimes be seen clearly through the insect integument. • Dissect parasitised insect to remove the nematode. • Transfer to 70% alcohol or 4% formaldehyde. • Examine under a dissecting scope and compound microscope. • Determine the shape of the mouthparts, presence or absence of stylus, shape of pharynx, and shape of the posterior end appendages.
2. Inferences and identification	• Mermitids are long white worms1–3 cm long but only 0.2 mm in width (Fig. 13). • Mermithids may parasitise singly, in 2s, 3s or higher. • *Mesomermis* larva has a long coiled caudal appendage. • *Gastromermis* has a needle-like cauda. • *Isomermis* cauda is only a short prickle.

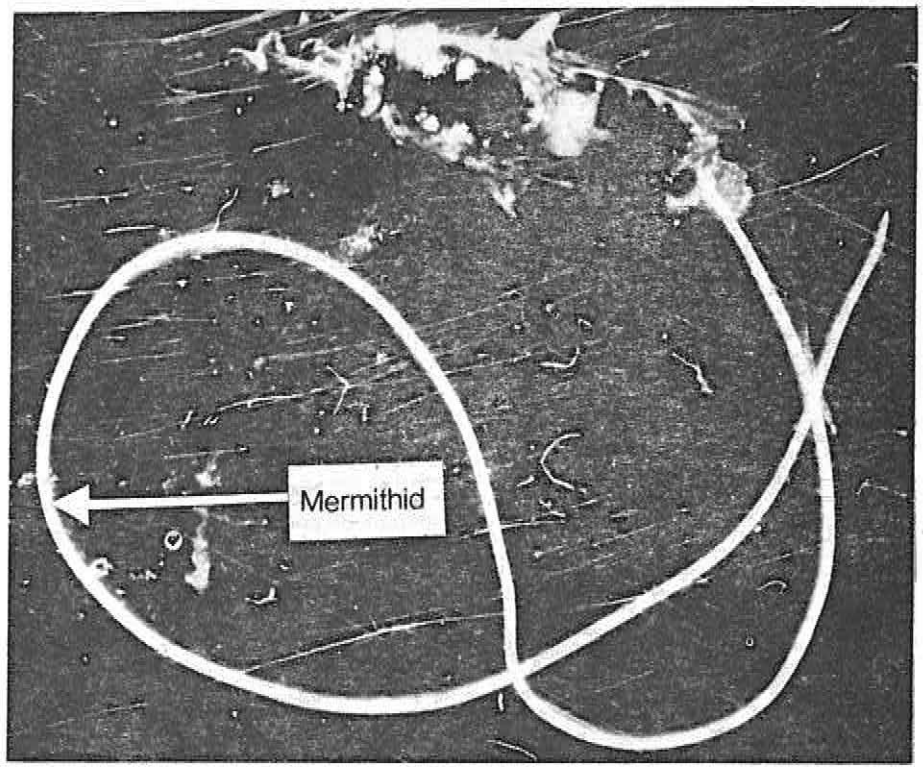


Fig. 13. A mermithid *Hexamermis glossinae* emerging from an adult tsetse fly *Glossina pallidipes*

Steps	Key points
	• Steinernematids parasitise terrestrial insects, especially the soil-inhabiting stages.
	• Generally the adults are 6.5–0.7 mm long.
	• Most significant is *Steinernema feltiae.*

E. Protozoa

In terms of variation in levels of association, protozoa probably represents the largest and most diverse association between insects and microorganisms. Such association can be symbiosis, phoresis, obligate parasitism, facultative parasitism, vectorial, to direct pathogenic.

In the insectary, they (together with viruses) are probably the most important group of pathogens to the personnel in an insect rearing facility, and a constant source of his/her headaches and heartaches. If a protozoan infection gets established in an insectary then it is wisest to eliminate the whole colony, and start all over again. This is due to two important issues.

1. Protozoa are transmitted transovarially and will pass from one generation to the next through the eggs or the ovary.

2. Protozoa often cause benign infections which, although they may not be evident in high mortality, will cause sub-lethal effects on the population; low fecundity, slow growth rate, shortened life span etc. all of which will affect the general performance of the colony.

It is therefore paramount that protozoan infections are avoided through hygienic conditions and care taken with introduction of seemingly healthy insects into the colony.

Steps	Key points
1. Identification of protozoa	• Protozoa rarely cause dramatic and noticeable symptoms of infection. Such symptoms associated with protozoan infection, including lethargy, loss of appetite, small size (Fig. 14), morphological deformity, reduced growth, difficulty in mating, loss of coordination, are all associated with other microorganic infections. • However, aquatic insects may show protozoa as white patches through the cuticle. • Insects die in large numbers, resulting in population collapse. • Insects infected with microsporidia may show body liquefaction as in viruses.
2. Microscope examination	• Protozoan diseases should be confirmed by examining insect haemolymph and other tissues in a compound microscope.

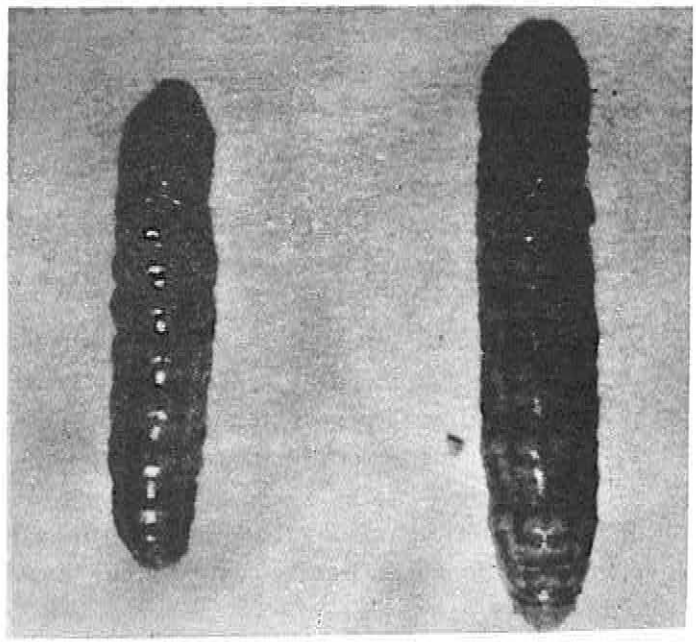


Fig. 14. Healthy (right) and infected larva (left) of the spotted stalk borer *Chilo partellus* at 7 days after inoculation with a sub-lethal dose of *Nosema marucae*

Steps	Key points
	• Dissect the insect in a clean glass-slide and remove portions of the fat body, midgut, and Malpighian tubules separately on a fresh glass slide. Macerate and prepare a smear.
	• Transfer several drops of the haemolymph onto a glass slide and prepare a smear.
	• Examine slides using phase contrast or bright field microscopy. The oval-shaped spores will be readily distinguished from insect tissues (Fig. 15).
	• Stain slides with Giemsa or haematoxylin and examine the mature spores as well as developing stages of the protozoa.

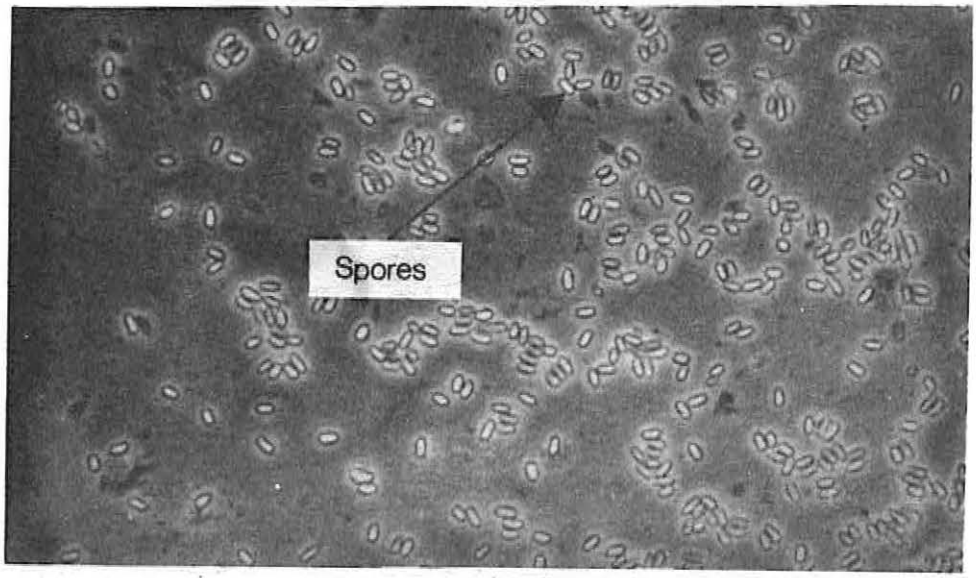


Fig. 15. Spores of *Nosema marucae* in a phase contrast microscope

Steps	Key points
	• To distinguish protozoa from other inclusion bodies, add a few drops of weak NaOH to a glass slide containing the suspension. Inclusion bodies will dissolve, protozoa will not.
	• Fungal spores will germinate in media (SDA), protozoan spores will not.
3. Staining	• Microsporidian developing stages stain well with Giemsa.
	• Air dry smear.
	• Fix in methanol (3–4 min).
	• Drain off excess methanol and allow to air dry.
	• Flood with Giemsa stain (stock solution diluted 1:1 with distilled water.

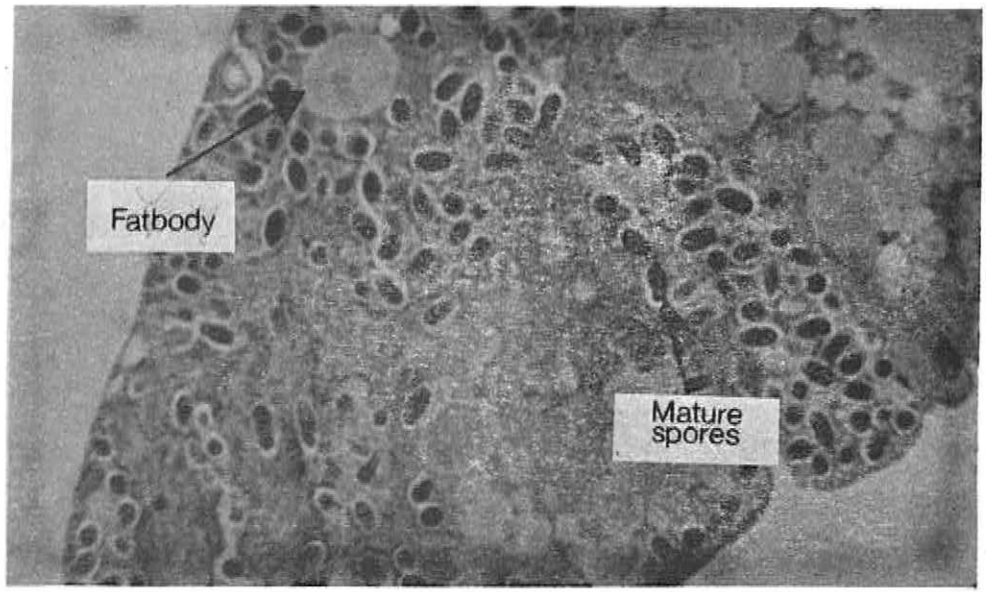


Fig. 16. Cross section of the fat body of the legume pod borer *Maruca testulalis* infected with *Nosema marucae*

Steps	Key points
	• Stain for 15 min.
	• Wash off excess stain in distilled water.
	• Air dry and examine under oil immersion.
4. Inferences and identification	• For the ultimate identification, thin sections of the tissues of infected insect have to be examined in an electron microscope.
	• Tissue specificity may be important in identification. For example microsporidia multiply profusely in the fat body (Fig. 16).
	• *Malamoeba locustae* occurs in the Malpighian tubules.
	• Microsporidia infect the fat body and integumental epithelial cells.

Steps	Key points
	• Presence or absence of locomotory organs may be important: flagella, cilia, or pseudopodia, are all important characters

Conclusion

Microorganisms are associated with insects at all levels, and many of them cause diseases resulting in extensive losses in the insectaries. Only through great care in insect rearing, under hygienic conditions, and away from factors that are likely to cause stress are we likely to succeed in avoiding the otherwise inevitable losses. Proper identification of the disease-causing micro-organism will remain an important prerequisite in this task.

Animal Health Implications in the Laboratory Breeding of Small Mammals (With Particular Emphasis on Rabbits)

I. G. Onyango

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

For worthwhile research to be carried out ecomonically, it is important to use uniform, disease-free animals. When available animals are not properly bred and therefore genetically an unknown entity; and/or those infected with a host of pathogens, researchers are forced to use excessive numbers of test animals. The solution therefore, would be to apply correct breeding methods and clean up the colony. This way, genetic differences as well as those caused by disease can be largely eliminated.

In order to maintain a healthy small mammal colony, it is important to ensure that the initial stock is free from disease and that contaminants are denied access to the colony. All incoming animals should therefore be examined for signs of disease and then kept under quarantine for three weeks during which their microbial status is monitored before they are allowed to mix with the rest of the colony. At the same time, efforts should be made to eliminate all ecto- and endoparasites.

Contamination is usually through beddings, which may harbour ectoparasites, or through feeds, which may have animal or vermin contamination. As far as possible, animal feeds should be purchased from reliable vendors and should be brought in clean sealed containers with the dates of manufacture clearly labelled. They should be stored in a cool dry place and fed to the animals as soon as possible after delivery. Whatever feed remains in the trough every time the cages are cleaned should be destroyed.

Animal Health

There are several factors that could influence the conditions of otherwise healthy well characterised animals. These include the types of cages and the population density: cramped cages are inhumane and quickly lead to an increased incidence of disease and to unthrifty animals. Floors of 16 mm mesh, 14 gauge wire is satisfactory and should eliminate incidence of sore hocks. The practice of keeping several species in one room should be avoided. Handling should be firm but gentle since rabbits sensing insecurity are likely to struggle and their backs may break.

The animal house should be located as far as possible from heavy machinery since some noises from machines that may be inaudible to man (ultra sound) may stress susceptible animals. In the construction of the physical environment it is important to consider the traffic patterns. In the layout the following areas should be identified: breeding area, experimental area, service area, and the staff facilities. Entry into the premises should be restricted and there should be a one way flow of traffic, the personnel should wear protective clothing and as far as possible each technician should handle only one species and avoid moving backwards and forwards from one animal room to another. They should disinfect their feet before they enter the animal houses.

The rooms should be constructed such that the temperature range is optimum for the animals:

Rabbits, guinea pigs — 18°– 20°C
Small rodents— 22°– 24°C

The relative humidity should range between 50–60% and there should be between 14–16 air changes per hour.

When there is an increased incidence of

- decreased litter sizes
- decrease in the overall productivity of the colony
- increased susceptibility to disease and infections
- greater mortality in the colony

we could suspect that there is an inbreeding problem.

Diseases that are commonly encountered in a rabbit colony are:

Bacterial i. Pasteurellosis: snuffles, enzootic pneumonia, otitis media, abscesses, genital infections, septicemias, conjuctivitis.	**Protozoal** i. Coccidiosis: intestinal, hepatic
This syndrome is mostly predisposed by stress. ii. Tularemia iii. Yersiniosis/paratuberculosis iv. Salmonellosis v. Tyzzers' disease.	**Arthropod parasites** i. Psoroptic mange ii. Sarcoptic mange
Inherited Defects i. Mandibular prognathism	**Others** i. Ulcerative pododermatitis/ sore hocks ii. Traumatic vertebral fracture iii. Mastitis/ blue breast iv. Mucoid enteropathy v. Pregnancy toxaemia.

Postmortem examinations are an excellent aid in preventive medicine programmes since by sacrificing one sick animal, we can quickly arrive at a diagnosis of the disease affecting the colony.

Bibliography

Joubert , C.J. 1978. Principles of care and management in the laboratory animal facility. *Journal of the South African Vet. Ass.* 49 No. 7: 153–154.

The Merck Veterinary Manual, 6th Edition.

Insect Rearing and Inhalant Allergies

Raml Kfir

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

Abstract

Many cases of allergic reactions that occurred through inhalation of insect-derived materials are reported in the literature, implicating insect species from at least twelve orders. The major groups are the Lepidoptera, Orthoptera, Diptera and Coleoptera. Allergy surveys indicated that the incidence of allergic conditions among persons who were directly exposed to insects was about 30%. Respiratory hazards present in insectaries are airborne particles, usually odourless, which originate from insects. Insect parts that may contain allergens are hair, setae, and scales that cover many insects and that are shed readily, dried exuviae, fragments of dead insects, and metabolic products such as faeces and silk.

Annual allergy tests may be used to monitor workers to detect developing symptoms or susceptibility. Individuals unable to avoid exposure to allergens may consider hyposensitisation.

Preventing the transmission of allergens is the primary defence against respiratory diseases occurring in an insectary. The use of filters, blowers and hoods is the most important way of preventing respiratory disorders. Applying control measures near the source of contamination uses less energy, costs less and works best.

Introduction

Rearing of insects is a prerequisite for many research projects in entomology and related fields. There are, however, potential health hazards associated with exposure to insects. There is a steadily increasing body of literature indicating that insects are an important source of allergens that promote inhalant allergies in man. An occupational disease attributed to an insect was recorded as early as 1713 when cough and difficulty in respiration was noticed as a reaction to fragments of dead silkworms (Ramazzini 1940). Since then, much evidence has accumulated about insects as a source of inhalant allergens and the cause of occupational allergies. Insects were also found to be the cause of allergies in the general population (Kang and Chang 1985). Over 300 cases of allergic reactions that occurred through inhalation of insect-derived materials from at least twelve orders were reported by Bellas (1982). The major groups of insects that cause allergies in humans, namely the Lepidoptera, Orthoptera, Diptera and Coleoptera were discussed in detail by Wirtz (1984). These groups of insects possess unique characteristics that contribute to the release of large amounts of allergens that readily become airborne in dry conditions (Bellas 1990).

In this chapter emphasis will be placed on inhalant allergens derived from insects to which entomologists are exposed to during the course of their work.

Inhalant Allergens in Insectaries

Respiratory hazards present in insectaries are airborne particles and vapours from chemicals that harm humans. These particles are usually invisible and originate from insect waste products, mould spores, ingredients of insect diets, or cleaning and sanitising chemicals. Hazardous vapours from chemicals have detectable odours and provide a warning whereas insect particles are usually odourless.

From measurements in pink bollworm insect cages it was determined that most particles are less than 0.5 micron in diameter, ranging from less than 0.5 micron to several millimetres in length. The larger particles consist of scales and broken appendages. The source of the smaller particles are probably fragments of insect exoskeleton, exuviae, faeces, and mould and fungal spores. The small particles must be removed from the air in order to protect insectary worker's lungs (Wolf 1985). Removing only the larger, visible particles does not eliminate the problem and may create the illusion that the air is clean.

Allergic reactions may involve the skin, eyes, and respiratory tract. Prolonged exposure may produce irreversible pulmonary damage. Allergic reactions may develop after repeated exposure over a period of years, or after a short exposure. Some workers can become sensitive to very low concentrations. There is no way to predict if a particular person will develop an allergy.

Routine work in the insectary such as handling and cleaning insect cages, weighing, counting, dissecting, sexing etc. can increase the air contamination.

Particles larger than 2.5 to 3 microns in diameter are usually deposited in the upper respiratory system. The result in a susceptible person can be rhinitis, laryngitis, and bronchitis.

Particles of about 1 micron in diameter are usually deposited in the alveolar cells of the lungs causing emphysema, pleurisy, pneumonia and pneumoconiosis (Olishifski 1978). Deposition of particles on skin and in the eyes can also produce adverse allergic reactions.

Particles that cause respiratory problems settle slowly. For example, it takes over 75 hours for sawdust particles of 1 micron in diameter to settle in still air (McDermont 1977). Therefore, natural setting is not a solution.

Insects as a Source of Allergens

Most of the available information about allergies caused by insects became available from three surveys conducted among entomologists in North America and Australia (Wirtz 1980; Bauer and Patnode 1983; Bellas 1983). It was concluded that various Lepidoptera, mainly moths, were the predominant source of allergic responses as reported from surveys of universities as well as government and private institutions. In these three surveys, over 100 species of insects were listed as associated with work-related allergic reactions to employees working with insects.

Various species of locusts and grasshoppers have been identified as troublesome in many laboratories around the world, workers developing rhinitis and asthma (Frankland 1953). Several species of Diptera have been implicated. Screw-worm in the USA (Wirtz 1980), blowflies, vinegar flies, mosquitoes and houseflies. Granary weevils and dermestid beetles have also been recorded as sources of inhalant allergens in entomologists.

Allergy and asthma was developed by exposure to exuviae of the parasitic wasp, *Mormoniella vitripennis* (Walker), (Whiting 1967).

According to Bellas (1990), consulting the allergy surveys, the incidence of allergic conditions among persons who were directly exposed to insects was 33%. Similar figures were recorded for people working with locusts (Burge *et al.*, 1980) and with *Drosophila*.

The Source of Insect Allergens

For an insect to become the cause of inhalant allergies, the allergens must be capable of becoming part of some respirable dust or aerosol. Insect particles that can provide allergens are hair, setae, and scales that cover many insects and that are shed readily, dried exuviae, fragments of dead insects, and metabolic products such as faeces and silk.

Hair of caterpillars of some families in the Lepidoptera possess urticating properties. Contact with these hairs produces an immediate irritation that can be severe. Continued exposure can lead to sensitization and allergic condition. Usually the initial injuries are mechanical and toxins are present. Hairs of many other species do not possess any toxic effect but they still have allergic properties.

Scales that are so readily discarded by many species of moths are an obvious source of potential allergens, but many insects such as locusts, cockroaches, crickets and silkworms, which do not possess hairs or scales, are also highly allergenic.

Dried and fragmented *exuviae* of many insects including cockroaches, carpet beetle larvae and wasps have been shown as a major source of allergens.

Faeces as a source of allergens is well documented for dust mites, locusts, fleas and other insects. As faeces are the waste product of digested food, it is difficult to explain its allergenic properties. In the locust, *Schistocerca gregaria*, it was found by Edge and Berge (1980), that the main site of production of allergens are the epidermal cells of the midgut and the associated gastric caeca. These cells are also the site of production of the peritrophic membrane, which is produced by most insects and which passes through the gut and is expelled with the faeces. It is probably the peritrophic membrane that is the source of the specific allergens associated with the faeces.

Susceptibility of Humans to Insect Allergens

Different workers exposed to similar levels of contaminants respond differently. This variation is due to variations in the rate of clearance from the lungs, effect of cigarette smoking, existing pulmonary diseases, and genetic factors. Preemployment screening may prevent susceptible people from being exposed, and annual allergy tests may be used to monitor workers to detect developing symptoms or susceptibility. Individuals unable to avoid exposure to allergens may consider hyposensitisation which may benefit some workers but must be performed by trained medical personnel. Allergenic extracts for many non-stinging insects are commercially available, and efforts should be made to identify the responsible allergens. Hyposensitisation to a variety of insects, by injection of whole body extracts, resulted in desensitisation in 82% of a test population (Wirtz 1984).

Prevention of Inhalant Allergies

In many countries, employers must by law provide a safe working place for employees. The responsibility lies with the employer to recognise hazards and to provide proper equipment and work procedures. It must be recognised that respiratory diseases can be prevented. Preventing transmission of allergens must be the primary defence against respiratory diseases in an insectary (Wolf 1984).

The concentration of airborne substances in the working place which is safe for workers to be exposed to are called threshold-limit values (TLV). The most accepted TLV guides are those published by the American Conference of Governmental Industrial Hygienists (Anonymous 1979).

Measurements of concentrations of airborne particles are possible in various ways. Filtration with fine mesh filters provides quick, inexpensive sampling. The filter may be weighed before and after sampling to provide concentrations expressed as weight per unit of volume of air (Wolf 1984).

Mechanical controls such as filters, blowers and hoods are the most important steps in controlling respiratory hazards. Applying controls near the source of contamination generally uses less energy, costs less, and works best (Wolf 1984).

Insect cages do not provide protection unless air is exhausted from the cage and particles are removed before they mix with room air. If contaminated air cannot be exhausted outdoors, then it must be filtered before being recirculated in a room. The amount of air, dust-loading capacity, type of contaminant and smallest size of particle to be removed determine the type of filter needed. The most common filter medium is glass fibre because of its low cost and because the fibre diameter can be regulated during manufacture. In general, the finer the diameter, the higher the air-cleaning efficiency will be. HEPA (high efficiency particulate air) filters are recommended to adequately eliminate allergy, asthma, and pulmonary hazards (Zeterberg 1973).

In some work situations, such as transferring insect cages to cleaning stations, it may not have practical solutions from an engineering point of view. If these situations involve temporary exposure, then respirators may be necessary. Respirators must cover the mouth and nose and should be used in environments that would pose an immediate health threat to a worker without a respirator.

The aims for respiratory protection are to save workers from suffering and permanent injury and to prevent lost working time.

References

Anonymous. 1979. Threshold limit values for chemical substances in workroom air. *National Safety News* 119: 66–73.

Bauer, M. and R. Patnode. 1983. Insect rearing facilities. Agricultural Research Service, USDA. NIOSH, GHETA pp. 81–121. Springfield, West Virginia.

Bellas, T.E. 1982. *Insects as a Cause of Inhalant Allergies: A Bibliography.* Division of Entomology Report No. 25. 2nd ed. Commonwealth Scientific and Industrial Research Organization, Canberra City, Australia.

Bellas, T.A. 1983. *Australian Entomological Society News Bulletin* 19: 39–41.

Bellas, T.E. 1990. Occupational inhalant allergy to arthropods. *Clinical Reviews in Allergy* 8: 15–29.

Burge, P.S., G. Edge, I.M. O'Brien, M.G. Harries, R. Hawkins and J. Pepys. 1980.

Occupational asthma in a research centre breeding locusts. *Clinical Allergy* 10: 355–363.

Edge, G. and P.S. Berge. 1980. Immunological aspects of allergy to locusts and other insects. *Clinical Allergy* 10: 347.

Frankland, A.W. 1953. Locust sensitivity. *Annals of Allergy* 11: 445–453.

Kang, B. and J.L. Chang. 1985. Allergenic impact of inhaled arthropod material. *Clinical Reviews in Allergy* 3: (3) 363–375.

McDermont, H.J. 1977. Handbook of ventilation for contaminant control (including OSHA requirements). *Ann Arbor Science*, Ann Arbor, Michigan.

Olishifski, J.B. 1978. Lung function and respiratory protection. *National Safety News* 118: 47–56.

Ramazzini, B. 1940. *Diseases of Workers*. W.W. Wright (trans.), University of Chicago Press, Chicago, pp. 257–261.

Whiting, A.R. 1967. The biology of the parasitic wasp *Mormoniella vitripennis* [*Nasonia brevicornis*] (Walker). *Quarterly Review of Biology* 42: 334–406.

Wirtz, A.R. 1980. Occupational allergies to arthropods — documentation and prevention. *Bull. Entomol. Soc. Amer.* 26: 356–360.

Wirtz, A.R. 1984. Allergic and toxic reactions to non-stinging arthropods. *Annu. Rev. Entomol.* 29: 47–69.

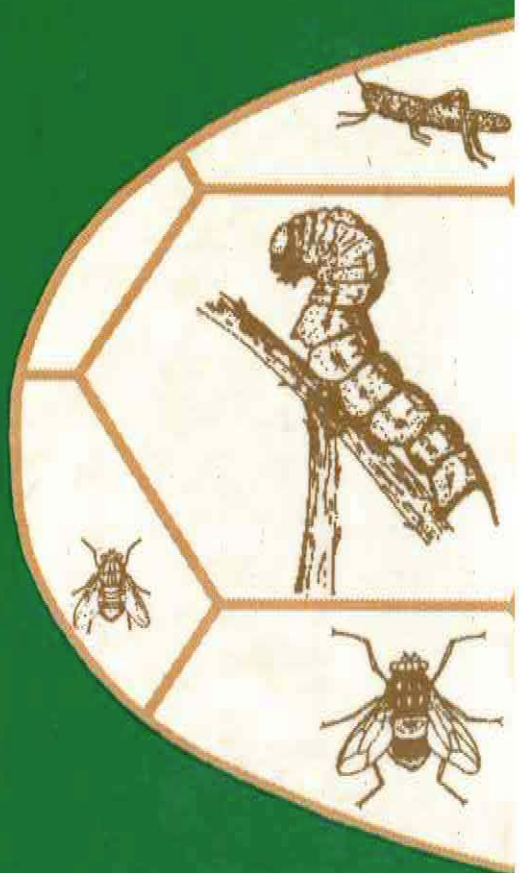
Wolf, W.W. 1984. Controlling respiratory hazards in insectaries, pp. 64–69. In: E.G. King and N.C. Leppla (eds.), *Advances and Challenges in Insect Rearing*. USDA-ARS-SR, New Orleans, La. 306 pp.

Wolf, W.W. 1985. Recognition and prevention of health hazards associated with insect rearing, pp. 157–165. In: P. Singh and R.F. Moore (eds.), *Handbook of Insect Rearing*, Vol. 1. Elsevier, Amsterdam.

Zeterberg, J.M. 1973. A review of respiratory virology and the spread of virulent

and possible antigenic viruses via air-conditioning systems. *Annals of Allergy* 31: 291–299.

ISBN 92 9064 065 X



ICIPE
Science
Press