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技術情報提供活動促進業務報告書

―林業分野プロジェクト国内委員会活動―

（下巻）

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個別技術情報支援のための質問書

1994 年 1 月 24 日

プロジェクト名　PNG森林研究計画

専門家名　　丸田　秀士

質問技術テーマ：天敵を利用した害虫防除の研究手法

1．質問技術テーマの具体的背景、及びそのプロジェクト活動の中での位置付け

当プロジェクトの主要研究トピックスの一つに挙げられている「森林昆虫および防除法に関する研究」の一環として、特に過去に大発生を起こした数種の昆虫に関しては、防除対策を確立すべく研究を進めている。

PNGのハイランド地方における主要造林樹種であるマツ類 *Pinus patula* の食葉性害虫として知られている *Lymantria ninayi* （マイマイガ）は、1976年から1978年にかけてゴロカ近郊のマツ造林地にて大被害を及ぼした。*L. ninayi* の大発生には周期があるため、発生予察を兼ねたモニタリングを定期的に行なっているが、今のところ再発の兆候は現われていない。従って、今のうちに防除策を確立することは、次回の大発生に備えて有効であると考える。

2．質問の具体的内容

L. ninayi の防除策として、その寄生性天敵である *Apanteles* sp. （コマユバチ）を利用する計画をたてた。現在は野外観察を行なっている段階であるが、実験を本格化するためには、害虫・天敵両種を累代飼育および大量飼育しなければならず、そのための施設が必要となってくる。そこで、新たに施設を設立する上での参考としたいため、日本を初めとする諸外国における、生物的防除の研究を行なっている機関が所有する施設について、その現状と問題点をお教えいただきたい。

3．期待する回答の範囲

施設の規模、必要な機材、人材（技術レベルおよび人数）

質問のキーワード；寄生性天敵、生物的防除法、大量飼育、マイマイガ、コマユバチ科

希望資料名；　　希望指導委員名；

1994年２月24日

「天敵を利用した害虫防除の研究手法」に対する回答

森林総研・北海道支所
昆虫研　前藤　薫

1．天敵昆虫を利用した害虫防除の手法と現状

天敵昆虫を利用した害虫防除法は、ごく大まかに①導入天敵の接種的放飼(inoculative releases of introduced natural enemies)と②土着・定着天敵の保護と機能増進(conservation and augmentation of native or established natural enemies)に分けられます。後者には、天敵の代替寄主（餌）の提供、天敵の大量放飼（inundative releases）などが含まれます。

①は有力な外来天敵を何処からか探してきて、定着するのに必要なだけの数を放飼する方法です。とくに害虫が侵入者（exotic invaders）である場合に有効であると考えられています。北米やオーストラリアに旧世界から侵入した森林害虫や果樹害虫に対して、原産地などから天敵昆虫を導入して被害の軽減に成功した例が数多くあります。日本でも中国から侵入した果樹害虫（カイガラムシ）などで成功例があります。

同じ天敵放飼でも、②の大量放飼は、①の接種的放飼とは大きく異なります。自然状態では不十分な土着天敵の数を人為的に補って害虫の死亡率を上げることが目的ですから、大量に増殖したきわめて多数の天敵を繰り返し放飼するのが普通です。集約的な害虫管理が可能な施設園芸などでは既に実用化されており、日本でもオンシツコナジラミの寄生バチやハダニを捕食するチリカブリダニなどが利用されています。中国や北米では食葉性害虫に対して卵寄生バチを大量放飼する試みが行われていますが、少なくとも林業害虫では実用化に至っていないようです。

一方、温帯の果樹園では、天敵の越冬場所となるよう生け垣の樹種を選ぶなどの工夫がなされており、これも②の一例と言えます。

①②いずれにしても、天敵の特性について、事前に十分な研究が必要であることは言うまでもありません。

2．Lymantria ninayiの場合

外来天敵であるApanteles sp.をこれから導入しようという計画なら①のケースになります。とくにLymantria ninayiが侵入害虫なら、是非とも試みるべき防除法と言えます。ただし、Apanteles sp.以外に導入することが望ましい天敵はいないのか、検討すべきでしょう。

Apanteles sp.は有力な土着天敵だが分布域が限定されているため、未生息地域に接種的放飼を行いたいという状況も、①のケースに準じて考えることができます。

以上のケースでは、生活環の同調など定着の条件が整っていれば、ひとつの地域に数千頭から多くても数万頭を放飼すれば定着が可能と思われます。逆に、定着に必要な条件が不都合だといくら多数の個体を放しても定着しません。

Apanteles sp.が土着天敵の場合、マイマイガのような突発大発生型の森林害虫に対して多少の数を一時的に「大量」放飼しても、防除効果はあまり期待できないだろうと思います。大発生時の虫密度がどれ位か分かりませんが、十分な死亡率を得るには莫大な数の天敵を放飼する必要があるはずです。十分な数の天敵をもっとも適切な時期に供給するには、天敵の大量増殖だけでなく保管技術の確立も肝要です。一般的には、幼虫寄生バチよりもむしろ卵寄生バチの方が大量増殖は容易です。また、大量放飼よりも、野外での代替寄主の確保など、土着天敵の活動しやすい林を造るほうが現実的かもしれません。

いずれにせよ、Apanteles sp.にこだわらず、天敵全般（寄生性だけでなく捕食者や微生物天敵も）の働きが野外でしっかりと評価されていることが前提であり、このことは大発生を予察するためにも不可欠です。また、ＢＴ剤（昆虫寄生細菌の毒素）のようにすでに実用化されている防除法の応用も検討されては如何でしょうか。

3．施設、機材、人材など

マイマイガの仲間は飼育が比較的容易で、１ｍ^{3}程度の空間があれば５百頭前後の飼育が可能です。①のケースなら大きめの飼育室がひとつあれば何とかなるでしょうが、大量放飼による防除を実用化するならもっと大きな施設が必要になります。寄生バチを飼育するための網ケージや羽化箱など、寄主と天敵の生活史に合わせて工夫します。寄主の卵や天敵の保存には低温庫が必要でしょうし、寄主や寄生バチが要求する発育条件によっては飼育室の温度や日長の調節が必要かも知れません。通常の昆虫実験器具のほか、微生物天敵も扱うとなれば滅菌器や隔離飼育室なども必要です。

管理・指導者として昆虫技術者がいてマニュアルが作成してあれば、飼育作業そのものは単純作業者に任せることができます。

DeBachら（1964）の古典的なテキストから生物的防除施設の実例が示してある部分を、また昆虫の大量飼育に関する最近のテキストから飼育室デザインと飼育管理の一部をコピーしておきました。参考になれば幸いです。

HANDBOOK OF INSECT REARING VOL. I

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INSECTARY DESIGN AND OPERATION

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1. INTRODUCTION

Every insect production program is unique in its purpose and available resources, including rearing facilities, equipment, and personnel. Organization and management of these resources determine program success that is measured by the ability to efficiently produce adequate numbers of quality insects (Chambers, 1977). To this end, the facility is a critical component that delineates environmental zones, confines insects, protects colonies from disease and other contaminants, establishes a route for the movement of materials, and provides an efficient and professional work environment.

The design and operation of a suitable facility are based on the purpose of the program, such as insecticide screening, sterile insect technique, or pathogen production; the biology, physiology, behavior, and ecology of the colonized species; the degree of insect quality desired; and the needs of personnel. These factors dictate the type of diet (i.e., natural vs. artificial), environmental conditions, rearing containers, pupal harvesting procedures, adult holding cages, and oviposition substrates. It is essential to consider the potential for microbial contamination of the diet as well as the susceptibility of insects to diseases and specific stresses such as overcrowding, dietary chemicals, and extremes in environmental conditions. Finally, consideration must be given to the number of employees needed, their health and safety, budgetary constraints, organizational unity, efficienty, and aesthetics.

The building design can be unique, duplicated from an existing facility, or derived by adopting some components and developing others (Leppla and Ashley, 1978). Unique designs, while sometimes expensive to develop, are justified because their precise function and improved flexibility are most likely to achieve program objectives. Conversely, when the design of an existing facility is copied without change, architectural and engineering costs are reduced, but shortcomings are retained and unique features precluded. Usually, though, a design is based on one or more successful facilities and incorporates unique program features. This approach requires caution because taking rooms or work areas out of context can significantly alter their functions. Thus, every effort should be made to integrate only those aspects that are required and that have been proven effective.

We emphasize multi-room facilities for rearing Lepidoptera as an example in which one or more employees conduct independent operations in specialized areas having specific functions and between which insects and materials are transported. However, the principles are applicable to smaller rearing programs and with modification to those involving other kinds of insects. Using insect rearing functions as design criteria, factors are identified that enhance the effectiveness of each area and ultimately contribute to a unified rearing facility.

2. ENVIRONMENTAL ZONES

An insectary consists of two major environmental zones based on the type of specialized area and the degree of environmental control required (Table 1). The first zone includes the operations center, quality control laboratory, work areas, and hallways that are maintained with temperature and relative humidity appropriate for human comfort. Conditioned air entering the clean work areas should pass through a series of increasingly finer-mesh filters ending with a High-Efficiency Particulate Air (HEPA) or absolute filter (Griffin, 1984). Because of potential contamination, air from dirty work areas is exhausted outside the building away from any fresh-air intake vents.

Table 1. Environmental zones and specialized areas of rearing facilities.

AREA	TYPICAL COMPONENTS	COMMENTS
Zone[a]		
Work Areas	1. Clean Work Rooms a. Diet preparation b. Surface sterilization of eggs and pupae c. Set-up (placement of insects on diet)	Highly restricted access; isolation from rest of facility; HEPA filtered supply air.
	2. Dirty Work Rooms a. Pupal harvest b. Container washing c. Waste disposal	Highly restricted access; isolated entrance; isolation from rest of facility with autoclave and passthroughs controlling flow of materials and products; air not recirculated to rest of facility.
Storage	1. Cold Storage (dietary ingredients) 2. Clean Storage (sterile, reusable containers) 3. Supply Storage (parts for equipment, filters)	Highly restricted access to clean storage.
Personnel	a. Showers b. Lockers c. Toilets d. Lunch room	
Mechanical Room	a. Boiler b. Water heater c. Motor-control center d. Phone board	

Shop	a. Construction b. Repair of equipment	
Operations Center	a. Environmental monitors b. Offices c. Meeting rooms d. Administration	Location for recorders, data logger, computer terminal, files, alarms, intercom to rest of facility.
Quality Control Laboratory	a. Quality assessment and control b. Process development c. Pathology	Restricted access; contains testing devices and environmental chambers; isolated area within laboratory for reverse photoperiod.
Zone 2		
Insect Holding	1. Maintenance of Reproductive Colonies a. Larvae b. Adults	Restricted access; precise control of environmental conditions; highly restricted access to quarantine.
	2. Mass Production 3. Treatment 4. Quarantine 5. Special Projects/ Process Development	

[a]Environmental conditions are maintained for worker comfort in Zone 1 and for optimal insect development in Zone 2.

The second zone consists of insect holding rooms in which the temperature and relative humidity are carefully controlled. The primary concern is to control conditions within rearing containers. Since containers inhibit the exchange of heat, moisture, and gasses their internal environment may not reflect ambient conditions within the holding room. Therefore, enough space should be provided to allow air flow between them while air within the room is circulated as uniformily as possible (Harrell et al., 1979; Owens, 1983). Special air filtration systems are often required, especially in adult holding rooms, to eliminate airborne body and wing scales (Wirtz, 1984). Air from holding rooms should not be recirculated unless it is filtered to remove microbes. Holding rooms, especially those with external walls, should be well insulated and capable of maintaining conditions during brief periods when the air conditioning system is inoperable.

3. ENVIRONMENTAL CONTROL

Environmental conditions should be continuously monitored in a centralized area. The master control panel for an air-handling system contains temperature and RH indicators, magnahelic and other gages, switches, and lights indicating normal and abnormal functioning of criticial components. Controllers, relays, valves, and other equipment are mounted inside the panel. Time switches that control photoperiod in holding rooms and other operations, such as after-hour illumination of UV lamps in clean work areas, should be mounted together near master control panel. The switches can then be easily checked for accuracy and reset if necessary. In some cases, automatic phone-dialing devices have been wired into alarm circuits to notify specified individuals in

case of equipment failure (Fisher, 1984a). Operation manuals for equipment and emergency trouble-shooting charts (Fisher, 1984b) must be available.

Humidification devices include air washers, steam injectors, evaporative pan systems, and centrifugal atomizers. Steam is the most effective for achieving relatively high humidities required by adults of many species. Whichever type of humidification device is used, regular cleaning and maintenance are required to remove minerals and other impurities (e.g. insect body and wing scales) that buildup and reduce performance.

Maintaining extremes of relative humidity in separate areas, e.g. 80% for adults and 40% for larvae, with one air-handling system is energy inefficient. Recirculated air from adult holding rooms must be dried by chilling it to a temperature that will provide 40% relative humidity for larval rearing. Air returned to adult holding rooms must then be reheated and rehumidified. This problem can be solved by installing separate air-handling systems for each humidification zone (Fisher, 1984a).

Lighting requirements correspond with specific activities conducted in insectary rooms. In most work rooms, if possible, natural lighting from windows should supplement artificial sources to improve light quality and relieve boredom. General lighting should probably exceed 30 ft-c while lighting at work benches or in hoods, where precise and careful activities are performed, should exceed 100 ft-c. Artificial lighting, maintained according to a regimen, should be the only source of illumination within insect holding rooms.

Light fixtures should be insect and moisture proof, and be flush-mounted or suspended at a distance from the ceiling that permits complete sanitation. In holding rooms with high ceilings, vertical banks of fixtures may be required for the most uniform distribution of light. Nightlights are sometimes used in oviposition rooms (Debolt and Petterson, 1978) and a time switch with dimmer circuit may be used to simulate twilight.

Air is circulated as uniformily as possible within rooms to avoid dead air space and vertical stratification of temperature. The location of supply and return ducts depends on the size and shape of the room as well as the arrangements of racks and other items that will disrupt air flow patterns. Installing these ducts too close together will result in supply air being exhausted prematurely without adequate circulation within the room. Temperature sensors are mounted about 60 inches above the floor on an interior wall so that outside conditions do not influence readings. These devices should sense "typical" air within a room and should therefore be located away from air supply vents and drafts caused by opening doors. Usually sensors are most effective when placed near return air ducts. In the case of RH control, humidistats are located almost anywhere in the room, because, unlike temperature, water vapor will not stratify or form dead spots. However, they should not be placed where airborne particulates, such as insect body and wing scales can acumulate and alter their function.

Air filtration within an insectary protects air-handling equipment, contains insects, and reduces the spread of microbial contaminants and unhealthful particulates. Primary filtration is accomplished with disposable roughing filters composed of oil-wetted or unwetted fiberglass and other synthetic fibers. This type of filter is often placed inside grills of return vents to prevent the movement of insects into ductwork and minimize the buildup of dust on the fan impeller and heat-exchange coils. Another roughing filter may be inserted just prior to secondary filtration devices used to disinfect the airstream. Filters are cleaned or changed regularly to maintain air flow and optimize the heating or cooling capacity of the system. In some cases, blockage may cause low pressure in the compressor resulting in coil freezeup. Operating without filters may cause clogging of heat transfer surfaces and result in the same problem. A magnahelic cage is often used to measure the pressure differential across a filter bank and indicate when it should be replaced.

Air filtration equipment includes ultraviolet irradiators (UVI), electrostatic precipators (EP), and high-efficiency mechanical filters. Sterilization using UVI requires direct contact between radiant energy and microorganisms that can be protected by the "shadows" of carrier particles. In addition, UV lamps loose effectiveness over time or when coated with dust. Electrostatic precipators are ineffective at removing large particles and insects. High (or ultra-high) efficiency particulate air (HEPA or UHEPA) filters are the most reliable and economical means of sanitizing the air. These filters are effective in eliminating particles greater than 0.3 μm, essentially sterilizing the airstream. To ensure their most effective use, these filters are located as close to supply vents as possible. HEPA filters offer considerable resistance to air flow so high-speed fans are required. To minimize the resultant increase in noise within the facility, silencers, anti-vibration mountings, and flexible connectors may be required between fans and ducts.

Regular maintenance and evaluation of the facility and its equipment aids the production of quality insects and the prevention of contamination by microorganisms. Fume hoods, the autoclave, and components of the air conditioning system such as filters, blowers, compressors, pumps, and controllers are maintained by service contract. Spare parts like filters, fan belts, and sensors are stored within the facility or in an adjacent building. Space allocated for this purpose depends on the distance from suppliers, availability of parts, replacement schedules, and reliability of equipment. Continuous monitoring of temperature, relative humidity, and lighting shows anomolies and identifies problems with equipment. Finally, all alarm circuits, including those that monitor temperature, pressure, and equipment function should be tested routinely.

4. CONTAMINATION CONTROL

A successful facility excludes, or provides an environment for elimination of microorganisms that can adversely affect insect colonies (Table 2). Even if not lethal, pathogens and dietary contaminants may alter insect quality. Their prevention and control depends on effectively containing and protecting the insects, providing optimal environmental conditions, establishing a unidirectional flow of materials and products, and restricting the movement of employees.

Table 2. Potential contamination sources in a multi-species insect production facility (Fisher, 1984b).

Potential source	Relative risk	Control measures
1. Air conditioning supply ducts		Absolute filters eliminate >99% of all microorganisms <0.3 micrograms
A. Outside air	Moderate	Magnahelic gages indicate effectiveness of filters
B. Air recirculated within building	Significant	Ceiling-mounted germicidal lamps reduce air and surface contamination during nonwork hours
2. Auxiliary air from fume hoods	Moderate	Hood design insures auxiliary air will not enter rooms

		Hood safety switch will not allow auxiliary air to flow when exhaust fan is not operating
3. Personnel	Very significant	Critical rooms are restricted to authorized persons
		Three-way intercom eliminates unnecessary employee traffic
		Personnel training in microbiological concepts and sanitary techniques
		Static foot mats to collect dirt
		Protocols (hand washing, clean lab coats) required before entering critical areas
4. Dust and dirt build-up on floor	Moderate	Covered floors for easy clean-up
		Regular sweeping, vacuuming, and mopping with germicidal detergent
5. Leakage of contaminants through passthroughs and double-door autoclave	Significant	Germicidal lamps in pass-through doors
		Well sealed passthrough doors
		Passthrough use training
		Factory installed contamination barrier around autoclave
6. Leakage of contaminants around doors	Moderate	Refrigerator-type, magnetic seals
7. Miscellaneous materials taken into critical areas (dietary ingredients, rearing cups, etc.)	Moderate	Materials removed from shipping boxes before being moved into areas
		Wash containers with sodium hypochlorite
8. Reusable materials (larval containers, adult cages)	Very significant	Strict washing procedures
		Steam-sterilize with direct passage into clean storage
		Sanitation of most plastics in sodium hypochlorite

		Use "autoclave tape" to ensure that sterilizing conditions are met
9. General	Variable	Clean hoods and work surfaces with germicidal detergent after use
		Monitor contamination levels with agar plates
		Empty trash cans each day
		Pick up escaped insects immediately
		Design building to ensure isolation of activities

The general rearing environment, including artificial diet, is evaluated on a regular basis for the presence of microbial contaminants to ensure sanitation of the facility and proper functioning of insectary equipment (Sikorowski, 1975; 1984). Swabs taken from walls, floors, and equipment can be plated on general media such as potato dextrose agar and trypticase soy agar for fungi and bacteria. Results are used to indicate the level of contamination and effectiveness of sanitary procedures. The effectiveness of passthroughs is monitored by exposing open agar plates inside the unit for 15 min before and then after illumination of ultraviolet lights. Indicator tape that changes color upon exposure to sterilizing conditions should be used in the autoclave with every load. Experience with microbial assessment reveals the most important sources of contamination that can then be monitored more intensively.

Since diseased insects and contaminated containers represent the most concentrated sources of inocula, the facility design must provide for isolation, treatment, or expedient removal of these materials. The most critical part of this scheme is the dirty work area where pupae are harvested, containers are washed and sterilized, and spent diet and non-reusable containers are discarded. Negative air pressure, restricted access, an outside entrance, passthroughs, and strict sanitary procedures are required to effectively reduce contamination potential from this area. To further isolate contaminants and protect the health of employees, containers are harvested within a fume hood. Reusable materials such as cages, containers, glassware, and instruments are sterilized in a steam autoclave while heat labile items are sanitized by soaking in a tank of disinfectant solution. Wastes are placed in a receptacle lined with a plastic bag and immediately removed from the facility.

Isolation of specialized areas is the primary basis of contaminant control. To illustrate (Fig. 1A), the holding rooms (HR) of a multispecies rearing facility contained larvae that regularly experienced outbreaks of virus and dietary fungal contamination. In the adjacent room (DP), artificial diet was prepared, poured, and allowed to cool on the table while containers with pupae were taken from holding rooms to another room for harvest. As a result, microbial contaminants were spread to the diet cooling in open containers. Despite sanitary procedures, strict regulations, and the expenditure of an excessive amount of time and resources, outbreaks were not controlled until the diet was prepared in an area isolated from insect colonies (Fig. 1B). Installation of a wall and an ultraviolet light equipped passthrough (P) provided a barrier to intrusion of contaminants into the diet preparation room. To complement such an improvement, all holding rooms and the diet preparation area were thoroughly disinfected and insects were continuously monitored for quality and disease incidence.

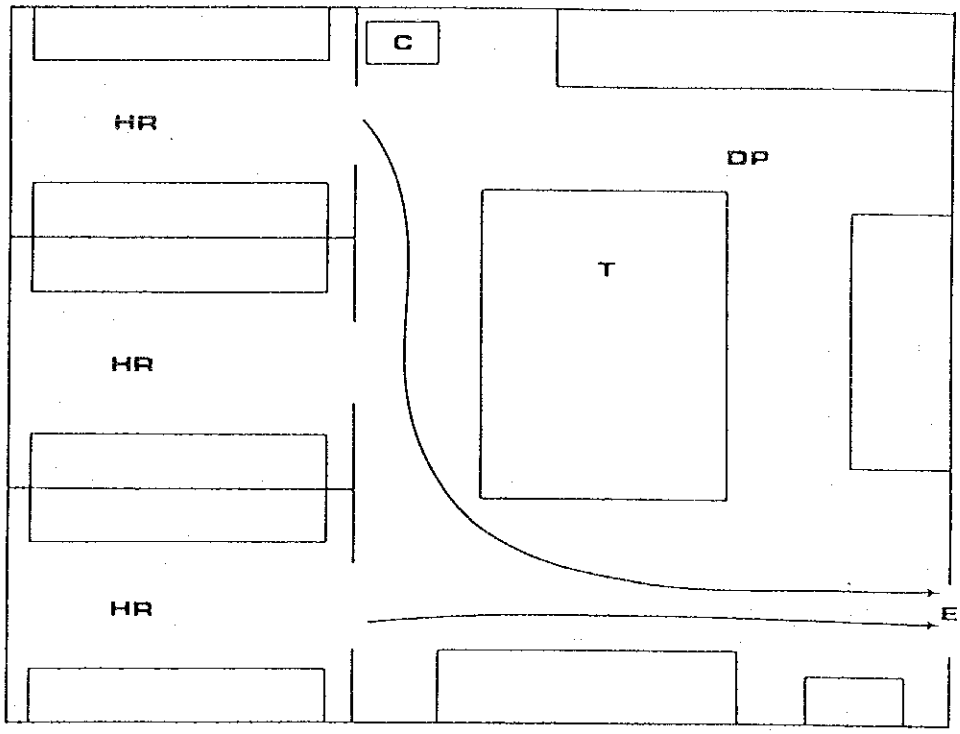

Fig. 1A. Uncontrolled route (arrows) of contaminated rearing containers from insect holding rooms (HR) through diet preparation (DP) and the unrestricted entrance (E) to site of pupal harvest. Prepared artificial diet is dispensed and allowed to cool in uncovered cups on a table (T) where it is exposed to airborne contaminants. Several hours later, cups are implanted with eggs, capped, and taken to holding rooms on a cart (C).

During the process of designing a facility, each stage of all species being reared should be evaluated for its mobility, handling requirements, susceptability to disease, and potential as a source of inocula. If a species poses particular problems, the design is modified (Fig. 2). Containers, with larvae that are unusually susceptable to disease or reared under conditions that promote the growth of dietary contaminants are isolated from other species and their transport is restricted within the facility. The larval holding room is located next to the pupal harvest room and a passthrough is placed between them for direct transfer of rearing containers. Illumination of germicidal lamps within the passthrough prevents backflow of contaminants from the harvest room into holding rooms. In addition, the entrance to the harvest room is installed so that it opens directly to the outside.

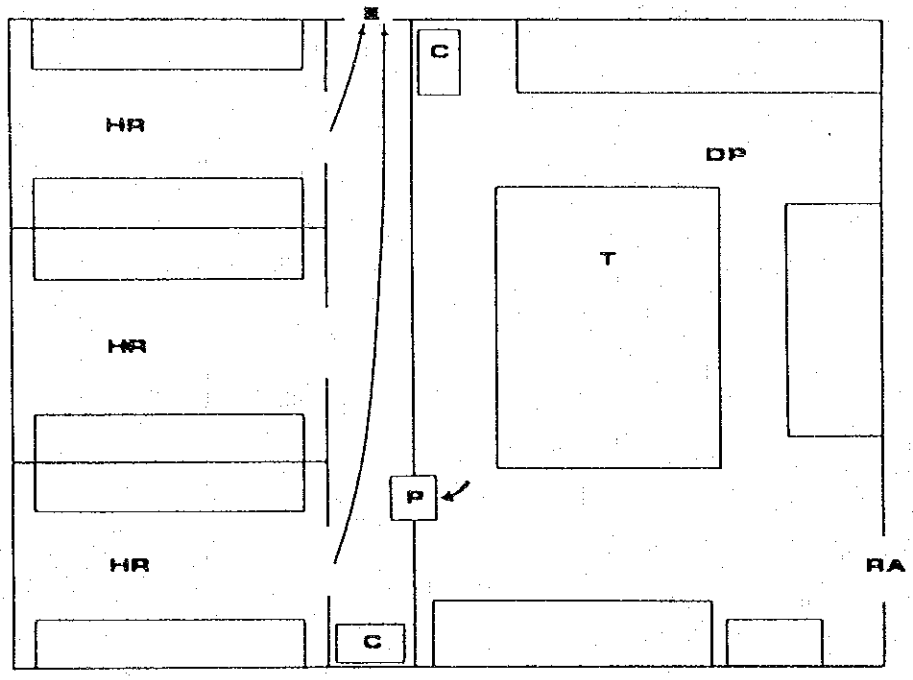

Fig. 1B. Controlled route (small arrows) for rearing containers from holding rooms (HR) to site of pupal harvest. In the diet preparation room (DP), diet is dispensed into cups and allowed to cool on a table (T). Afterward, cups are implanted with eggs, capped, and transferred to the larval holding area (large arrow) on a cart (C) via a passthrough (P). The passthrough is illuminated with germicidal UV light for 15 min to sterilize the interior and prevent contamination from entering the diet preparation room. Entrance (E) provides passage to holding rooms and restricted access (RA) is maintained to the diet preparation area.

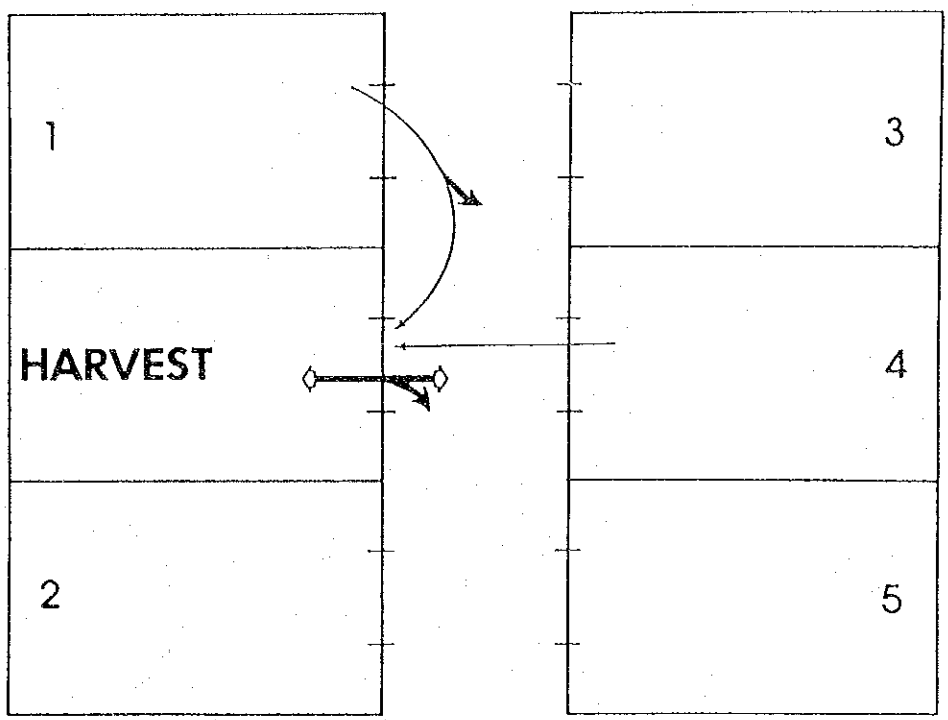

Fig. 2A. Movement of materials (small solid arrows) and employees (open arrows) between an inadequately isolated harvest room and adjacent insect holding rooms (numbered). Large solid arrows indicate uncontrolled potential sources of contamination.

Other specialized rooms should be considered in the design to help eliminate microbial contamination (Fig. 3). Eggs and pupae used to maintain the reproductive colony are routinely surfaced-sterilized with sodium hypochlorite or formalin in a room accessable from a hallway that services the holding rooms. Eggs are taken directly into this room while pupae are received via a passthrough after they have been separated from containers and spent diet in the harvest room. After treatment, eggs and pupae are passed into a set-up room where they are placed on diet or in emergence cages. Then, they are distributed to their respective holding rooms via passthroughs. Access to the set-up room can be gained only through the sanitary section of the facility.

The storage of ingredients used in artificial diets presents a unique set of contamination problems including stored-product pests, dust, and nutritive residues that can support the growth of microorganisms. Therefore, the storage area should be adequately isolated from the room where diet is prepared. A walk-in regrigerator is often used for this purpose as well as for preserving ingredients. Ideally, food items are stored in plastic containers with tightly fitting lids for protection against moisture. If materials are stored in cardboard boxes or porous bags, they should be placed on pallets.

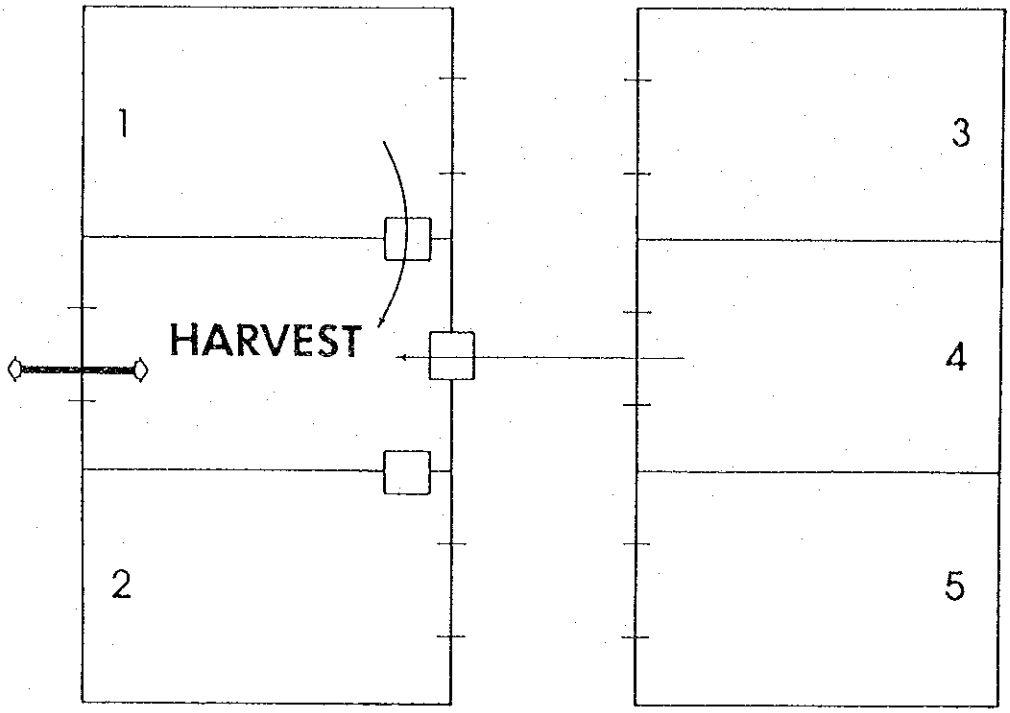

Fig. 2B. Controlled movement of materials and employees between the harvest and insect holding rooms. Employees enter the harvest room directly from outside the building.

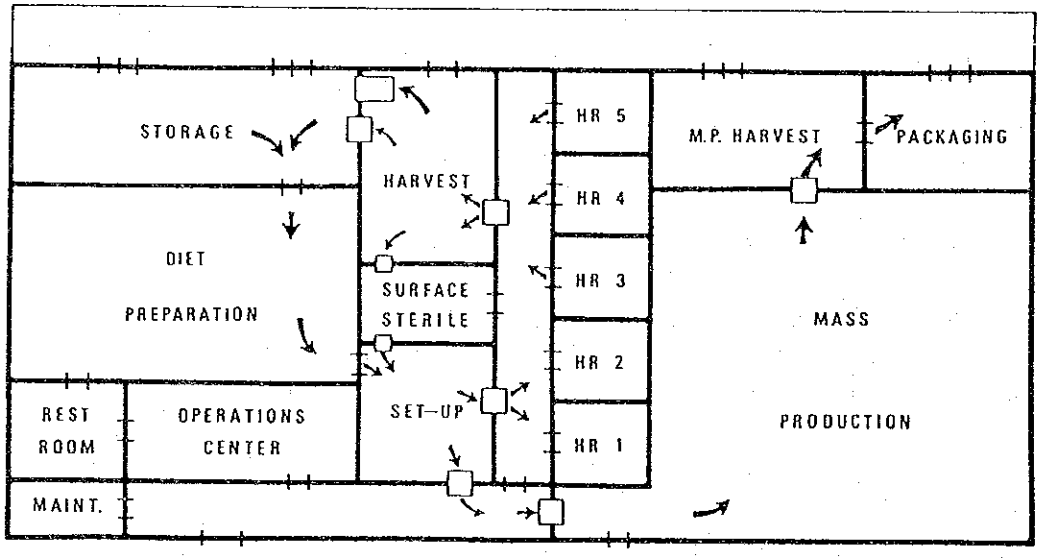

Fig. 3. Traffic pattern in a facility for mass rearing Lepidoptera (modified from Leppla et al. 1982). Arrows indicate unidirectional movement of materials and insects from storage (upper left) through diet preparation, set up, holding (HR) or mass production (MP), to the harvest rooms. Eggs and pupae are surface sterilized before being transferred to the set-up room and containers are recycled to storage. Cubicles in the walls are passthroughs and the rectangle between harvest and storage rooms is a double-door autoclave.

5. INSECT CONFINEMENT

Restricting insects from moving outside designated areas, reduces the potential for spreading contaminants and improves maintenance efficiency. Larval rearing containers and adult cages comprise the first level of confinement. Selecting the most appropriate ones depends on the ability of the insects to escape and on their acceptable communal density (Peters and Barbosa, 1977). Other considerations include the type of diet and the size, composition (gas exchange and light transmission properties), price, availability, and reusability of the containers (Burton and Perkins, 1984). The facility is designed to ensure that containers can be easily handled, washed, sterilized, and stored. Thus, consideration is given to appropriately sized and shaped passthroughs, dunk tanks, autoclaves, sinks, carts, insect-storage racks, and equipment to dispense artificial diet. In addition, flexibility is incorporated into the design to accomodate different containers that may be required in the future.

Insect holding rooms, the second level of insect containment, should be large enough to prevent overcrowding. There should be enough of them to separate closely related species, immatures and adults, and those susceptible to the same diseases. To impede the movement of insects from holding rooms, doors fit tightly into bucks. Insect-proof refrigeration type magnetic seals also have been used to secure the perimeter of steel doors. The wall and floor junction is an ideal location for harboring unwanted organisms and allowing their movement throughout the facility. Vinyl cove base cemented to the wall is inadequate in holding rooms where chewing larvae (e.g. Heliothis spp.) frequently escape the first level of confinement. These insects easily can chew under or into the base in search of an isolated pupation site. This behavior makes sanitation virtually impossible and, in an extreme case, has even undermined the structural integrity of wooden subflooring. Space around electrical receptacles, pipes, and light switches is filled with calking or expandable foam. Electrical receptacles are fitted with tight, weather-proof covers, and grills and prefilters are used in return ducts to keep insects from entering the air conditioning system and being spread to other areas of the facility. In addition, blacklight traps may be required in certain rooms to capture escaped adults. These considerations are especially important in quarantine facilities where exotic species are reared.

The final barrier to insect movement is the isolation of rooms that require a sanitary environment. A double-door system is effective in isolating an internal room, especially if insect traps are placed in the adjacent external room or transition vestibule. Positive pressure generated by the air conditioning system repels small organisms. The room where eggs are larvae are placed on diet is thoroughly cleaned and sanitized after each use. Equipment, supplies, or funishings are decontaminated before being moved into these rooms. Traffic between areas is restricted and all employees are completely trained in sanitary techniques.

6. CONSTRUCTION MATERIALS

Construction materials and techniques are an integral part of environmental control capabilities (Griffin 1984). Smooth and uncluttered walls and ceilings should be protected with a high-quality, impact resistant material that will prohibit intrusion of moisture and the growth of microorganisms, and that will withstand scrubbing and treatment with steam or germicidal solutions.

Most paints, while easy to apply, are unsatisfactory because they provide little protection against moisture, impact, and cracking or peeling over time. Catalyzed epoxy resins with high solid content offer much greater protection and may be used over drywall, masonry, metal, or wood to a thickness of 8 mil or more. When using these materials, care must be taken to avoid loss of adhesion from improper mixing or application under adverse conditions. Even

after application, delamination may result when water enters cracks or joints and dissolves the substrate.

Ceramic veneer applied to concrete, concrete blocks, masonry, and wood over wire lath is an excellent surface for reducing impact damage and maintaining sanitary conditions. However, it is expensive and, unless properly installed, is not impervious to the intrusion of moisture which can create an environment that supports the growth of microorganisms. Fiberglass-reinforced panels mounted to any substrate are economical, durable, and easily cleaned. Care is taken to ensure that seams are properly sealed and that the surface is not abraded during sanitation. To protect the integrity of the wall system and facilitate sanitary procedures, insect cages are held on mobile racks rather than on shelves mounted to the walls. Bumper strips on walls and steel or plastic corner pieces prevent damage by carts and racks.

Ceilings in an insectary should not be of the suspended type as the panels are not sealed to "T" supports, are difficult to keep clean, and provide spaces that harbor insects and allow them to move from one area of the facility to another. Conduit and utility pipes are run inside the walls, ceiling, or in a corridor isolated from the rest of the facility. If piping must be exposed in any room, it is separated from the wall or ceiling so that the area behind it can be cleaned and sanitized.

Insectary floors should be non-porous, resistant to chemicals, easily cleaned, waterproof, non-slip, monolithic, hard enough so insects cannot chew through them, and capable of supporting point loads from carts and racks. Further, floors should be free of depressions where water may accumulate and slightly pitched for efficient drainage. Floor drains, for the use of hoses and steam for cleaning, are covered if not used regularly and primed with water periodically to avoid contamination of the facility by sewer gases. If drains are not installed, a wet/dry vacuum may be used.

Concrete flooring is durable and can be treated with abrasives, metallic aggregates, and other admixtures to reduce dust and prevent moisture intrusion. Quarry tile, unglazed floor tile made from clay, is durable, impervious to water and staining, and resistant to abrasion. But, tiles are slippery when wet and are often jointed with grouting that discolors and provides areas for bacterial growth. Asphalt resilient flooring is not recommended because it separates from the substrate when regularly exposed to moisture and indents with point loads. Linoleum also indents, but it is durable, resilient, and resistant to the intrusion of moisture from above. However, it is not recommended for applications where dampness can penetrate from underneath such as in concrete slabs below grade. Perhaps the best floor-covering materials are the synthetic resins such as epoxy, urethane, and polyester mixed with fillers to add color or surface texture for improved traction. These materials can be sprayed, rolled, or trowelled in a monolithic layer that is easily extended up the walls to provide a coved junction for easy cleaning. Floor resins applied to concrete, wood, and other base materials in various thicknesses are durable, and resistant to moisture, chemicals, and indentation from point loads. However, catalyzed resins require carefully controlled mixing and application over properly prepared substrates.

7. TRAFFIC FLOW PLAN

Traffic flow plan is defined as a route among and through rooms for the movement of insects, materials, and employees (Fig. 4). It is designed to facilitate efficiency and ensure isolation of specialized areas such as clean and dirty work areas, insect holding rooms, and quarantine. Incorporating a workable plan into the facility design is most effectively achieved by preplanned placement of rooms rather than by forcing a route after rooms have been located. It can only be achieved with a thorough knowledge and understanding of what operations and activities will be performed in each area.

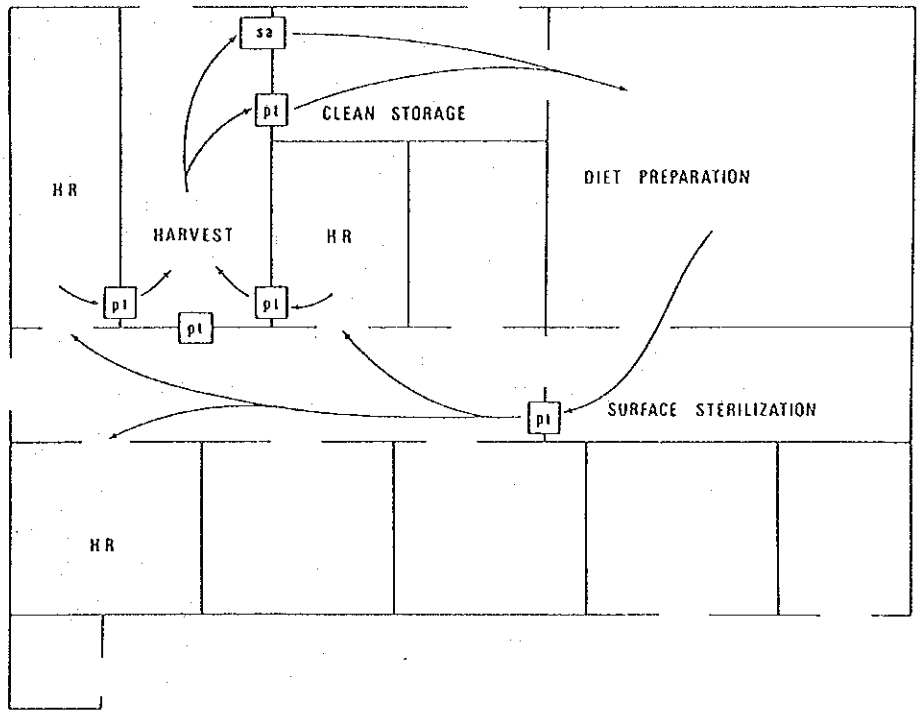

Fig. 4. Flow plan for materials and insects within a medium-sized multispecies insect rearing facility that supports insecticide screening (Fisher, 1983a). Containers from clean storage are filled with diet, planted with eggs in the clean work area (rooms in the upper right) and distributed to appropriate holding room (HR). After pupation, containers are passed directly into the harvest room via passthroughs (PT) where they are harvested, washed, and sterilized in a double-door autoclave (SA). Nonautoclavable materials are soaked in a sodium hypochlorite solution before being placed in the PT leading to clean storage where they are removed and stored until further use.

The flow of materials within a facility must also be regulated. All reusable items such as containers and cages that have been exposed to insects or other sources of microbial contamination are sterilized, preferrably in a steam autoclave. These materials are not exposed to contamination before being reused. To ensure this, a double-door autoclave is installed with the control end in the dirty work area and the remote end opening into the clean-storage room. Contaminated materials are put into the unit in the harvest room, sterilized, and taken out in clean storage. Non-autoclavable materials such as polyethylene or acrylic are sterilized in a disinfectant solution and transferred to clean storage via a dunk tank or UV-equipped passthrough.

Laboratory carts are frequently used to transport materials and insects within a facility. Their use should be restricted to avoid transmission of contaminants from one area to another. It is recommended that each area have its own cart and that sanitary transfer points be designated between areas. Laboratory coats are likewise restricted to specific areas and not worn throughout the facility. Protocols are adopted requiring regular laundering of coats.

Ultimately the effectiveness of the flow plan and isolation scheme depends on their acceptance and use by insectary personnel. All systems can be disrupted if the design is inadequate or if employees are improperly trained. Physical barriers can be used to prevent excessive employee traffic within the facility. Primary doors to restricted areas should have locks and signs indicating limited access. Other doors to these areas, often required by safety codes, should remain locked from the outside and be used only in emergencies. Static or other types of footmats are placed at entrances to clean work areas. A passthrough is inserted where the flow of materials cannot be interrupted and the movement of employees must be restricted. In addition, regulations can be developed to limit access to specific individuals or certain times of the day. Phones equipped with intercoms and placed in the diet preparation and harvest rooms, the control center, and in the hallway are used to avoid unnecessary employee traffic, aid in coordinating activities, and assist during emergencies. For sanitary purposes, an intercom system is used which does not require handling.

Scheduling of insectary activities throughout the day organizes employee traffic, distributes demands for equipment, and reduces contamination potential (Table 3). For example, it is inadvisable for a person to harvest pupae prior to preparing artificial diet. Daily schedules progress from activities conducted in clean areas to those performed in rooms where the contamination level increases.

Table 3. Generalized hierarchy for scheduling insectary activities.

1. Prepare facility for use (unlock doors, warm up autoclave, check environmental conditions, etc.).
2. Prepare artificial diets and cool in set-up room.
3. Sanitize diet preparation and clean storage rooms (restrict access).
4. Harvest, surface sterilize, and quantify eggs.
5. Place eggs or larvae on diet.
6 Sanitize set-up room (restrict further access).
7. Move containers to larval holding rooms.
8. Harvest, surface sterilize, and quantify pupae.
9. Place pupae in emergence cages.
10. Sanitize surface-sterilization room (restrict further access).
11. Put pupae in adult holding rooms.
12. Sanitize insect holding rooms.
13. Wash and sterilize reusable containers.
14. Sanitize harvest room.
15. Record production data, insect quality; materials, equipment service and attendance.
16. Prepare to close facility (turn off equipment, check environmental conditions, etc., lock doors).

8. WORK ENVIRONMENT

The environment in which insect production occurs influences the attitudes and performance of employees. Thus, because the most important component of quality insect production is the insectary employee, the effectiveness of the entire program may be jeopardized by a poor work environment that includes health and safety hazards, inadequate space, illogical organization of equipment and supplies, and a lack of cleanliness. In a recent example, airborne wing scales in a room containing ovipositing moths forced an employee to hurry through egg harvest to avoid prolonged exposure. In the process, moths escaped, egg sheets were dropped and stepped on, and the sheets from some cages were not harvested. In another example, walls of a diet preparation room were splattered with dried diet, dust covered most surfaces,

and cockroaches occurred in large numbers. Under these conditions, employees showed little care in the performance of their duties and were unconcerned about maintaining sanitary conditions.

Health and safety problems often associated with insect production include insect wing scales and other body parts, microorganisms, chemical powders and fumes, dusts from dietary ingredients or vermiculite, aerosols, and sanitizing agents. Additional hazards involve steam or heat from diet preparation equipment, ovens, and autoclaves, radiation, electrical shock, and slipping on wet floors. These and other potential problems should be reflected in the design of the facility and development of procedures. Fume hoods, chemical storage cabinets, pressure-relief valves, scale collectors, safety glass, dust masks or respirators, fire extinguishers, and eye baths are used to minimize these problems. Employees are made aware of these hazards through proper training.

Work space, equipment, and access to materials and supplies should be organized in the most efficient and practical manner (Goodenough, 1984). By so doing, employees are encouraged to work within the system and not try to circumvent it. To avoid unnecessary employee traffic, supplies and materials are readily available where they will be used. Developing a functional design necessitates knowledge of procedures that are to be carried out in every room and an understanding of the requirements associated with these procedures, such as equipment, storage, and utilities. Enough work space is provided to comfortably accomodate equipment and personnel. Cramped conditions cause mistakes; excessive space encourages laziness and unnecessarily increases costs.

Aesthetic aspects of a rearing facility are often over-looked during its design. The internal environment must be pleasing and relaxing to promote maximal employee effectiveness. A color, such as light blue, that does not hide dirt or insects is preferred to stark white. A professional atmosphere is achieved by coordinating aesthetic characteristics throughout the facility. For example, two-tone colors of laboratory cabinets and hoods can be matched with wall and floor colors. This not only breaks the monotony, but also tends to signify unity of effort within the facility. Windows in areas other than insect holding rooms relieve boredom and provide a source of natural lighting. They should be double or triple glazed for security, insulation, and prevention of condensation.

9. CONCLUSIONS

We have described some of the variables that should be considered in designing and operating a generalized insect production system. The degree of importance that each variable receives depends on the objectives and limitations of individual programs. But every effort should be made to consider as many of them as possible, as early in the planning and design phase as practical. By considering the relationships of these factors, a truly integrated facility will evolve.

Planning and designing an insectary is a dynamic process that often takes months to become finalized. During this period, an experienced designer should be at the focal point of all discussions and receive suggestions from managers, contractors, equipment manufacturers, insectary personnel, and the scientists who will be using the insects. The designer should have a thorough understanding of rearing concepts, knowledge of the basic components, broad practical experience, and an ability to combine all of these into a functional plan that will satisfy the program objectives.

The most sophisticated, practical, and organized facility design is ineffective if insectary personnel have not received orientation and are poorly trained in rearing operations (Ertle and Day, 1978; Fisher, 1984b). Employees must know what the objectives are and what role they play in achieving them. They must understand the basic biology of insects, especially the species with which they will be working. They must be aware of the significance of microorganisms in insect production and understand the procedures used to eliminate them. Finally, facility design and function must be

explained to all employees so they will realize the consequences to the program of circumventing design intentions.

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INSECT REARING MANAGEMENT*

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1.0 INTRODUCTION

Insect rearing is the maintenance or culture of insects in captivity through one or more stages or generations. As a distinct discipline it is not new. Insects have been reared for the benefit of mankind for thousands of years. Silk technology and sericulture in China date back 4500-7000 years. Mulberry arboriculture and the indoor rearing of silkworms were recorded in the 4th century from the Xia Dynasty and by the 9th century many books on the insect's bionomics and rearing were available (Chou, 1980; Tsai, 1982). Apiculture in China dates back as far as sericulture (Tsai, 1982) and the commercial breeding and teaching of beekeeping practices flourished 1800 years ago (Anonymous, 1980). Methods for distributing immature lac insects on trees to produce lac and the development of the shellac industry date back about 7000 years in India (Venugopalan, 1956). The increase in demand for the natural materials produced by these beneficial insects and others (e.g. the leaf galls produced by *Melaphis chinensis* for tannin extraction, insects for food, insects in medicine) gradually necessitated an increase in production of the insects themselves. The rearing of these insects required a degree of organisation resulting in true domestication and thus a degree of management.

It is not until this century that other important reasons for culturing insects have become apparent (see Knipling, 1966,1984; Singh, 1977). Modern pest control strategies require supplies of test insects for release and research. Millions are irradiated for the sterile insect technique (SIT), and biological control by augmentation often requires considerable numbers of parasites, predators or insect vectors for distribution of bacteria and viruses. Behavioural control strategies rely on supplies of synthetic sex attractants originally isolated from laboratory-reared insects. Constant supplies of insects are often needed for initial biochemical assays. Fumigation programmes depend upon the availability of test insects for post-harvest disinfestation research. Insects reared under different conditions are used to test the activity of toxins at concentrations that produce lethal, sublethal and chronic genetic and behavioural effects on individuals or populations. Insects are also often used as biological, ecological and physiological research models.

Different degrees of insect rearing may be distinguished. Insects are reared for pleasure on a small scale involving perhaps one or two generations. Rearing in this category is usually carried out by hobbyists and in schools. Laboratory (medium-scale) rearing is undertaken by researchers and educators for experimental use and may involve the production of a few hundred to several thousand individuals of any given species per month. Factory (large-scale)

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* For an explanation of the terms used, the authors advise the reader to refer to the glossary included at the end of this chapter.

rearing usually involves the production of millions of individuals of a single species for extended periods of time, e.g. in control programmes using SIT. The two last-named types of rearing are generally accompanied by varying degrees of facility specialisation since colony establishment and continuous disease prevention require particular equipment and techniques. Both types require varying degrees of organisation and management.

Insect rearing management (IRM) is the efficient utilization of resources for the production of insects of standardized quality to meet programme goals. Good management implies organising, monitoring, forward research, planning, liaison with other involved groups and eventual accomplishment by the judicious use of resources. Programme goals are defined by the overall needs of the 'client' or 'user', i.e. the group requesting the insects for its particular purposes. The objectives set by the user group for the laboratory production group must be clearly outlined well in advance, particularly where the programme goals are long-term. This allows the production group to carefully assess its own objectives. Resources are the finance, personnel (time and labour), materials and space available to the production group.

Organisation in insect management, insect production management and the role of the insectary manager have been discussed by Leppla (1984), Schwalbe and Forrester (1984) and Fisher (1984) respectively. In this chapter, management principles are directed toward facilities that rear multiple species for multiple users. Although this requires different levels of management in comparison with large-scale, single-species facilities, most of the concepts that follow are applicable to both. IRM, as it applies to laboratory-scale rearing for the supply of experimental insects, can be divided into several component elements.

2.0 ELEMENTS OF INSECT REARING MANAGEMENT

We have identified seven major elements in IRM that are associated with any production programme leading to the eventual supply of insects. These are: 1) objectives; 2) colony establishment and maintenance; 3) design of the rearing laboratory; 4) research and the development of techniques; 5) resources; 6) quality control; 7) production.

As shown in Fig. 1, each element is linked with each of the others through management. For a new rearing programme, the elements should be considered approximately in the order in which they are numbered. The initial emphasis on any particular element will of course depend upon the objectives and an investigation may begin on two or more elements simultaneously. A change in emphasis may occur as rearing methods develop and when user-group objectives change. The integration of elements through management leads to production efficiency, the conservation of resources and a high yield of quality insects.

The pathway from an initial request for insects to their production and eventual supply is shown in Fig. 2, where the position of each key element is indicated by the number assigned to it in Fig. 1. The relative importance of any one element depends primarily on whether or not there is an already established colony, experience in rearing, and the eventual use of the insect. The left half of Fig. 2 is mainly concerned with those elements (1, 2, 3 and 4) which are involved in setting up a colony, perhaps by introducing a field-collected population into the laboratory. The right half is more concerned with the development of techniques, resource management, quality control, production and the supply of an established species. The relationship between elements shown in Fig. 1 should be kept in mind. Note that control of microbial contamination plays an important role in management throughout. Contamination sources are the air, the laboratory, food and the insects themselves. Strict precautions should be taken to suppress contamination during all phases of production.

Establishment of a species new to rearing involves new research on biology and the development of a rearing technique. It may take 1-2 years to set up such a laboratory colony. In this respect, forward research is

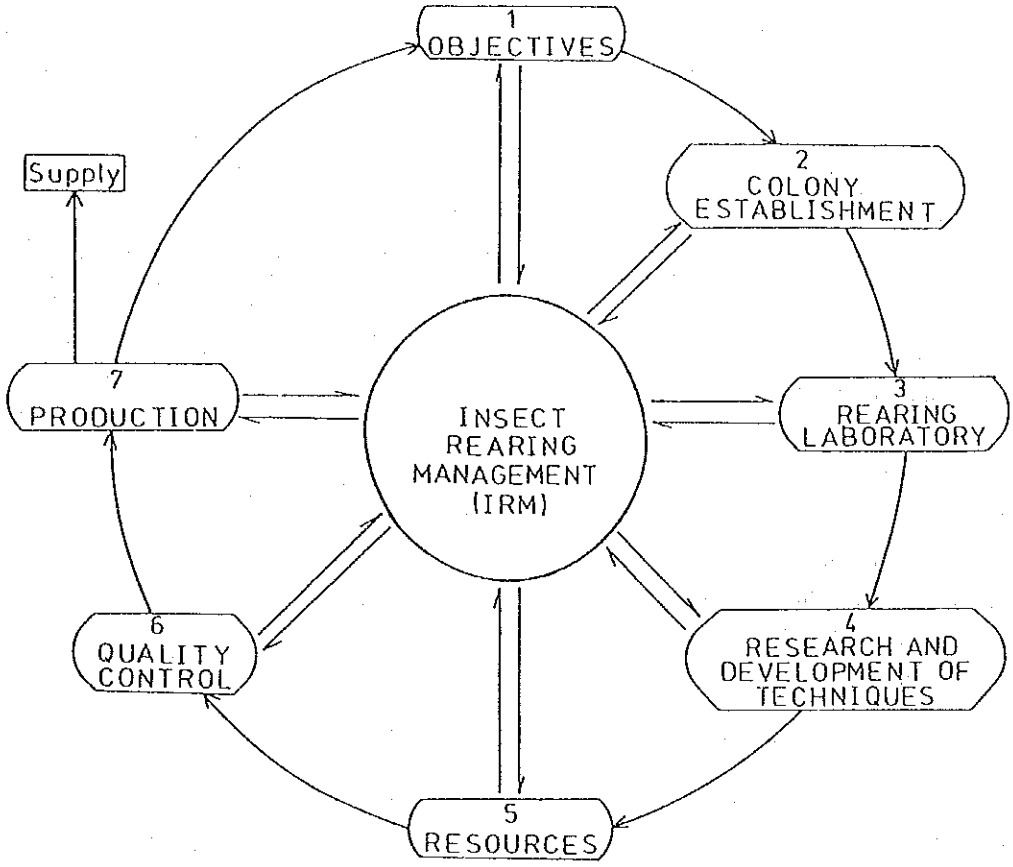

Fig.1 Elements of Insect Rearing Management

the key to successful and regular insect production. The direction a rearing programme takes depends upon its initial objectives and programme goals.

2.1 Objectives

Any rearing programme begins with a user request for insects. The objectives of the user group must be clearly defined and understood at the outset. There should be full early discussion between the production and user groups as to the purpose for which the insects are to be used. This prevents waste of time and resources later. Interfaces between the two groups are shown in Fig. 3. Pending a feasible programme, there should also be meetings between the two groups when objectives are modified or programme goals are changed.

Some programmes will have special requirements of insect quality which must be outlined. For example, the production of synthetic pheromones or other biochemicals may depend on extraction from laboratory-reared insects for initial identification. These insects must be capable of producing the required natural substances. Objectives relating to physical or behavioural quality may also be important. Insects reared in the laboratory for SIT programmes must be capable of mating and aggressive enough to compete with their wild counterparts. More specific production objectives should include an assessment of future user requirements relating to insect developmental stages

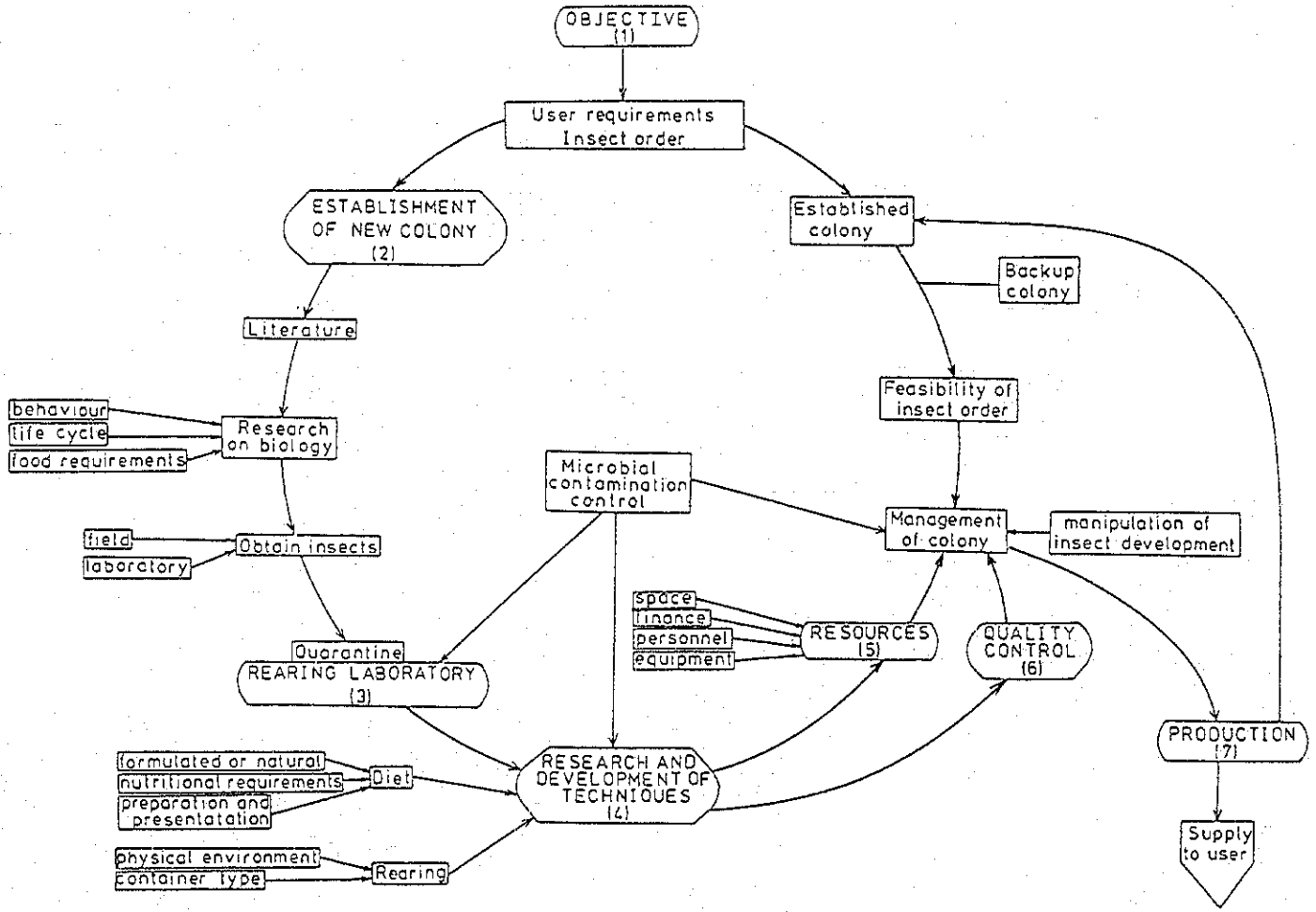

Fig. 2 Pathways of insect rearing management
(Key elements are numbered)

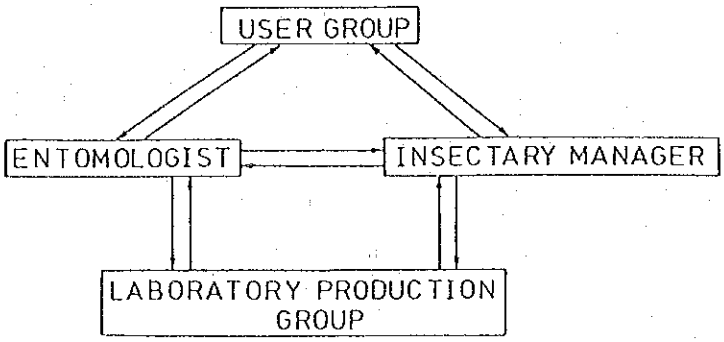

Fig.3 Insect production and user-group interfaces

(eggs, nymphs, larvae, pupae, adults), or ages (hours, days, day degrees), and sex.

All requests for insects and brief programme objectives should be detailed on an insect order form. On this should be included the name and location of the user group, what the insects are required for, species, strain, developmental stages required, numbers of each stage, dates of requirement, frequency of supply and any special instructions such as quality. Requests should be placed at as early a date as possible to allow for feasibility studies and forward planning. Where the laboratory holding does not include the species required, enough time must be allowed for colony establishment. This is especially important if a species has not been reared previously or is known to be difficult to rear. Advance notice for large orders allows plenty of time for manipulation of laboratory colonies.

The ability to meet any insect order depends on the size and vigour of the laboratory colony and a uniform rate of development during the various developmental stages. The colony should be disease-free, genetically diverse and large enough to provide sufficient numbers of insects to prepare for the order. Complex orders or orders requiring specific stages can be met only if the colony is well established and its biology well researched in advance.

2.2 Establishment of a laboratory colony

The success of a rearing programme depends upon obtaining a disease-free and genetically diverse parental stock. These insects may be collected from the field and placed under quarantine or transferred from other established laboratory colonies (see Fig. 2). Field acquisition assures a good genetic base but there are real dangers of introducing viral, bacterial or fungal pathogens into the laboratory. The egg is usually the best stage with which to start a colony since it is least likely to carry disease micro-organisms. Fungal and bacterial contaminants carried externally can easily be eliminated by surface-sterilization. Introduced larvae should be reared individually to prevent contamination and allow later separation of parasitized individuals. Captured adults must be induced to mate and lay eggs on a suitable oviposition substrate. For the introduction of all stages into the rearing laboratory, the importance of strict hygiene and quarantine standards cannot be stressed enough. Well established, confined, laboratory colonies are especially prone to microbial and viral epizootics of extrinsic origin. Methods by which microbial disease can be eliminated, removed or confined are discussed by Singh (1980) and Sikorowski & Goodwin (Vol. I, p. 85).

The establishment of a filial laboratory colony from a field-collected parental colony is dependent upon development of a sound rearing method involving the use of a natural or artificial alimentation. In the case of insects that feed only on a natural host or for which no suitable artificial diet has been developed, it may be necessary to grow host plants or maintain a second colony of insects as food. Although the use of a natural host would appear to be desirable, the problems sometimes associated with it make the use of an artificial diet more practical. Such diet can provide uniform insects whose precise nutrition and quality are known. Moreover, the collection of data pertaining to life history, behaviour, colony management and manipulation of development is facilitated. Unlike a natural host, an artificial diet can always be made available in sufficient quantity and is not subject to environmental vicissitude.

If an artificial diet is used, its texture and consistency may be important with respect to feeding induction. Special nutritional needs of previously unreared species can be gauged only by experimentation, and multiple-species rearing diets are useful in this respect (Singh 1983, and p.19, this Vol.). The formulation, preparation and evaluation of artificial diets have been reported in detail by Singh (1977, 1983). During laboratory colonization, unless specimens are readily available in the wild, a few field-collected individuals should be maintained on natural host material for the duration of tests of the artificial diet and initial rearing research. This provides a back-up in case of colony collapse due to infertility or total mortality or due to mechanical breakdown in the facility.

The insect's natural habitat can provide clues to the direction that the method should take. In initially choosing a suitable artificial environment for a previously unreared insect or one with an undeveloped rearing method, field conditions should be considered. Such conditions continually vary in temperature, light intensity, humidity, ventilation, and photoperiod. Daytime cycles of temperature (thermophases) and light (photophases) and night-time cycles of temperature (cryophases) and darkness (scotophases) undergo regular diel and seasonal patterns of change. These natural variables should be considered only as guidelines for laboratory rearing as it is of course not practically possible to reproduce all their permutations.

2.3 Design of rearing laboratory

The rearing laboratory or insectary layout and design is of crucial importance to effective insect rearing management, and reliable environmental control is probably the single most important key to a successful rearing programme. An insect's growth, development and behaviour can be controlled and manipulated and a thorough knowledge of development under different environmental conditions allows fine tuning of production for supply to user groups.

The design of an insectary should take account of future requirements for insect species and numbers, and stringent hygiene standards. The air flow in rearing rooms and circulation through rearing containers is not only important for the insect's well-being, but is also crucial for the maintenance of good diet condition. Contamination by fungal spores and bacteria can spread very quickly when ventilation is poor, resulting in high insect mortality and consequent low yields. The flexibility of environmental control must be considered with regard to the behavioural and physiological requirements of the species to be reared. Temperature, humidity, light intensity and photoperiod should all be variable.

Equipment failure is a serious problem during rearing programmes. Environmental control units should be equipped with alarms which operate audibly and visibly and if possible should be linked to a telephone priority system. Whether or not units are equipped with alarms or failsafe units, small

back-up colonies should always be maintained away from main colonies to prevent total colony loss caused by equipment failure. Equipment reliability is more important than sophistication.

Depending on size, the insectary should have separate washing facilities incorporating soaking and rinsing tubs, a dishwasher, an adequate drier for glassware and plasticware and a waste-disposal unit. A media preparation room should contain a fume hood, sinks, refrigerators for natural foodstuffs and chemicals, a supply of hot, cold, distilled and/or deionized water and plenty of shelf space with cupboards that close to prevent dust contamination. This room should also house all cooking equipment, including an autoclave, and chemical balances for fine and gross weighing. A small store or larder attached to the media preparation room facilitates bulk storage and there should be access to a freezer or chiller unit. Incoming foodstuffs should be dated. Facilities such as a sterile room or laminar flow should be available where possible. Contamination control is particularly important in the assaying of new diets and during the inoculation of new diets with insects.

Rearing laboratories should be well stocked with shelf and cupboard space. Everything necessary for the day-to-day rearing of a given species should be available at hand. Bulk supplies of containers should be kept in stores and transferred to the rearing laboratories as required. Sets of insect handling tools should be made available to each worker in the insectary and spare sets should be made available in any room where insects are handled. The effective spatial and temporal utilization of resources should be the responsibility of the insectary manager. Further discussion on insectary design and type of layout can be found in Fisher and Leppla (Vol. I, p. 167) and in Leppla and Ashley (1978).

2.4 Research and development of techniques

Extensive knowledge of an insect's life cycle under laboratory conditions is an essential prerequisite to colony management. Insects are representative of a wide variety of feeding types, habitats, behaviour and developmental regimes. Their taxonomy, corresponding types of mouthparts, and the behaviour (flying, crawling, burrowing; predatory, herbivorous, detritivorous, parasitic) of adults and immature stages should be taken into consideration when developing a rearing method. Information on behaviour and feeding can usually be obtained from textbooks and scientific papers, or from specialists.

The fertility, mortality and hatchability of eggs under laboratory conditions must be known and a knowledge of any colour changes associated with their development is useful. The number of larval instars in each generation, the corresponding head capsule dimensions for each instar, and the duration of each stadium should be determined under different conditions of temperature and humidity for rearing programmes that will require supplies of a particular stage. Larval development time is sometimes associated with the degree of crowding of individuals, and under some rearing conditions the number of instars may vary. Pupal development times should also be determined under various conditions, and fresh pupal weights are often used as indicators of insect quality in laboratory colonies. It may be possible to use dimorphic traits for sex determination in pupae. Developmental data concerning all stages, collected under various conditions, not only allow effective colony management but can also be used to develop rearing techniques and quality control procedures.

An extremely valuable tool in IRM is the use of 'day degrees' (D°) or 'centigrade heat units' (CHU), widely employed as developmental indicators. For the purposes of management, day-degree data obtained should be used after standardization of a laboratory colony. The physiological age in day degrees

for any particular insect stage can be calculated by:

$$D^\circ = D\,(T-K)$$

where D is the duration of development (days), K is the lower developmental threshold temperature or developmental zero (°C) and T is a constant rearing temperature. A minimum of 3 (but preferably 5) rearing temperatures should be used for each stage to obtain a developmental rate *vs* temperature curve, and other factors that affect development (humidity, light, etc.) should remain constant at each temperature. Examples of the use of day degrees can be found in Gutierrez *et al.* (1981), Guppy (1981) and Stevenson (1981).

The total development time of the pre-adult stages is ascertained at adult emergence. This can be shown on a graph as percentage adult emergence over time and contributes important information to laboratory colony management. Many species have a post-emergence pre-mating period, and at this stage, provided that adults are visibly dimorphic, the sexes may be separated for controlled mating later. The pre-oviposition and oviposition periods should be determined together with daily rates of egg deposition during the oviposition period. Fecundity is usually expressed as eggs per female per day.

Physical, behavioural and biological variables are interactively involved in the development of a rearing method. The most important physical factor to consider initially is temperature. Even moderate temperatures may desiccate a neonate larva or nymph in a few hours before feeding begins, but the same temperatures may considerably speed development during later stages. Temperatures which contribute (together with other variables) to the induction of high levels of oviposition may be considerably lower than those required for a satisfactory rate of development. High or low temperatures during development often influence adult size and may have an effect on adult lifespan, fecundity and fertility.

Newly laid eggs of some species are far more sensitive to higher or lower temperature than those that have sclerotised. Egg development is also sensitive to humidity. Low levels can cause desiccation, whereas high levels can encourage the growth of moulds and fungi at any stage of development. Inadequate or high humidity also affects the quality and texture of the artificial diet.

The quality of light (wavelength and intensity) and length of photoperiod produce physical, behavioural and biochemical responses in insects. Many exhibit local or diel taxic responses to light and dark. Development may be arrested (as in quiescence or diapause) or augmented, and reproductive patterns may change. Although diapause can be a problem in continuous rearing programmes, it can often be induced, terminated or prevented altogether by the manipulation of photoperiod and temperature either separately (e.g. Sieber and Benz, 1980) or in combination (e.g. Wildbolz and Riggenbach, 1969), or by simulating a certain thermoperiodic regime (e.g. Beck, 1982). Quiescence whereby development or activity ceases temporarily during adverse conditions, is not usually a problem. Mating and oviposition (and indeed the development of reproductive organs) can be partially under the control of light quality and photoperiod and the effects of these variables should be tested during the development of a rearing technique.

Much of an insect's natural behaviour is associated with its response to light, and phototaxic responses are extremely useful in insect management. The stimulation of movement in a certain direction by suitable placement of a light source can be used to advantage, e.g. during neonate colonization of artificial diet, during pupal movement just before emergence and during collection of adults.

The choice of rearing container is very important and depends primarily on the insect's feeding habits and other behaviour. Where practicable individuals undergoing laboratory establishment are reared singly in plastic, glass, cardboard, or perspex containers to reduce microbial contamination and eliminate competition. Group rearing, where possible, should not begin until the second or preferably third filial generation. However, competition for space and food can prolong and desynchronise development. Good colony management later is dependent upon suitable numbers of individuals arriving at the same stage at the same time. It may therefore be advisable initially to use smaller containers with a lower density of individuals. The containers used to house adults require particular attention since conditions must be suitable for mating and oviposition. Plastic containers are easily washed and handled and can be fitted with gauze to provide suitable ventilation. Hard plastic or perspex may be more suitable for some species since those with biting mouthparts can considerably damage soft plastic (Singh and Jerram, 1975). Translucency allows inspection at any time and stackable containers of the same size are space-saving. A discussion and practical guide to insect rearing containerization may be found in Burton and Perkins (1984).

Where it is necessary to handle large numbers of insects individually, mechanisation should be a serious consideration and short cuts are often extremely practical. Mechanisation reduces labour requirements and microbial contamination. Consider each insect stage in turn, dissect the handling process involved with them and decide if mechanization is practicable. It may be possible to avoid hand inoculation of diet with first instars by allowing eggs to hatch directly on the diet instead. Adult handling may be avoided by placing pupae or nymphs directly into mating and oviposition containers. With any change in technology or methodology, however, insect quality should be monitored very closely.

2.5 Resources

A major goal of IRM is to meet objectives by the most judicious use of financial, personnel, material and spatial resources. Each resource must be available as and when required and distributed according to the needs of a particular programme. Efficient overall utilization of resources is primarily the responsibility of the insectary manager. During the day-to-day operation of the insect rearing laboratory, five major areas of resource consumption can be identified (see Fig. 4).

1) Internal and external insectary building maintenance including the monitoring and adjustment of environmental control facilities.
2) Equipment design, maintenance, production and upkeep.
3) All aspects of insect rearing for research, colony maintenance, supply of orders and associated activities such as diet preparation.
4) Consultation and information processing including the processing, documentation and discussion of insect orders, production of research papers and methodological notes, and staff meetings.
5) Public relations including the preparation of display boards and pamphlets, and press, radio and T.V. interviews.

The involvement by any particular member of staff in any of the areas of resource consumption varies. Each area is a function of finance, and all five areas use personnel.

The production of insects for user groups is often the primary objective of the rearing group, and as such a greater proportion of time is adjusted continuously according to daily priorities and the particular programmes in operation. Systematic operations are consistent with careful planning. During periods of lower demand, more time may be spent in processing of research data, development of equipment, and public relations. Equipment maintenance (particularly washing and sterilization) and building maintenance are ongoing

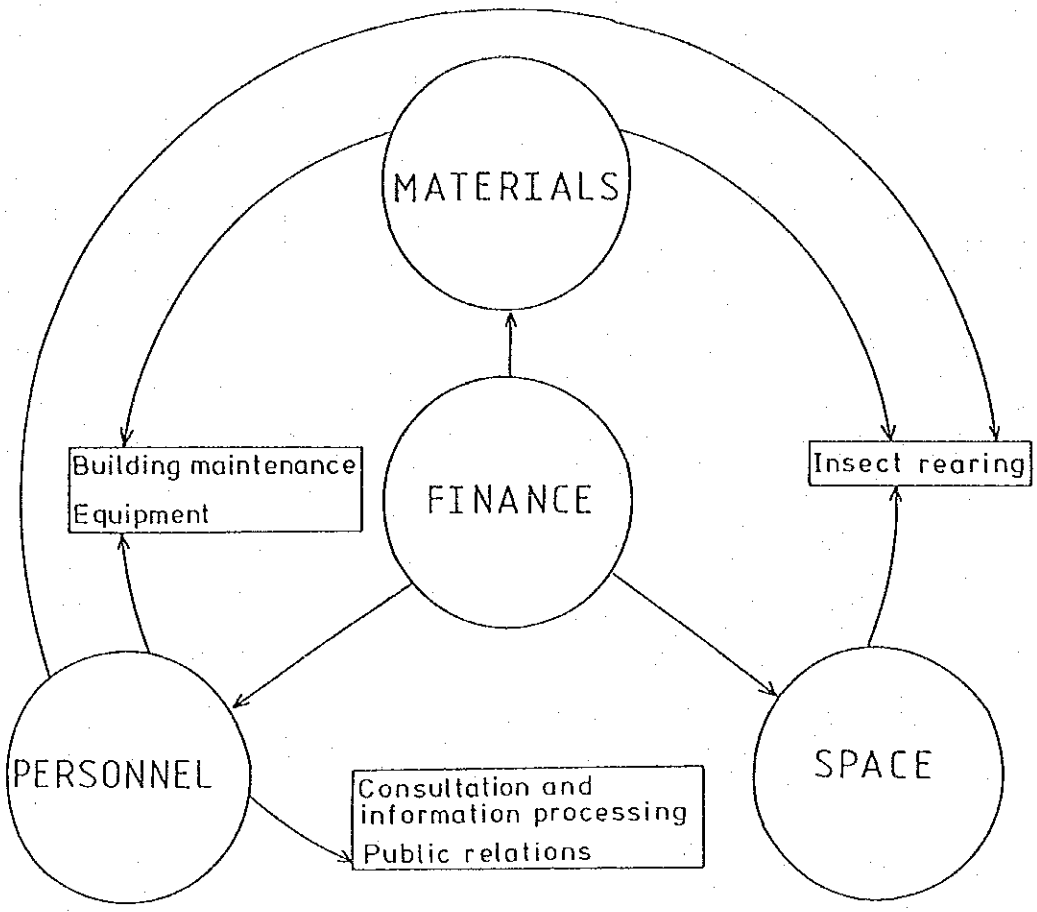

Fig. 4 Resources and main areas of resource consumption

activities.

Labour is the major budgetary item and insect rearing is a seven-day-a-week operation. The organisation of personnel depends upon the size of the facility, the number of species being reared and the number of personnel available. In multiple-species rearing laboratories, the proportion of the labour pool allotted for the rearing of each insect species depends mainly on the numbers and stages required, the frequency of supply, artificial diet requirements, and the rearing method. These should be organised by the insectary manager.

The availability of material resources, especially rearing containers and dietary ingredients, should be carefully considered at the outset of a programme and reassessed throughout. Forward ordering is essential, particularly before the production of large insect orders. The same applies where containers have been specially designed or constructed and are not made commercially. Inventories of diet supplies and containers are useful in planning, and back-up equipment saves time in the event of mechanical breakdown.

The allotment of rearing space should be considered in the long term with respect to existing and future production programmes. Rearing on a small and

medium scale may require only minimal amounts of space, particularly during experimentation, and in these situations insect growth cabinets or incubators may effectively replace rearing rooms. The allocation of rearing rooms depends on the requirements for species and the numbers of each, and space may from time to time be required for quarantine purposes. Laboratory space should always be available for routine activities such as media preparation, insect handling and equipment cleaning.

The key to good resource management lies with the insectary manager, who should be aware of the day-to-day and longer-term material requirements in the laboratory, and should be sensitive to the needs of the other personnel.

2.6 Quality control

Huettel (1976) distinguishes two types of quality with respect to laboratory colonies. The first involves the performance of laboratory-reared insects in a field-release situation with respect to the intended role of the insects at release. The second is defined in terms of a particular trait or set of traits whereby laboratory-reared insects are compared to field-collected ones.

We have identified a third factor which concerns the comparison of a laboratory colony with itself over time. The monitoring of specific traits gives a good indication as to its continuing performance. Detailed developmental measurement of the parental and first filial laboratory generations is essential, since these data set a natural baseline for future comparison. Developmental periods change over generations as colonies become more standardized. Individual insects with a particularly long life cycle may eventually be excluded by virtue of their larval, nymphal, pupal or adult lifespan. This selection for an optimum length of life cycle involves a degree of genetic exclusion which may or may not affect overall quality. This type of standardization is common practice in many insect rearing laboratories since colony management is simplified as a result. Although selection begins with the parental generation, it becomes more intense in the first few filial generations. The exclusion of some alleles due to selection of developmental traits thus occurs early; behavioural, biochemical and physiological selection follow. Performance in a new laboratory colony should be quantified early, when the insects are closer genetically to the wild population. However, as Huettel (1976) points out, the quality of a laboratory insect population is important in relation to the insects' end use.

Boller and Chambers (1977) list adaptability, motility, orientation to habitat, sexual activity, physiology and reproduction as major components of overall quality. In practical terms, measurements of fecundity, fertility and adult and pupal weight may be used as basic indicators of the ongoing biological and physiological performance of a laboratory colony.

Quality control data are useful and meaningful only if several conditions are fulfilled. (1) Rearing conditions must be reproducible. (2) If an artificial diet is used, it must be reproduced consistently both in preparation and composition. (3) Artificial dietary ingredients must maintain stable quality in order to produce disease-resistant and physiologically complete individuals. (4) Filial laboratory populations must be capable of completing repeated life cycles under specific conditions.

2.7 General principles of production

Insect production encompasses all steps in the process of rearing insects for research, colony maintenance and supply to user-groups. The first two are prerequisites for the latter, and all three are ongoing. Laboratory production for supply to user groups cannot begin until the other six elements of IRM have been integrated to a satisfactory degree (see Fig. 1). The facilitation of production occurs through the use of work schedules and through forward

planning relating to the utilization of resources. The mechanics of basic production are outlined for economically important species in this handbook.

A few guidelines that should be carefully observed during the production process are as follows: (1) Continuously examine each step in production for ways to reduce the time and labour input and consider mechanisation where applicable. Methodological changes should be gradual, experimental and replicated several times with small subcolonies before incorporation as an established major colony procedure. Rearing group production failures may result in considerable setbacks to user-group programmes. (2) Maintain good hygiene and cleanliness and monitor microbial contamination. (3) Keep biological records of development times for all species and a log-book of numbers produced during each generation. All biological information such as fecundity, fertility, and adult and pupal weights is useful in insect management. (4) Check insect quality as frequently as user-group requirements demand or as time is available to production personnel.

A formal user-group request for insects is called an insect order. This is a form on which is detailed the species, numbers, stages, dates of supply, purpose of supply, and any special instructions, such as quality, for the laboratory production group. The ultimate aim of insect production for supply is to make sure that user groups receive their orders on schedule and in the correct numbers. Before production begins, the insect order must be evaluated by the laboratory production group.

Fig. 5 outlines the steps that must be taken during evaluation, from receipt of the order to the beginning of production. A practical application of this follows below, using the codling moth as an example. This insect has been selected because we have had 14 years' experience of rearing it in the laboratory. During 140 continuous generations insects have been used for pheromone research, parasite and virus production and post-harvest disinfestation research.

3.0 PREREQUISITES TO A CODLING MOTH PRODUCTION PROGRAMME

The basic prequisites required to plan the production of insects for supply to user groups are as follows:

1) established rearing procedures;
2) data pertaining to the duration of developmental stages and fecundity at various temperatures, relative humidity and light regimes;
3) adequate material and labour resources; and
4) a rearing facility with several environmental control units for manipulation of the colony.

To initiate a production programme leading to a supply of codling moth larvae, developmental data obtained from rearing at 25°C, LD 18:6 h and LD 12:12 h photoperiods and 60 ± 5% RH are used. Data pertaining to the pre-mating, pre-oviposition, oviposition, fecundity, fertility, egg development, duration and mortality of each stage during development are outlined in Table 1 for the two separate artificial diets. Table 1.1 lists the numbers of fertile eggs (fertility being almost 100%) produced per female from the first to the fourth days of oviposition, with running totals after day 2. These figures are used to gauge production of first instars for insect orders.

Table 1.2 shows the time elapsed when 98-100% of insects have reached a given stage during individual rearing and the day numbers on which each stage occurs. Days are numbered from the day of oviposition. Mortality at each stage is given, allowing a detailed calculation of expected yields. It can be seen that 9-12 days after oviposition 100% first instar larvae are in evidence and their mortality is less than 2%. Larvae are all second instars on days 13

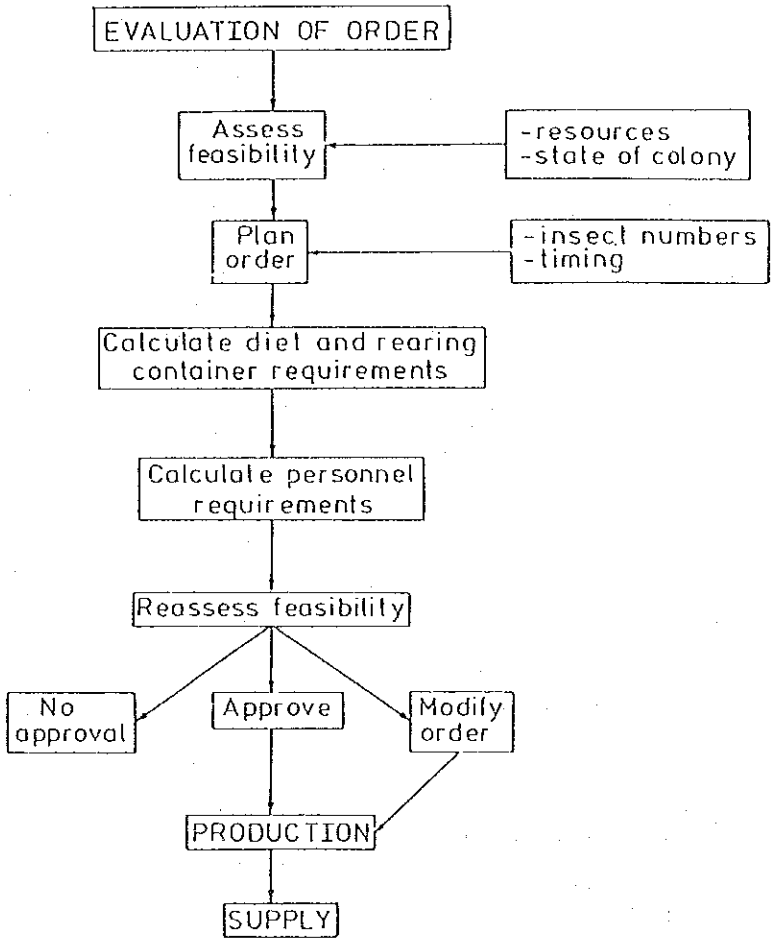

Fig.5 Steps in the evaluation of an insect order

and 14, third instars on day 16, fourth instars on day 19 and fifth instars from day 23 to 27, and they have all pupated by day 32. Total mortality under individual inoculation is less than 11%.

Table 1.3 lists the developmental periods and emergence of *C. pomonella* under group rearing. Development is less uniform than under individual rearing since there is competition between larvae, particularly first instars. Furthermore, larvae colonise the diet themselves from eggs placed directly onto it. These eggs vary from 3-7 days old (if mating and oviposition have occurred over 3-4 days), so development is more advanced for some insects than for others.

Figures given in Table 1.3 for larval and pupal periods and adult emergence indicate when 90% of the insects are in these respective periods. These are referred to as usable insects. The other 10% are referred to as

Table 1 Development of codling moth at 25°C, LD 18:6 and 60 ± 5% RH

Table 1.1

Fecundity of adults group-reared as larvae on diet of Brinton *et al.* (1969), expressed as mean numbers of eggs per female

Day:	1	2	∑	3	∑	4	∑
Mated pairs	81.5	30.3	111.8	21.2	133.0	12.5	145.5
Group mating	38.0	20.0	58.0	17.0	75.0	11.0	86.0

Table 1.2

Development and mortality data - individual rearing[a]

Day No.[b]	Stage or Developmental Period	Mortality (%)
1	first egg batches	
2-8	egg development	<2.0
9-12	1° larvae	<2.0
13-14	2° larvae	<1.0
16	3° larvae	<1.0
19	4° larvae	<1.0
23-27	5° larvae[c]	<1.0
32	pupae	<3.0

Table 1.3

Developmental data - group rearing[d]

Pre-mating period, 1 day
Pre-oviposition period, 1 day
Oviposition period, up to 4 days
Egg development, 7 days
Larval period, 22-26 days
Pupal period, 8 days
First adult emergence, 29 days from neonate larva
Adult emergence, over 6-10 days

Footnotes

(a) Reared individually in polyethylene tubes on diet of Singh (1983).
(b) Except for day 1, the day numbers listed are those on which 100% of the stage shown in the second column occur. Individual insects vary slightly in development, and on days not listed (15, 17, 18, 20-22, 28-31) more than one stage is in evidence.
(c) Fifth instars destined for diapause will cease development and begin cocooning on about day 30.
(d) From rearing containers (Ashby *et al.*, 1982) on diet of Brinton *et al.* (1969).

non-usable since their development is either too advanced or, as is usually the case, too retarded. Only the usable insects are employed in the remainder of the programme. Most moths begin to emerge 30 days after first-instar colonization of the diet (see Fig. 6).

The number of days over which a significant number continue to emerge depends upon the factors discussed above, i.e. the extent of larval competition and the age difference between the eggs. Once all the prerequisite information is available, individual insect orders can be processed.

4.0 THE APPLICATION OF IRM IN THE PRODUCTION OF CODLING MOTH (*Cydia pomonella*)

Two rearing methods are used (Vol. II, p. 237 on codling moth). Group rearing, in large rearing containers (Ashby *et al.* 1982), on the diet of Brinton *et al.* (1969), is used exclusively for adult production since this method requires the least labour.

All larval stages (except the first instar) requested for orders are reared individually on the diet of Singh (1983) in 75 x 12 mm polyethylene tubes. Under this method larval development is uniform and the insects are easier to extract from the tubes when their required stage is reached.

We have identified 10 steps in the evaluation of an insect order from its receipt to actual production and supply.

4.1 The insect order

The order shown in Table 2 was received on 23 February 1983. The various stages outlined were requested for post-harvest disinfestation research on Granny Smith apples. The larvae were required for artificial infestation of the fruit. After insect establishment, the fruit was to be fumigated at various dosages and the kill rate was to be assessed. The complete order was labelled CM7/83, i.e codling moth order No. 7 for 1983. Each individual request for larvae or eggs was then designated a suborder and labelled in square brackets (Table 2, column 1). This helps avoid confusion during the detailed planning phases.

4.2 Order feasibility

The order is a relatively complex one composed of 20 suborders. Insects are required on various dates between 21 June and 5 September. Before it is approved by the rearing group, two questions are asked. Is it feasible? Can it be met? The question of feasibility relates to whether the laboratory colony can be physically manipulated to supply the user group with the required stage on the required date. Whether it can be met depends upon the availability of resources, especially personnel. All larvae for supply (except first instars) have to be reared individually, and this involves considerable labour input. Although the first date of supply is 21 June, planning begins immediately the order is received in order to test its feasibility.

4.3 Planning the order

The insect order (Table 2) is composed of a wide spread of suborders from June to September. The supply of first-instar larvae required for inoculation onto diet in order to produce the stages requested in the insect order will depend in turn upon the availability of eggs and therefore of adults. An essential set of data is therefore the present state, and thence the projected state, of the laboratory colony during the time that the first instars are needed. These data must then be coordinated with the requirements for first instars and the laboratory colony manipulated accordingly. Yearplanner charts are especially useful in this respect since the state of the colony at any given time in the near future can be predicted at a glance.

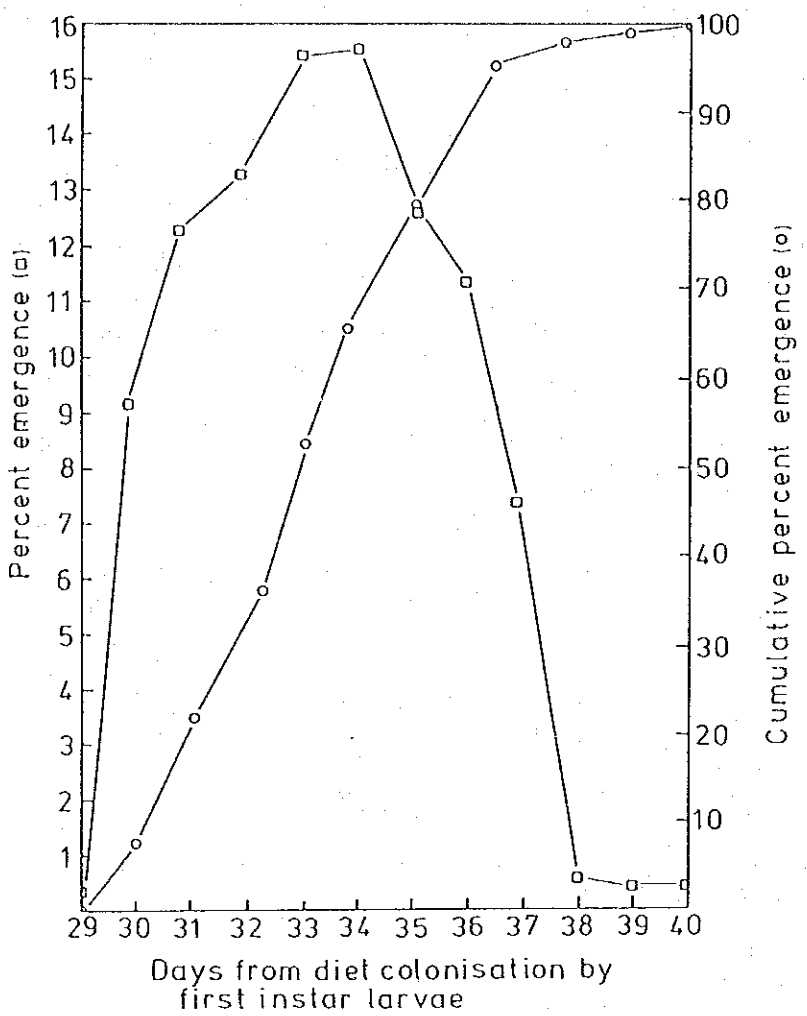

Fig. 6 Distribution of Cydia pomonella emergence

4.4 Initiation of the projected moth production schedule

Table 3 outlines the laboratory colony projection. In February, when the request for insects came in, the laboratory colony was already split into two subcolonies, A and B. On 2 March the legend 130/A30 appears (see a in Table 3). The first number, 130, refers to the laboratory generation number, each new generation beginning with eggs from adults of the previous one. The letter A refers to subcolony A (of generation 130), and the number 30 following it refers to the number of days to adult emergence since the colonization of artificial diet by first-instar larvae. This and subsequent labelling (A31, A32 ... A39; B30, B31 ... B39) refers to the days (and hence dates) on which moths are available from their respective subcolonies. It can be seen from Table 1.3 and Fig. 6 that few moths emerge on day 29. Moths actually become usable on day 30 because sufficient numbers have emerged by then. Emergence continues over 9 more days up to day 39 (11 March). On day 37 (9 March) moths collected on the previous days are to be mated, and will continue to oviposit

Table 2 Insect order form

INSECT ORDER NO. CM7/83

Name: T.A. Batchelor Group: Horticulture; Entomology
Date order placed: 23 Feb 1983

Species: *Cydia pomonella* Required for: Post-harvest disinfestation research on Granny Smith apples

Suborder No.	Stage required	Date required	No. required
[1]	4° LARVAE	21 JUN 83	600
[2]	5° DIAPAUSE LARVAE (+ CONTROL)	22 JUN 83	1200 (+100)
[3]	3° LARVAE	28 JUN 83	600
[4]	4° LARVAE	29 JUN 83	600
[5]	1° LARVAE	05 JUL 83	600
[6]	5° DIAPAUSE LARVAE (+ CONTROL)	06 JUL 83	1800 (+100)
[7]	4° LARVAE	08 JUL 83	700
[8]	4° LARVAE	11 JUL 83	700
[9]	2° LARVAE	12 JUL 83	600
[10]	4° LARVAE	18 JUL 83	700
[11]	5° DIAPAUSE LARVAE (+ CONTROL)	21 JUL 83	1800 (+100)
[12]	3° LARVAE	25 JUL 83	700
[13]	4° LARVAE	28 JUL 83	700
[14]	2° LARVAE	01 AUG 83	700
[15]	5° DIAPAUSE LARVAE (+ CONTROL)	04 AUG 83	1800 (+100)
[16]	4° LARVAE	10 AUG 83	700
[17]	1° LARVAE	05 SEP 83	700
[18]	1 DAY OLD EGGS	22 JUN 83	1800
[19]	1 DAY OLD EGGS	27 JUN 83	1800
[20]	1 DAY OLD EGGS	09 JUL 83	1800

Special instructions: Rear all larvae individually except 1°. Maintain 100 additional 5° larvae in LD 18:6 photoperiod as controls for diapause larvae. Supply diapause larvae before cocooning.

Rearing group comments: Increase requirement by 10% for larvae, 25% for eggs to allow for mortality. Use LD 12:12 for diapause.

Order approved by: M.D. Ashby Date: 26 Feb 1983

up to day 39 (11 March). Moths collected on day 36 are used for mating to allow for the 1-day pre-mating period required (see Table 1.3). Both moth emergence and egg incubation occur at 25°C. Eggs laid on 10 March will hatch on 16 March (allowing 7 days development; see Table 1.3). On this date eggsheets containing well developed eggs (at the blackhead stage) and neonate larvae should be placed on trays of fresh Brinton diet which has been conditioned for 24 hours at 25°C. Eggs on these sheets that were laid a day later (on 11 March) will hatch on 17 March. The eggsheets remain on the diet for another day (18 March) to allow late-oviposited eggs to hatch, for first-instar wandering and for late penetration of the diet.

The same developmental rules must apply to subcolony B, which is projected to begin emergence on 21 March. However, looking forward particularly to June, July and early August, moth emergence and availability must be staggered so that eggs and hence first instars will be available for inoculation for suborders [1] to [4] and [6] to [16]. To find out exactly when these inoculations for first instars must be done, it is necessary to prepare a

Table 3 Codling moth production schedule March-August 1983

DATE	MARCH	APRIL	MAY	JUNE	JULY	AUGUST
1		MATE 130C	INOC 132A B37	MATE 132A A34	C40	
2	(a)130/A30		MATE 131B B38	MATE 132A A35	C41	
3	A31		MATE 131B B39	MATE 132A A36	C42	
4	A32		MATE 131B 131/C30	A37	C43	
5	A33	INOC 131C	MATE 131B C31	A38	C44	
6	A34	INOC 131C	C32	A39	(c)END COLONY 132C C45	
7	A35	INOC 131C	C33	132/B30	133/A30	
8	A36	INOC 131C	C34	INOC 133A B31	A31	
9	MATE 130A A37		INOC 132B C35	INOC 133A B32	A32	
10	MATE 130A A38		INOC 132B C36	INOC 133A B33	A33	
11	MATE 130A A39		INOC 132B C37	MATE 132B B34	MATE 133A A34	
12			INOC 132B C38	MATE 132B B35	MATE 133A A35	
13			C39	MATE 132B B36	MATE 133A A36	
14		131/A30	C40	B37	A37	
15		A31	C41	B38	A38	
16	INOC 131A	A32	MATE 131C C42	B39	A39	134/A30
17	INOC 131A	A33	MATE 131C	B40	133/B30	A31
18	INOC 131A	A34	MATE 131C	INOC 133B B41	INOC 134A B31	A32

19				A35	MATE 131C		INOC 133B	B42	INOC 134A	B32	A33			
20				A36	MATE 131C		INOC 133B	B43	INOC 134A	B33	A34			
21	MATE 130B	B30		A37				132/C30	MATE 133B	B34	A35			
22	MATE 130B	B31	MATE 131A	A38				C31	MATE 133B	B35	A36			
23	MATE 130B	B32	MATE 131A	A39	INOC 132C			C32	MATE 133B	B36	A37			
24		B33	MATE 131A	131/B30	INOC 132C			C33		B37	A38			
25		B34		B31	INOC 132C			C34		B38	A39			
26	INOC 131B	B35		B32	INOC 132C			C35		B39	134/B30			
27	INOC 131B	B36		B33	INOC 132C			C36		B40	B31			
28	INOC 131B	B37		B34		132/A30		C37	INOC 134B		B32			
29	(b) BEGIN SUBCOLONY	B38	INOC 132A	B35		A31		C38	INOC 134B		B33			
30	MATE 130C	B39	INOC 132A	B36		A32		C39	INOC 134B		B34			
31	MATE 130C	B40				A33					B35			

Footnotes

(a) 130/A30: 130 is the laboratory generation number; A refers to the 'A' subcolony of generation 130; 30 refers to the number of days to adult emergence since the inoculation of artificial diet with the first instar larvae. See text for complete details.

(b) New subcolony numbered 130C begun on 29 March with mating of moths from generation 130B. This will eventually ensure that moths become avalable throughout June from the three subcolonies. Also note that mating and oviposition are allowed to continue for 4 days, as is inoculation, in order to extend moth emergence to 42 days in May. This also occurs for generation 131B from 2-5 May and 9-12 May respectively to extend emergence in June, when moths are required for suborder [20].

(c) Subcolony C no longer required. Any moths remaining from this colony held at 15°C until assimilation into colony A on 11 July.

Table 4B. Programme summary of insect order

1	2	3	4	5
Suborder number	Stage required	Number required	Actual number of larvae to produce	Actual number of eggs to produce
[1]	4°	600	660	825
[2]	5°D	1300	1430	1788
[3]	3°	600	660	825
[4]	4°	600	660	825
[5]	1°	600	660	825
[6]	5°D	1900	2090	2600
[7]	4°	700	770	963
[8]	4°	700	770	963
[9]	2°	600	660	825
[10]	4°	700	770	963
[11]	5°D	1900	2090	2600
[12]	3°	700	770	963
[13]	4°	700	770	963
[14]	2°	700	770	963
[15]	5°D	1900	2090	2600
[16]	4°	700	770	963
[17]	1°	700	770	963
[18]	Egg	1800	-	2250
[19]	Egg	1800	-	2250
[20]	Egg	1800	-	2250

The programme summary is a reference that incorporates information from the insect order form (Table 2) and the actual inoculation work to be done in the laboratory (reckoned by increasing the number of larvae requested by 10% and the number of eggs by 25%).

programming schedule (Table 4A). The projected moth production schedule can then be completed later.

4.5 Preparation of the programme schedule

Table 4A is an outline of the schedule of actual work to be done in order to fill the 20 suborders requested in the insect order. Each month encompasses all columns or a combination of columns for placement of mating, inoculation and supply dates as necessary. The suborder supply dates are all taken from the insect order form and entered as suborder numbers both onto Table 4A and column 1 of the programme summary (Table 4B). The stage and insect number requirements are also entered onto Table 4B in columns 2 and 3. The actual numbers of larvae and eggs that must be produced at the outset are then calculated. It is known from Table 1.2 that mortality rates of eggs and each larval instar range from less than 1% to less than 2% per stage. Thus for suborder [1], for example, a maximum of 5% of larvae will die between the first and fourth instars. For suborder [2], a maximum of 6% mortality will occur between the first and fifth instars. However, although an increase by these percentages over the original requirement may be sufficient, all original requirements (i.e. 600 fourth instars for [1]; 1300 fifth instars in diapause for [2]; etc.) are in fact increased by 10% (i.e. to 660 fourth instars for [1]; to 1430 fifth instars in diapause for [2]; etc.) to allow a safety margin in case of increased mortality. This is done for all suborders of larvae (column 4 of Table 4B).

Eggs are far more susceptible than larvae to changes in temperature and humidity and although under constant conditions mortality is low, the numbers of eggs actually produced are increased by 25% (column 5 of Table 4B). This allows for both physical (handling) damage and possible environmental oscillation in rearing rooms. Thus, to supply 1800 eggs in each of suborders [18], [19] and [20], initial production is aimed at 2250 per suborder. An overestimation of 25% for eggs is also used i.e. over and above the actual production numbers for larvae shown in Table 4B: to produce 660 first instars for suborder [1], 825 eggs are required; to produce 1430 first instars for suborder [2], 1788 eggs are required. The remaining actual egg number requirements for other suborders are outlined in column 5 of Table 4B.

Once the egg numbers required are known, the numbers of adults that will be needed to produce them can be calculated. Fecundity drops considerably when adults are held prior to mating for more than five days, and some females will actually lay infertile eggs in the holding containers. It is thus preferable to use fresh moths if possible. Moths are mated and oviposit in cardboard mating boxes (MB) at a ratio of 20 pairs per box. From this, 38 eggs per female (or 760 eggs per MB) are expected over the first 24 h, and 20 eggs per female (or 400 eggs per MB) over the second 24 h of oviposition (see Table 1.1).

To produce the 825 eggs required to fill suborder [1], 2 mating boxes are needed if oviposition occurs over only 1 day (since 1 box will yield only 760 eggs). Two boxes should produce 1520 eggs. Although less than 20 pairs could be placed in the second box, it is good practice to have an excess of eggs since, during the individual inoculation of diet, a good supply of first instars is required. Personnel do not therefore have to search for larvae, and time is conserved.

In suborder [2], 1788 eggs are required to produce 1300 fifth-instar larvae destined for diapause. To produce this number of eggs, 3 mating boxes are theoretically required (producing 2280 eggs). However, taking into consideration the high investment in labour required to inoculate 1430 first instars individually onto artificial diet, this order may be more conveniently inoculated over 2 days. For this reason, 4 mating boxes of adults are used and

oviposition is allowed to continue for 2 days. Thus, 7 days after the first day of oviposition, half of the actual number of larvae required, i.e. 710, can be inoculated. The remaining 710 can be inoculated on the following day from healthy first instars obtained from the second day of oviposition. The second day of oviposition should produce 1600 eggs (see Table 1.1).

The type of planning discussed above should be used throughout the remaining suborders for calculation of adult numbers required. If excess adults are available (and at this stage it is assumed that they will be) eggs are an abundant resource that is relatively easy and economical to obtain. The success of the entire programme rests primarily on egg production. If adults are not in abundant supply at the time they are required, it may be necessary to re-use them.

4.6 Timing of suborders

So far, the supply dates from the 20 suborders have been entered on the programme schedule (Table 4A) and the numbers of insects required to generate this supply have been calculated. It now remains to work out (backwards in time from the supply dates) the exact dates of inoculation of first instars onto artificial diet in order to obtain the correct stages required on the supply dates. From these dates, the dates on which mating and oviposition must occur can in turn be worked out. All the data necessary for these calculations are contained in Tables 1.2 and 1.3.

The supply date for suborder [1] is 21 June, when 600 fourth-instar larvae are required. From Table 1.2 it can be seen that it takes 19 days from oviposition for larvae to mature to the fourth instar under the specified conditions. On day 19, 100% of larvae will be in the fourth instar. By deduction, the larval period from first to fourth instar is 11 days, since the first instar begins on day 9 and all larvae are fourth instars on day 19 (the days being inclusive). Now taking 21 June as day 11, 660 neonate first-instar larvae must be available on 11 June, the first day of the larval period. In order to produce first-instar larvae on this date, eggs must be hatching. It is known that egg development takes 7 days and that 1 day is needed for mating and pre-oviposition. One day pre-mating is also needed before this. Working backwards from 11 June as day 7 (i.e. completion of egg development and larval hatch) it can be seen that eggs must be laid on 5 June. Mating and pre-oviposition must therefore occur a day earlier, on 4 June. The pre-mating period must occur on 3 June or before, and it is during this period that adults must be available for collection.

The actual numbers of larvae to be inoculated (660) and the suborder number [1] can now be placed on the table (as in Table 4A) for 11 June in the inoculation column. The number of mating boxes (2MB) calculated previously and the suborder number [1] can then be placed on the table for 4 June in the mating column.

The timing of calculations for all suborders involving larvae in the second, third and fourth instars are worked out in exactly the same way as for suborder [1] except that larval periods are different. The larval period for 100% second instars occurs 4-5 days after neonate inoculation and for 100% third instars 8 days after neonate inoculation. The requirements for fifth-instar diapausing larvae are more general, since the original order requested only that larvae had not yet cocooned (Table 2). Cocooning usually begins about 30 days from neonate. Fifth instars in this order will be supplied 18-22 days from the neonate stage since they have usually finished feeding by this time. To induce diapause in these larvae a short-day photoperiod is used, e.g. LD 12:12. The 100 controls also requested will be kept under LD 18:6.

Orders for first-instar larvae can be supplied from eggsheets. Supply must occur on day 7 of egg development, since eggs will be beginning to hatch at this stage. Mating and oviposition must therefore occur 8 days before the supply date to allow for the pre-mating period.

One-day-old eggs are required for suborders [18], [19] and [20]. Thus for suborder [18], for example, which must be supplied on 22 June, development must begin 24 hours earlier on 21 June. Mating boxes should be set up with adults on the afternoon or evening of 20 June to allow mating and ovipositon to occur overnight. Adults used should be at least 1 day old.

4.7 Completion of the projected moth production schedule

Once the inoculation and mating schedules for all suborders have been completed, they should be double-checked and entered on the programme schedule (Table 4A). The task of completing the projected moth emergence schedule (Table 3) can now begin. The completed mating schedules in Table 4A show that 80 adults that are at least 1 day old are required to fill 4 mating boxes for suborder [2] on 28 May ((a) in Table 4A). Also adults will be needed throughout most of June, with large numbers required from 24 June to 30 June. Adults will also be needed throughout July. A single requirement for 120 moths for suborder [17] occurs at the end of August.

It is necessary initially to produce a rough draft of the projected moth emergence from subcolonies A and B. These colonies can then be manipulated in order to make adults available on dates leading up to when they are required for mating (Table 4A) to fill all suborders. Thus moth emergence (Table 3) should by synchronous with the mating schedule (Table 4A). The mechanics of timing by the use of life history data for group-reared insects has already been explained.

In Table 3, the aim has been to initially stagger both subcolony A and subcolony B so that the moth emergence of B begins the day after the last usable moth has emerged from subcolony A. This juxtaposition of subcolonies was made possible in April by mating moths from subcolony A at the end of emergence (on 9, 10 and 11 March) and by mating moths from subcolony B at the beginning of emergence (21, 22 and 23 March). This effectively closed the 9-day gap between the end of emergence of subcolony A (11 March) and the beginning of emergence of subcolony B (21 March), and produced a steady emergence of moths from 14 April to 3 May. From then on, provided the chosen day from colony mating is the same in each consecutive generation, this juxtaposition can continue. Thus, for generations 131A and 131B mating is initiated on day 38 (22 April for A, 2 May for B) to produce continuous emergence beginning on 28 May and running through to 20 June. Note that mating (and inoculation) is allowed to continue over 4 days for 131B so as to extend emergence to day 43. This allows moths to be made available for mating in suborder [18]. In the same way, both colonies are mated on day 34 in each generation in June and July to produce moths from 7 to 27 July and from 16 August to the end of August.

The availability of moths from subcolonies A and B over the dates shown in Table 3 is not sufficient to provide moths for all suborders. The large requirement at the end of June and beginning of July is not catered for. A new subcolony must therefore be started to produce moths during this period, beginning on 21 June to meet the requirement for suborder [7] on that date. Because large numbers of fresh moths are required from 7 July (Table 4A), this new subcolony, designated C, must be maintained up to 6 July. This can be done by extending the inoculation period, and hence the mating period, over 5 days (inoculate from 23-27 May and mate from 16-20 May). Working back a further generation, this new colony can be started by splitting subcolony 130B on 29 March (see (b) in Table 3). Moths can now be made available to fill all the suborders in order CM7/83.

The subcolony that moths will be supplied from for mating for each suborder can now be entered on Table 4A in the mating column as A, B or C. Thus, for suborder [2] on 28 May moths will be available from colony A; moths for mating on 14 June for suborder [3] will be supplied from subcolony B; and on 21 June moths will be used from both subcolonies B and C for suborder [7]. This system also aids in the calculation of diet requirements later. It is only at this point, after considerable planning, that the colony is known to be biologically manipulable and the order is known to be feasible. The order is not accepted, however, until several resources have been considered.

4.8 Calculation of artificial diet and rearing container requirements

Table 5 summarises the total numbers of adults to be used from each generation, the corresponding number of rearing containers needed to produce them, the amount of diet to prepare and preparation dates.

Table 5 Rearing container numbers and diet preparation schedule

Generation number	Subcolony start date	No. of adults needed	No. of rearing containers	Amount of diet (kg)	Preparation date
131A	16 Mar	400	1	4.0	14 Mar
131B	26 Mar	400	1	4.0	14 Mar
131C	05 Apr	400	1	4.0	14 Mar
132A	29 Apr	640	3	12.0	27 Apr
132B	09 May	1100	4	16.0	27 Apr
132C	23 May	1120	4	16.0	27 Apr
133A	08 Jun	940	3	12.0	06 Jun
133B	18 Jun	640	2	8.0	06 Jun
134A	18 Jul	400	1	4.0	13 Jul
134B	28 Jul	520	2	8.0	13 Jul

The amounts of Brinton diet needed for group rearing can be gauged quite simply. By adding together the total numbers of moths that will be used from each subcolony from the mating schedule in Table 4A, the number of rearing containers required can be found. Under conditions of medium larval density, a rearing container will produce about 770 moths.

Using generation 132A (see Table 4A) as an example, 4 mating boxes (80 pairs) are needed on 28 May and 2 mating boxes (40 pairs) are needed on 4 June. In addition, at least 200 pairs should be drawn from the colony for mating on 1 June (see Table 3) to produce eggs for the next generation (133A). A total of 640 moths are therefore required from this generation. Although this number of moths can easily be produced from 2 rearing boxes, the days of emergence on which moths are required must be considered. It can be seen from Fig. 6 that only about 9% of the total emergence occurs by day 30. A box producing a total of 770 moths will therefore yield only 69 moths on that day. Thus, the 80 pairs (160 moths) needed on 28 May (day 30; see Table 3) can be obtained only by collection from 3 rearing boxes, which should yield about 207 moths. On day 34 (1 June) over half of the adults will have emerged (see Fig. 6). From 3 boxes, this will provide moths - i.e. more than the 200 pairs needed - for the next generation, and mating can occur as planned in Table 3.

The last requirement for adults from generation 132A occurs on day 37 (Table 4A; 4 June), when 40 pairs are needed for suborder [1]. This requirement can easily be met since 95% emergence will have occurred by day 37 (see Fig. 6). Three rearing boxes of Brinton diet will hence be sufficient to provide moths for generation 132A. Moth numbers and rearing box numbers for all subcolonies of each generation are worked through by deduction in the same way, beginning with the number of moths required on each day of emergence. This is particularly important where large numbers of moths are to be used during the

initial stages of emergence.

The total production of Brinton diet from March to July is estimated at 88.0 kg. The diet keeps 3-4 weeks under refrigeration, so a single large batch can be produced at the beginning of each generation, to be used as necessary on each subcolony start date (Table 5). Diet preparation should be restricted to days when there is less work, e.g. no mating or inoculation, to be done.

The total number of polythene tubes required for individual inoculation of Singh diet with first-instar larvae for suborders [1] to [17] (excluding [5] and [17], which are for first instars) is 15730 (from Table 4B, column 4). The amount of diet dispensed into these tubes varies according to the degree of larval development occurring. For suborders of second, third and fourth-instar larvae, 1.5-2.0 g of diet per tube (about quarter full) is sufficient. Fifth-instar larvae destined for diapause require 3.0-4.0 g of diet (tubes about two-thirds full).

The number of tubes produced should be increased by about 2% to allow for errors in dispensing. The total number is thus about 16000. Considering the shelf life of the diet, it may be dispensed in two batches of 8000 tubes, one on 2 June and one on 30 June. Each batch will require about 20 kg of freshly prepared Singh diet.

4.9 Calculation of personnel requirements

The final resource to be considered is the personnel available to carry out the insect rearing and diet and equipment preparation necessary for the order. The numbers involved are summarised in Table 6.

Table 6 Minimum personnel requirements for Insect Order No. CM7/83, June to July only

Task	Approximate person hours
Brinton diet preparation (including waxing)	8
Singh diet preparation (2 batches)	16
Larval inoculation of diet	126
Moth collection and sexing	69
Mating box preparation complete with moths	40
Egg collection	8
Equipment washing and sterilization	28
Total	295

One experienced person can prepare and dispense a batch of Brinton diet (13.0-39.0 kg) in 3-3.5 hours. Painting a paraffin wax coating over the diet takes about 10 minutes per tray. The total time needed to produce each complete batch of 8000 tubes of Singh diet is about 8 person hours.

An experienced person can inoculate about 750 neonate larvae individually onto diet in test tubes in 6 hours. The total complement of tubes for this order (15730) will therefore take 126 hours. Collection of individual moths from rearing boxes is necessary in order to sex each moth and place the correct sex ratio in each mating box. The amount of time spent in collection varies, depending on the emergence day (see Fig. 6). Sixteen rearing containers of moths will be collected from generations 132A, B and C and 133A and B during June and July, with a yield of about 770 per container. An experienced person can collect on average about 3 moths per minute. Thus, the total expected emergence (12320) will take 69 hours to collect. The 64 mating and oviposition boxes (from Table 4A) required for suborders in June and July and the 32 required for subcolony mating (2 eggsheets per rearing container) will each

take about 25 minutes of preparation (including placement of moths therein). The collection of eggsheets is estimated at 5 minutes per mating box. Washing and sterilizing eggsheets is estimated at 5 minutes per mating box. Washing and sterilizing of rearing boxes and diet preparation equipment should take about 28 hours.

All the figures cited are for a person experienced in insect rearing. Inexperienced personnel may initially take consideraby longer to complete a given task. The total minimum amount of time required to complete the June-July section of this order (i.e. the labour intensive period) is about 300 hours. Thus, if one person works 7 hours per day on the order, it will take about 43.0 working days (almost exactly 2 months) to complete. Realistically, two persons should be available during this period to cope with the order, and a third may be needed on days when there is a heavy workload or some other commitment. To undertake this order, the personnel resources are adequate in our laboratory.

4.10 Approval of the order

At this stage the order is known to be practicable in terms of both colony manipulation and resources, and it is approved on the insect order form. The user group is then informed of its confirmation.

The transfer to a daily diary of all tasks shown in Tables 3 and 4A facilitates work distribution by the insectary manager. This also allows the advanced planning of material and personnel resource distribution, particularly for periods of heavy workload. Work schedules for other species being reared for supply should all be combined in the same diary.

Although the example used for laboratory codling moth production is a relatively complex one, the methodology is not definitive and there are several alternatives to the procedure outlined. These alternatives, particularly with respect to temperature variation, become apparent both during the planning of the order and during the production of insects. However, short cuts are best avoided by inexperienced personnel or where methods are not fully researched. Even minor changes in technique can result in underproduction, and supplies to user groups may suffer.

The example has detailed how one insect order has been planned and executed. There may be several orders like this for codling moth in progress at any given time. We also rear several leafroller species for various research programmes and hence cater for several user groups. Their requirements may each extend to more than one species and may involve a number of stages and supply dates. The use of insect production management in the organisation of multiple-species rearing is thus essential in our laboratory and the application of IRM provides a high degree of exactness and efficiency in the production and supply of experimental insects.

5.0 CONCLUSION

Insect rearing management (IRM) is a complex field that encompasses science, art and business management practices. This requires abilities in forecasting, planning and organisation, personnel management, and the application and use of flow charts and schedules in production. A broad knowledge of entomology is a prerequisite for the understanding and development of insect rearing methodology. This includes a theoretical and practical familiarity with an insect's behaviour, food requirements and biology. A background in biochemistry, nutrition and food technology is also desirable. Insect rearing is a continuous learning experience involving the application of techniques that are always developing. Effective management of any insect colony can be developed only through patience and practice.

The most valuable assets in any IRM programme are its personnel. Whether an insect rearing laboratory is large or small, the quality of its workers determines its daily operation and overall efficiency. Insect rearing is a seven-day-a-week occupation that requires skill, care and dedication.

We have established seven basic elements involved in IRM: objectives, colony establishment, research and the development of techniques, design of the insect rearing laboratory, resources, quality control and production. Each element may be limited or modified by any one or more of the others. It is only after their integration through management that production can begin, leading finally to the supply of high-quality insects. We have attempted to present an overview of the elements and how they are involved in both establishing a laboratory colony and producing and maintaining an already established colony as a more efficient production unit through management. Using the codling moth as an example, we have presented a workable model of laboratory insect rearing management which can be used to plan and conduct a rearing programme intended for research needs. The basic prerequisites and requisites for a programme using IRM may be summarized as follows:

1) Discussion of long-term objectives with user group.
2) Adequate knowledge of insect's biology under laboratory conditions.
3) Ability to manipulate insect development.
4) Reappraisal of objectives and receipt of insect order.
5) Preparation of management schedules for feasibility study.
6) Approval or non-approval of insect order.
7) Insect production and supply.

We recommend that insect rearing laboratories engaged in producing insects for research should practice IRM. The advantages are:

1) It is efficient.
2) It is economical.
3) It utilizes resource management and conservation.
4) Its successful use inspires confidence in personnel.
5) It leads to the reliable supply of insects on demand through accurate planning.
6) It allows production of high-quality insects.

The degree of success of an IRM programme can be measured by whether or not all the objectives of the user group have been met; if they have, the programme may be considered successful.

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7.0 ACKNOWLEDGEMENTS

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8.0 GLOSSARY

COLONY MAINTENANCE The rearing of a number of insects which will sustain a laboratory population at a required continuous level.

ESTABLISHED COLONY A population that has been reared successfully in the laboratory for several generations.

INOCULATION The act of introducing an insect, often at the egg or larval stage, onto a food source in a rearing container.

INSECT ORDER A formal user group request for insects detailing the species, numbers, stages, dates of supply, purpose of supply, and any special instructions, such as quality, for the laboratory production group.

INSECT REARING The propagation or culture of insects in captivity through one or more generations.

INSECT REARING MANAGEMENT (IRM) The efficient utilization of resources for the production of insects of standardized quality to meet programme goals.

INSECT USABILITY Insects that have been reared for a particular purpose and have reached a desired stage within a specified period are referred to as 'usable'. Insects that have not reached a desired stage during a specified period are referred to as 'non-usable'.

INSECT YIELD The percentage difference in numbers of live insects occurring between a starting stage and a required stage; e.g. if 80 pupae are obtained from development of 100 first instars, then the pupal yield is 80%.

LABORATORY PRODUCTION GROUP All personnel involved in insect rearing.

MANIPULATION Modification of the duration of development of individual insects, populations or entire colonies by control of environmental variables.

SUBORDER Any part of an insect order (q.v.) that can be supplied as a biological unit meeting specified criteria, e.g. stage, age, date of supply or sex.

USER GROUP The personnel receiving insects for a particular purpose from the production group.

Biological Control of Insect Pests and Weeds

Edited by Paul DeBach
Assistant Editor Evert I. Schlinger

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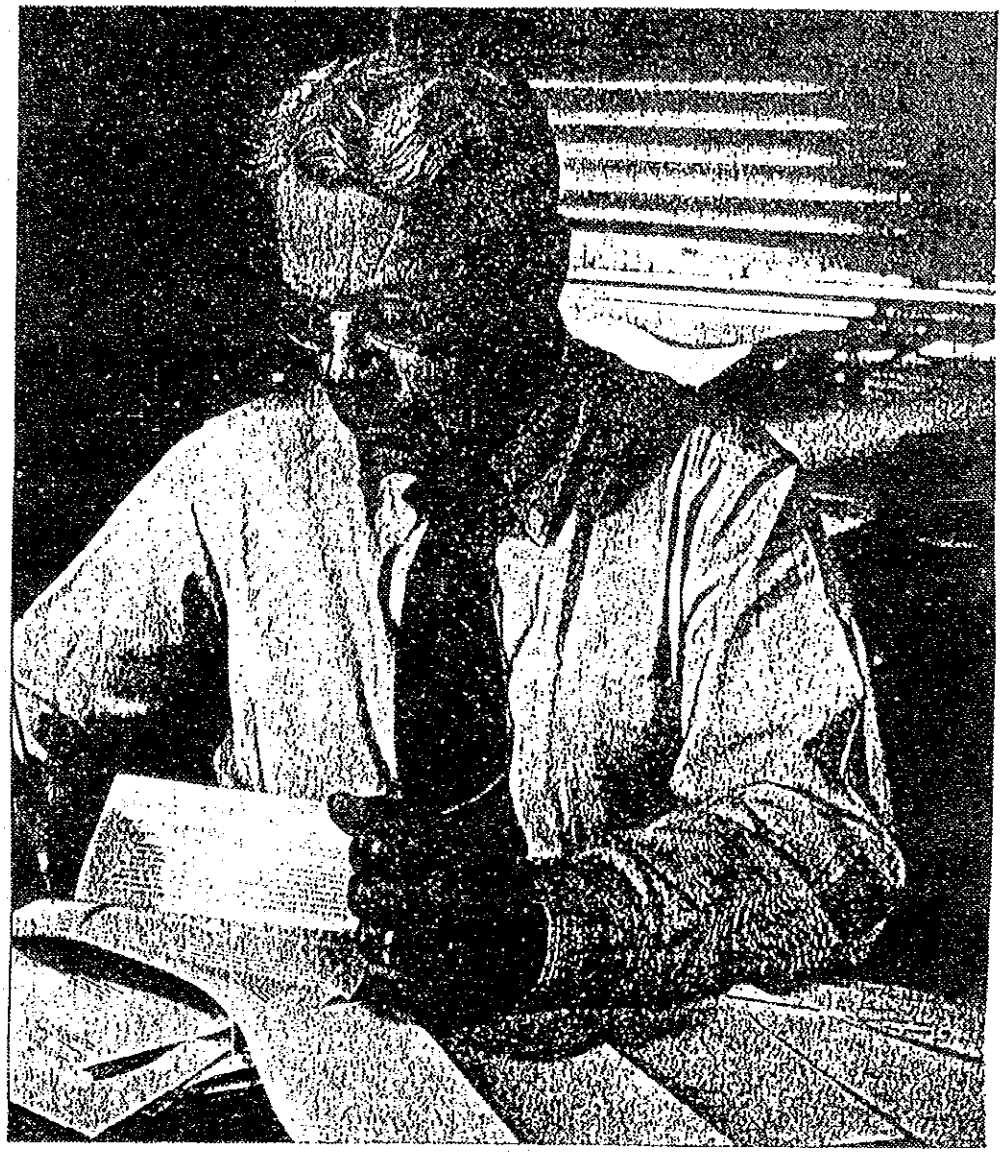

DEDICATION

This book is dedicated to the late Professor Harry S. Smith, the mentor, inspiration, and former chief of nearly all of the chapter authors. For many years Professor Smith was recognized as one of the world's outstanding authorities on biological control. He was in charge of biological control work in California from 1913 *to* 1951, *first in the State Department of Agriculture from* 1913 *to* 1923, *then in the University of California from* 1923 *until his retirement in* 1951. *Under his guidance the first American continental projects on biological control of weeds and on insect pathology were developed in California. Royalties from this book go to the Harry S. Smith Memorial Fund of the University of California, which is used to help students and to promote progress in the field of biological control.*

First published 1964

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Preface

THIS BOOK has had a long gestation period. About thirty years ago the late Professor H. S. Smith and Professor C. P. Clausen laid plans for such a book and did considerable initial work on it. However, not until the mid-fifties was the idea decided upon of having as authors various specialists in different phases of biological control within the Department of Biological Control of the University of California. This is the result.

We think the reader will find this book to be different from the usual symposium-type, multiple-author book. It was planned for cohesiveness and continuity much as a single author would plan his book. The volume was purposely divided into sections whose chapters form a related group of subjects. Section 4, for example, is organized in the manner in which a typical project in applied biological control might be carried out; ranging from foreign exploration and importation to the final field evaluation of the effectiveness of introduced natural enemies.

Section 1 defines biological control and discusses its scope, importance, and historical development. Section 2 covers the ecological basis of biological control with emphasis on the fundamentals of population ecology and natural control and also treats of some common concepts and questions in biological control work. Section 3 lays the basis for working with and understanding the organisms employed in biological control research. It treats of their biologies, habits and identification. Section 4, as mentioned, essentially describes the 'bread and butter' work of biological control—the importation and establishment of new natural enemies. Section 5 discusses ways and means of improving the effectiveness of already established natural enemies or of preventing their effectiveness from being reduced. Sections 6 and 7 were considered separately because they deal with the specialized phases of insect pathology and biological control of weeds. In conclusion, the last chapter reviews over 220 successful cases of biological control, discusses current trends and considers future possibilities. The Table of Contents serves as an index to the various subject matter specialities. A complete index to scientific names is given at the end of the book as are over 2500 literature references. The survey of literature was completed by the various chapter authors by June, 1961.

The general outline of the book and most of the eventual authors were determined by a committee consisting of Paul DeBach, R. L. Doutt, T. W. Fisher, C. B. Huffaker, E. I. Schlinger, and E. A. Steinhaus. C. P. Clausen was originally scheduled to be editor but upon his retirement in 1959 he was succeeded by the current editor. Later, E. I. Schlinger was named assistant editor. Professor

inexpensive pollen substitutes for bees. Since some parasitic and predatory insect species require pollen in the adult stage at least, research concerning pollen substitutes is important to biological control workers. Haydak and Tanquary (1942) reviewed such attempts and from their tests found that a mixture of soy-bean flour and dry skim milk can be advantageously used in an emergency. Haydak (1949, 1958) obtained better responses when worker honey-bees were fed soy-bean flour fortified with both niacin and riboflavin or dried brewers' yeast. Weaver and Kuiken (1951) compared essential amino acids of royal jelly and six pollens and found no marked difference in essential amino acid composition. They also found that soy-bean protein, casein, and whole egg were very similar in amino acid make-up in pollen substitutes that explains the lack of success in finding a highly effective substitute. However, de Groot (1953) believes the concentration of the amino acid, methionine, in soy-bean flour to be a limiting factor for growth of the honey-bee. The same author (1953) reviews adult dietary studies and gives the amino acids essential for the adult bee in reference to longevity and weight increase. Longevity is substantially increased by adding protein foods to a carbohydrate diet. Pollens from different plants are not the same value for bees, and supplementing various pollens with nutrients offers clues to identifying deficiencies which influence longevity and fat body development (Maurizio 1954) and growth (Levin and Haydak 1958). Honey-bee larvae have been reared on royal jelly in the laboratory (Weaver 1955, 1958).

Formicid nutrition has been studied in only a few species. Smith (1942, 1944) working with colonies of *Camponotus* found that reduced food supply influenced the size of adults produced, and qualitative differences in food composition affected number and size of the progeny. Gösswald (1940, 1951) was able to culture *Formica rufa* L. by providing various insects—i.e., sawfly larvae, *Musca domestica*, and *Tenebrio molitor* L. larvae—or horse meat, plus a carbohydrate such as honey or a sugar solution, and in the field solid sugar placed on artificial nests to aid in colony establishment.

CHAPTER 13

Insectary Facilities and Equipment

T. W. FISHER AND G. L. FINNEY

INTRODUCTION

PRINCIPLES of culturing entomophagous insects were presented in chapter 11. Included for clarity were certain specific facilities or items of equipment relevant thereto. The purpose of the present chapter is to describe and discuss basic features thought to be integral parts of the physical means whereby entomophagous insects can be cultured. Subject matter in this chapter will emphasize development and use of buildings and facilities wherein an attempt is made to alter the existing local climate.

Fundamentally, the word 'insectary' means a place wherein insects are housed or propagated. Therefore, technically the insectary concept would embrace the entire range from caged individual limbs or trees, as the vedalia beetle, *Rodolia cardinalis* (Muls.), was first propagated in California, or *Cryptognatha nodiceps* Mshll. in Fiji, to the opposite extreme of modern climate-controlled installations such as the insectaries of the Canada Department of Agriculture, Entomology Research Institute for Biological Control, Belleville, Ontario, Canada, one of the Commonwealth Institute of Biological Control laboratories at Bangalore, India, and at Rawalpindi, Pakistan, and the insectaries of the University of California, Department of Biological Control at Albany and Riverside.

If justification for the existence of insectaries is necessary, it is clearly given by Beckley (1956) who stated '... the primary reason for the existence of the Associates Insectary is to promote biological control.' This statement takes on added significance because Mr. Beckley is responsible for pest control on 10,000 acres of oranges and lemons in or near Santa Paula, California, and therefore views biological control strictly as an effective and economical means of controlling mealybugs and soft scales, particularly *Saissetia oleae* (Bern.). Another growers' co-operative insectary exists in Fillmore, California, and here *Metaphycus helvolus* (Comp.) is propagated for release against *S. oleae* on over 7,000 acres of citrus. These are only two examples of proved commercial insectaries and they clearly represent the ultimate goal of insectaries, namely, to provide safe, economical pest-control service for the grower.

Before discussing the physical facilities helpful in the propagation of entomophagous insects, it would be well to consider the personnel who will use the facilities, for it goes without saying that the success or failure of the programme

will depend largely on the capabilities, training, and interest of the personnel involved. Therefore, careful selection of insectary workers is particularly important since the propagation of insects demands highly specialized and extremely varied work. Consequently, no amount of formal training can prepare a person completely for the work. A high degree of interest, curiosity, and enjoyment in working with living animals usually will indicate capability in this direction. Of course, formal training in entomology is highly desirable and usually necessary at the supervisory level. True, practical training is of great benefit, but a somewhat broader background of insect behaviour, physiology, taxonomic affiliations, and biologies will better equip personnel to cope intelligently with insectary problems as they arise.

Further, it is very important that all persons working in the insectary realize the need for the faithful performance of their particular jobs in the over-all propagation programme, and in so far as it is practical, all personnel should develop a basic understanding of all operations within the insectary. Large programmes require a higher degree of specialization among personnel than do programmes where perhaps two or three persons perform all the duties associated with insect propagation—insect handling as well as mechanical knowledge and ability adequate to keep insectary equipment functioning properly.

If the insectary serves several projects under different project leaders or is of commercial scope, it seems desirable to have a superintendent who is responsible for the physical facilities as well as for co-ordination of the various aspects of the propagation programmes, such as making decisions regarding assignment of space in order to minimize intercontamination of cultures.

LOCATION OF INSECTARY

From the standpoint of climate control within the insectary, an area of temperate climate offers the best location. In tropical and subtropical regions, with the exception of certain insular areas, the climatic area chosen should be cool, as in the higher elevations, for it is far easier and cheaper to control heating requirements than to provide facilities for adequate cooling. Experience has shown that inadequate temperature control during climatic extremes usually results in loss of cultures and can neutralize the work of several months.

The ground area occupied by an insectary is determined by the projected purpose and the value placed on such a development by persons interested in it. One or two rooms in an existing laboratory may be considered adequate for certain programmes. By way of contrast, there are over 6,000 square feet in the insectary at Riverside, California (figure 60), and over 61,000 square feet in the quarantine building and new (1955) laboratory building at Belleville, Ontario, Canada (figure 61).

Easy access to the insectary is a necessity. This includes roads, approaches, ramps, and loading areas, which are necessary for convenient handling of host-plant material, lumber, refuse removal, and miscellaneous items of large bulk.

Where possible, consideration should be given to locating the insectary away from the immediate vicinity of agricultural areas. A minimum buffer zone would

be perhaps a quarter mile for these reasons: (1) reduces hazard of insecticidal drift from a crop area entering the insectary; (2) reduces chance of contaminant species of parasites or hosts entering the insectary; and (3) reduces chance of hosts (pest insects) moving from the insectary to the crop area. The few preliminary tests which have been concluded to date in order to determine the effect of air pollutants on insects indicate deleterious results to certain aphelinids and coccinellids. Therefore, unless adequate filter systems are contemplated, it would be well to avoid locating an insectary where urban or industrial atmospheric contamination (smog) is a problem.

The terrain of the building site need not be perfectly level. Because of the prime importance of climate control within the insectary, the insulation gained by going below ground may be worth considering. Where contour, water table, and subsoil conditions permit, a basement or cut into a hillside may offer tremendous savings in future air-conditioning costs, as well as permit more accurate climate control.

If the above ground portions of an insectary can be oriented with the greatest length arranged in a true east–west direction, especially in areas with hot summers, natural lighting gains uniformity, the wall on the sunny side can be shaded by an overhanging roof or louvres, and only the short west wall is exposed to the greatest heat source and can therefore be shaded by awnings, louvres, or trees.

Landscaping in the immediate vicinity of the insectary should exclude plant species which serve as hosts of phytophagous insects scheduled for propagation within the insectary. It is well to keep all foliage away from walks and entrances in order to reduce the possibility of carrying contaminant organisms into the insectary on clothing.

BUILDING SPECIFICATIONS

This discussion applies primarily to permanent insectaries. A permanent insectary is one whose projected use will extend over several years. Temporary insectaries are those with a projected short-term use such as for a few seasons.

Exterior Design

Local materials, construction regulations, and architecture will largely decide the exterior features of the permanent insectary. However, facilities of a temporary nature may be needed under certain circumstances and so will be discussed briefly later in this chapter. It is again emphasized that the primary insectary problem is climate control, and many aspects of design revolve around this one necessity regardless of the permanent or temporary status of the insectary. As a consequence of the insectary's highly functional nature, such appurtenances as coolers, louvres on windows, and reflective roofs may be expected to alter somewhat the exterior appearance of the insectary from that of conventional buildings. This need not always happen, however. A field station of the Commonwealth Institute of Biological Control at Fontana, California, is an example of a highly functional insectary in keeping with its residential surroundings (figure 62).

Interior Design

The floor plan will be determined by the proposed use of the insectary. If the intended use is for research and limited mass culture, a quarantine area should be provided and the main insect-handling area may consist of one or more large rooms which may be partitioned into smaller rooms as needed. The insectary at Albany is an example of this type (figure 63).

If the intended use is for large-scale mass-culture programmes, a different design will be called for. The commercial insectaries at Fillmore and Santa Paula illustrate this type (figure 64). Figure 65 shows some of the insectaries given in the listing at the end of this chapter (table 5).

A suggested modest floor plan which meets basic requirements of routine mass production as well as research is shown in figure 66. Because of their varied programmes, federal, state, or university institutions probably would be concerned with both aspects. This concept guided the design of the recent addition (1960) to the insectary at Riverside. Figure 60 shows exterior view and floor plan.

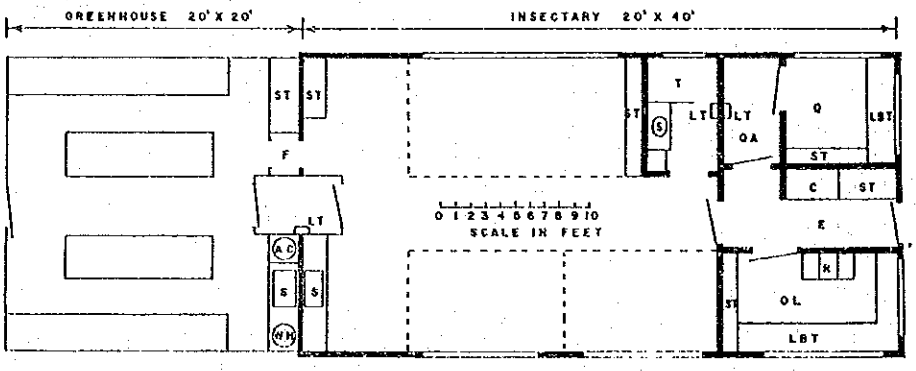
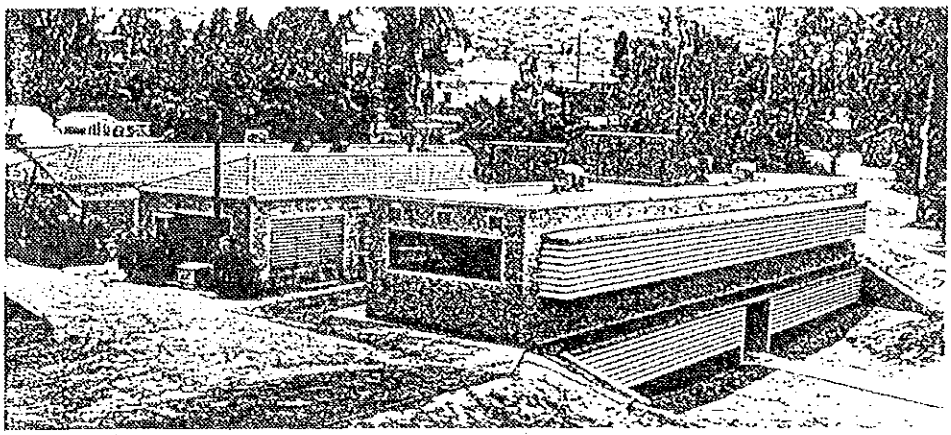


FIGURE 66. Suggested modest floor plan which meets basic requirements of routine mass production as well as research. (Dotted line indicates location of temporary partitions.)
AC Air conditioning. C Clothes closet. E Entry. F Fumigation. LBT Laboratory table. LT Light trap. OL Office-laboratory. Q Quarantine. QA Quarantine anteroom. R Records. S Sink. ST Storage. T Toilet. WH Water heater.

In order to provide ample space for manœuvring bulky items such as carts and racks, corridors are at least 4 feet wide and doorways at least 3 feet wide. Ceiling height is recommended at 7 feet. This height can be reached easily by a person 5 feet 10 inches tall, thus facilitating collection from the ceiling in open-room cultures. Cost of air conditioning is reduced considerably by eliminating excessive head space.

Because fine dust is harmful to beneficial insects, dust control in the insectary is very important, particularly in open-room culture. Since a concrete floor is a major source of dust from the scuffing of its surface, it usually requires covering, at least in rearing rooms. Painting and waxing are adequate only if they are maintained properly. A more satisfactory solution is to cover concrete floors with linoleum or asphalt-vinyl tile. Wooden floors do not present a critical dust problem, but they



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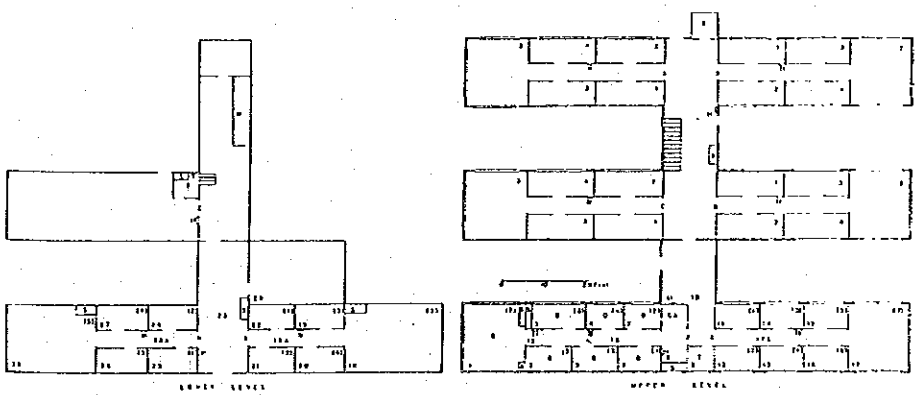


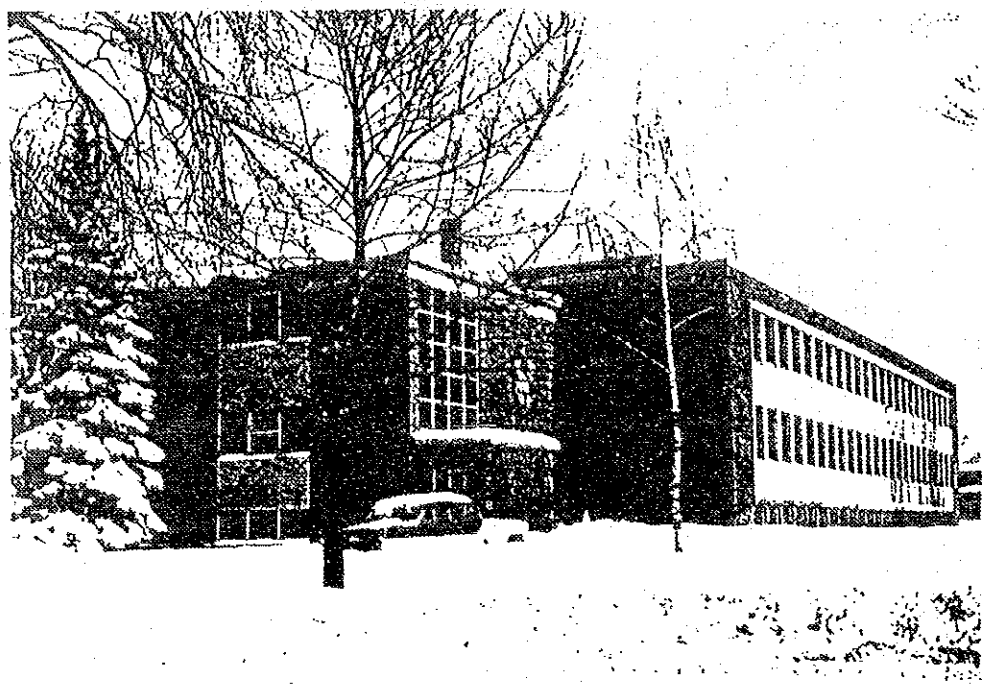
B

FIGURE 60. Facilities of the Department of Biological Control (University of California) at Riverside, California.

A. Exterior aspect as viewed from the north-west. The two wings with the tile roof were erected in 1930. The new addition was completed in 1960.

B. Floor plan. Large rooms for mass culture and small rooms for isolation are provided.

Legend:	A	Autoclave	M	Machinery	S Sink
	I	Incinerator	Q	Quarantine	T Toilet
	It	Light trap	QA	Quarantine anteroom	



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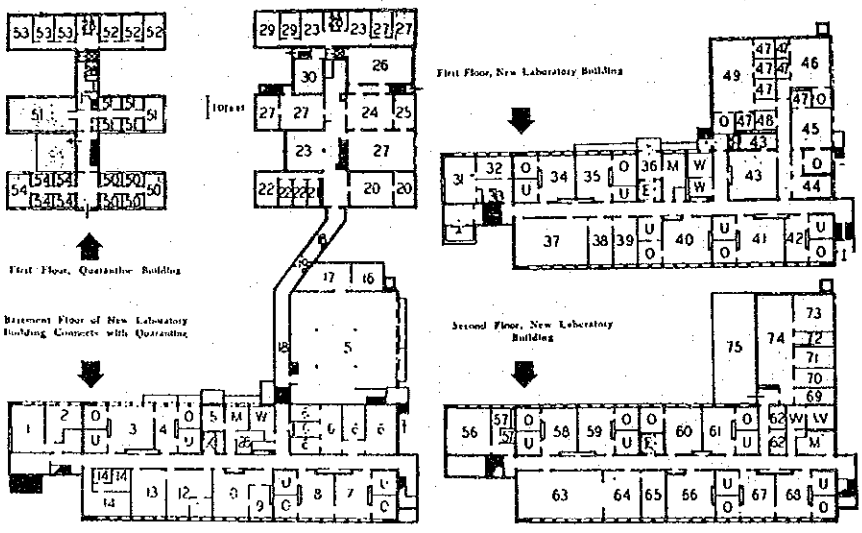
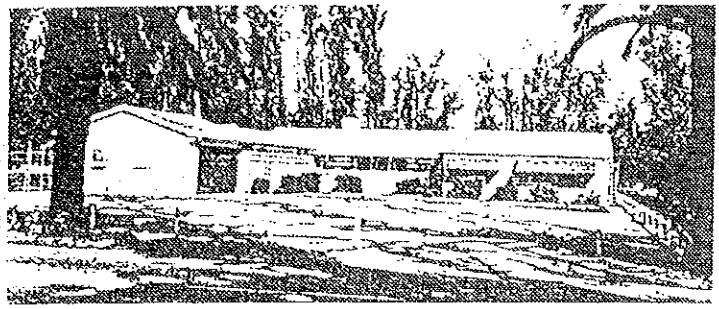


B

FIGURE 61. Canada Department of Agriculture, Entomology Research Institute for Biological Control, Belleville, Ontario, Canada.

A. Exterior of laboratory building. Quarantine building not shown.

B. Floor plan. Details may be obtained from *Agricultural Institute Review*, November–December, 1955.



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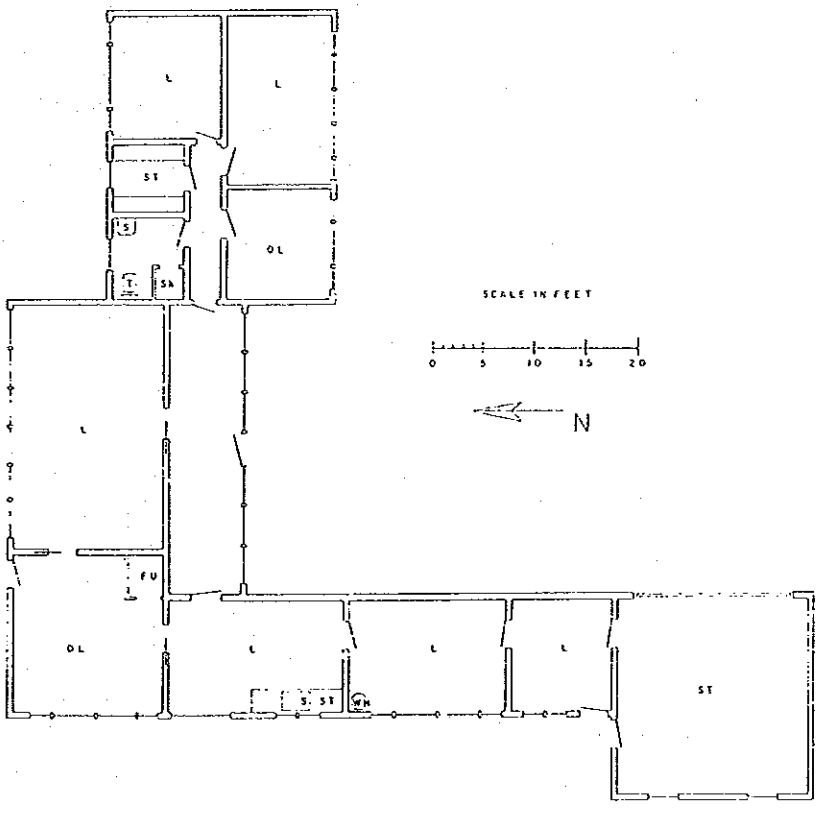
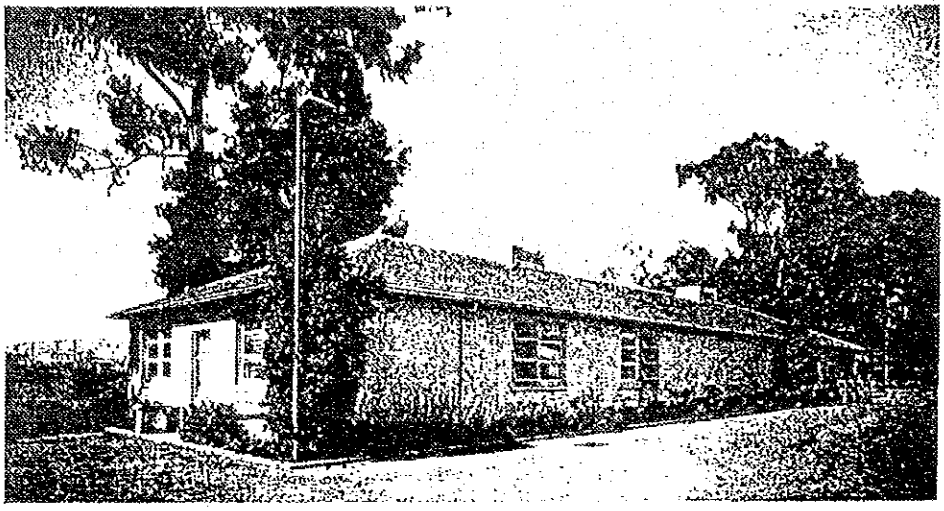


B

FIGURE 62. Commonwealth Institute of Biological Control insectary located at Fontana, California, is an example of a highly functional insectary designed in the motif of its surroundings—in this instance, a residential area.

A. Exterior—south aspect.

B. Floor plan. Cooling is provided by refrigerated room air conditioner placed in window openings. The larger storage area is cooled by an evaporative cooler.



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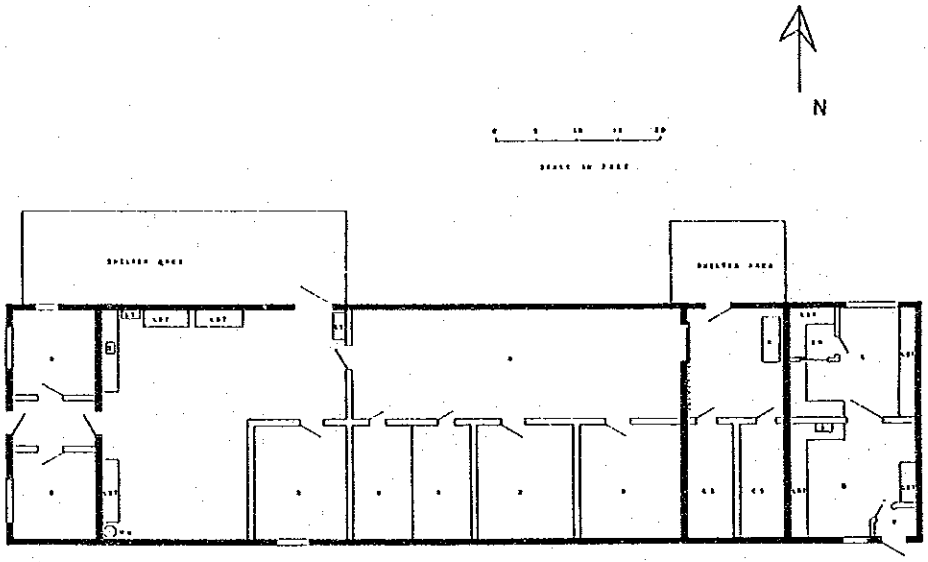


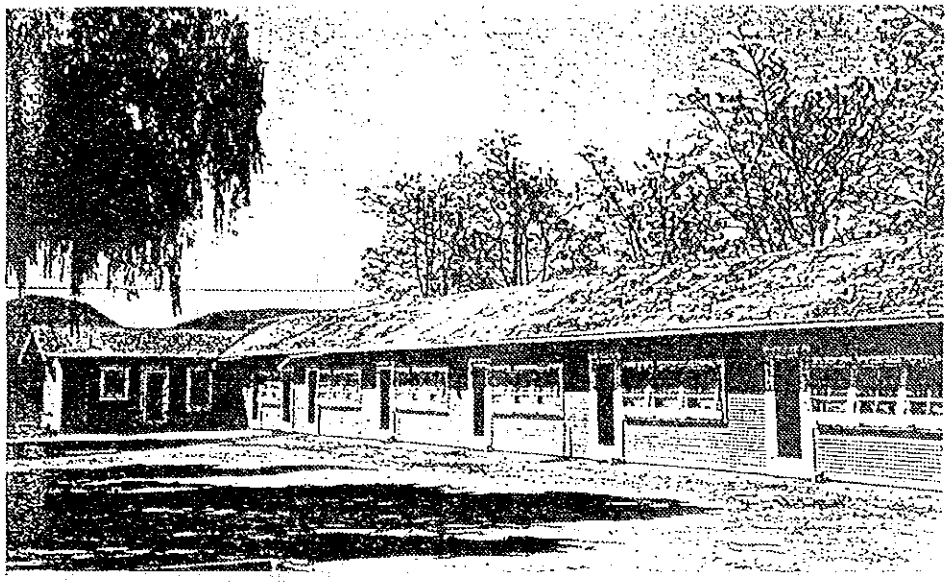
B

FIGURE 63. Insectary of the Department of Biological Control (University of California) located at Albany, California—an example of open planning.

A. Exterior: south aspect.

B. Floor plan. Most of the partitions are temporary and can be moved as propagation needs dictate. The quarantine laboratory is in a separate building (see figure 49 B, chapter 10). Shelter areas are roofed over only and serve as storage for cages, melons, etc.

Legend:	CS	Cold storage		R	Rearing	ST	Storage
	L	Laboratory		S	Sink	V	Vestibule
	LBT	Laboratory table		SR	Sterile room	WH	Water heater
	O	Office					



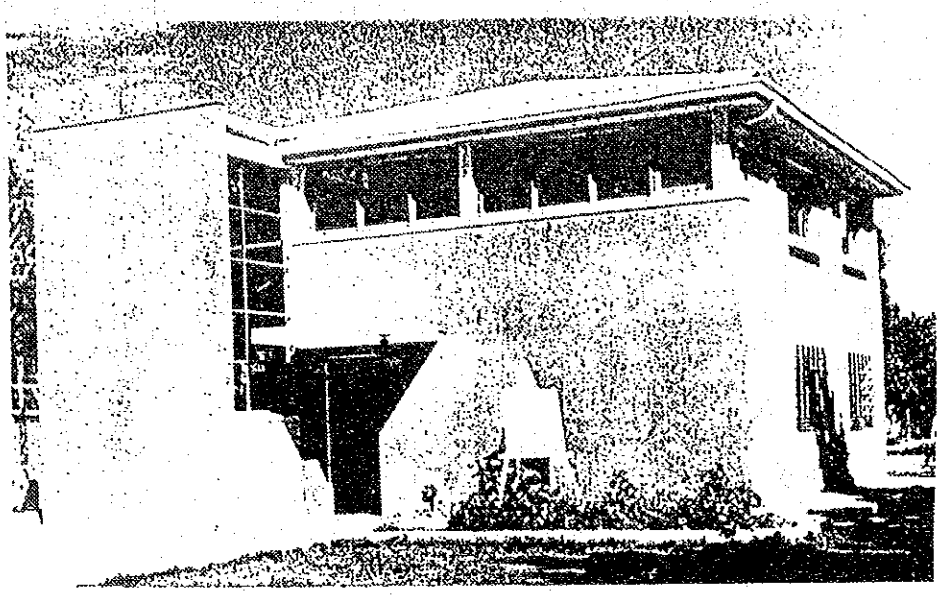
A

B

FIGURE 64. Commercial insectaries. The buildings shown plus other similar structures at each location serve the acres indicated.

A. Fillmore Citrus Protective District, Fillmore, California, serves 7,000 acres of citrus.

B. Associates Insectary, Santa Paula, California, serves 10,000 acres of citrus.



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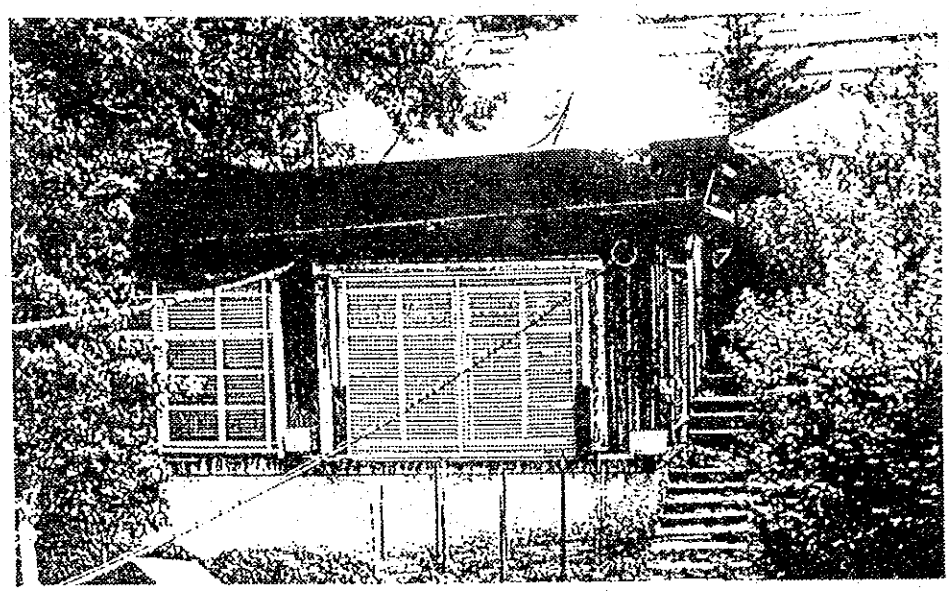
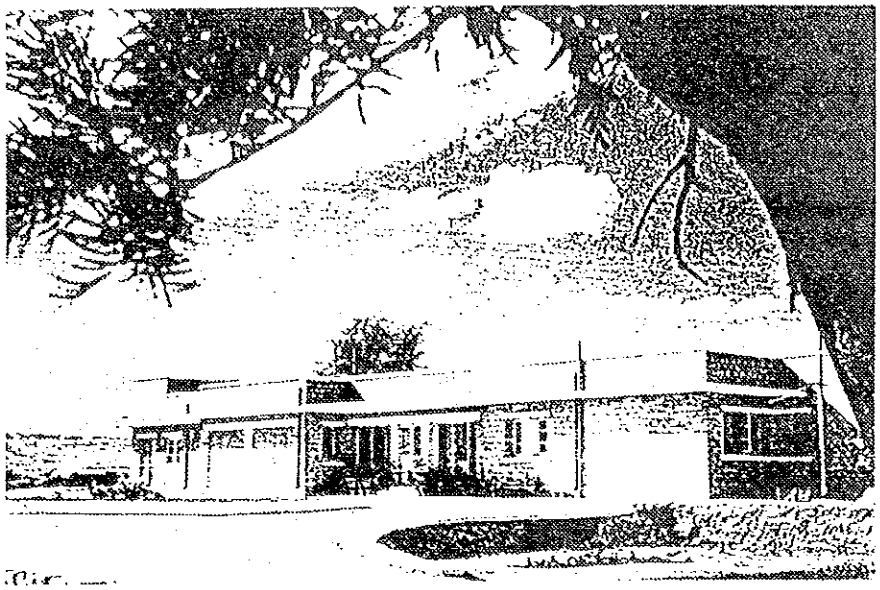


B

FIGURE 65. Research insectaries selected from the listing at the end of this chapter (table 5).
A. Ministerio de Agricultura, La Cruz, Chile.
B. Landesanstalt für Pflanzenschutz, Stuttgart, Germany.



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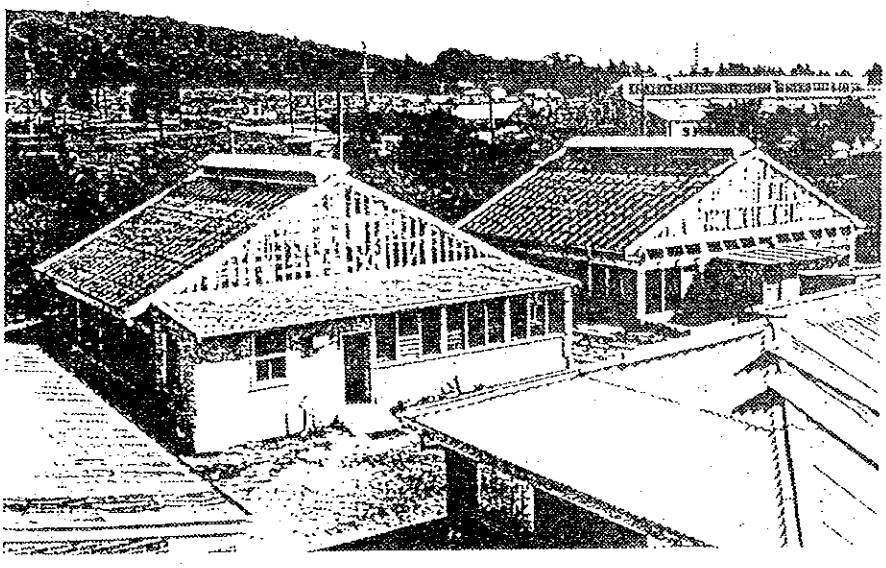
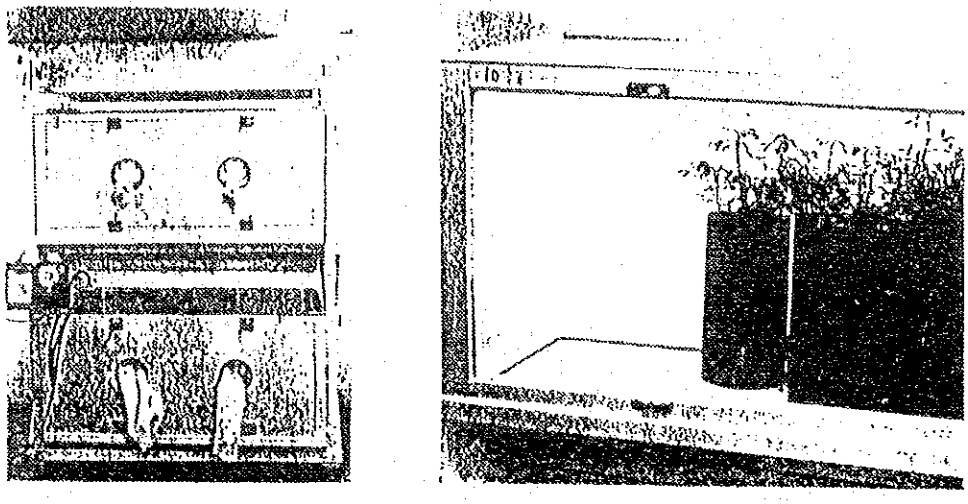


D

C. Commonwealth Institute of Biological Control, Bangalore, India.

D. Commonwealth Scientific and Industrial Research Organization, Division of Entomology, Canberra, Australia.



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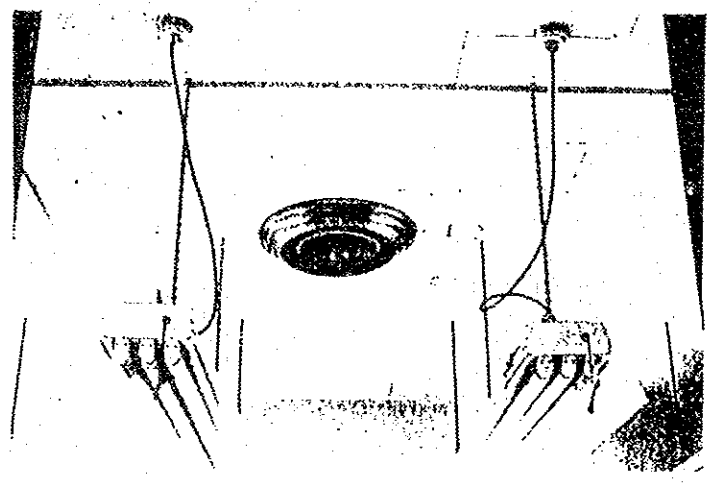

B

FIGURE 67. Methods of providing light for plant growth.

A. Portable lighting. Left: The lighting fixtures above each cage contain two (cool white) fluorescent tubes. A time clock automatically controls hours of light for four cages (two racks). The upper right photo shows the interior of a cage designed for use with this equipment. The sides and back are covered with organdie cloth, the top is heavy cellulose acetate, the door (not shown) is ¼" plywood. This cage sits on a removable bottom of ¼" plywood to which a tight seal is assured by soft rubber gasketing.

B. Fixed lighting. Where space and programming permit, banks of tubes 8 feet in length can be raised or lowered on chains attached to hooks in the ceiling to come within 12 inches of the plants, an optimum distance for many plants.