20. Data Recording and Analysis

Keeping accurate records consistently is essential in maintaining satisfactory production and quality. Management must insist on the maintenance of a system of record keeping, even when operations are going well and it appears to be unnecessary. Examples of forms used in recording quality-control data are provided in FAO/IAEA/USDA (2003). Data management is a vital component of mass-rearing operations. Akey et al. (1984) reviewed data processing. Modern computer systems have simplified data collection and analysis (Parker 2005).

20.1. QUALITY CONTROL

The computer-controlled environmental systems available today make record keeping rather easy, but this should not allow managers and operators to become complacent that machines will keep things running smoothly. Daily checks of the previous day's records must be made to ensure that production systems are functioning as planned. Environmental systems are amenable to automated controllers which monitor environmental conditions according to a pre-programmed plan (Parker 2005).

Preventive equipment maintenance activities must be kept on schedule. The costs in loss of production or in poor insect quality are too high to omit preventive work.

Rogers and Winks (1993) provided examples of control charts with data on fecundity, egg hatch and pupal weights over many generations (one point on a curve for each generation). In the charts, each parameter is bounded by control limits, set at three standard deviations from the mean. Charting these parameters shows natural variation within the control limits, non-random drift towards one of the limits and when a value has exceeded a control limit. These authors showed a table (with mean values \pm standard errors of the mean) to enable a comparison of parameters among several colonies.

Control charts (section 16.1) are a routine but very useful tool in monitoring the status, and especially the trends over time, of quality parameters. Calkins and Parker (2005) provided a good description and examples (values of fecundity) of Shewhart control charts; Leppla and Ashley (1989) showed a chart for pupal production.

Indices of competitiveness and compatibility obtained from field-cage mating performance tests are discussed in FAO/IAEA/USDA (2003) and Calkins and Parker (2005), providing formulae and graphic representations.

20.2. SAMPLING INSECTS FOR QC TESTS

The quality of insects will inevitably vary somewhat due to variations in the rearing environment and in the insects themselves. Such variations in insect quality need to be accounted for when sampling insects for QC testing so that the data obtained in QC tests are an accurate reflection of the overall quality of the insects produced, and means can be estimated with a reasonable degree of precision. It is necessary to have a statistical basis for deciding on the number of samples needed to achieve a predetermined level of precision (Cohen 2004).

Two approaches are described in FAO/IAEA/USDA (2003) — a stratified sampling scheme and pooling of data. In the case of codling moth mass-rearing, where process QC and product QC are separate activities and may be done by different persons and where shipment of insects to other projects is not common, stratified random sampling would appear to be the appropriate approach. To enable the tracing of any quality problems to a particular date of diet production, QC samples should be taken from diet and insects produced from that diet prepared on one day. (Taking samples from every day's production may not be necessary — once a week may be enough.) However, in a situation where the product from several days becomes mixed in the rearing system, e.g. emerged adults, samples will have to be identified by a particular week rather than a particular day.

21. Health Hazards and Safety in a Rearing Facility

Employers are responsible to recognize health hazards and provide maintained preventive equipment and training in safe working procedures, and employees need to work in a safe manner (Wolf 1984, 1985; Fisher and Leppla 1985; Cohen 2004; IAEA 2008). This section deals with allergies and safety issues; however, some micro-organisms that live in diet or in insects may cause disease in humans, e.g. *Aspergillus, Pseudomonas, Rhizopus, Serratia* and *Streptococcus* spp. (Sikorowski 1984a; Sikorowski and Lawrence 1994a, b).

21.1. ALLERGIES

Insect mass-rearing can pose a significant health hazard through inhalant and contact allergies, causing respiratory problems, dermatitis, etc. (Wirtz 1984; Wolf 1984, 1985; Kfir 1994). Besides respiratory allergies to moth scales in the air, allergic reactions to dust (from dry diet ingredients or spent diet), mould spores, mites and pheromones also occur. Preventing allergic reactions involves recognition and documentation of the problem and correction of the problem through appropriate air-ventilation and filtering, coupled with protective clothing and filter masks or respirators (Owens 1984; Wolf 1984; Ashby et al. 1985; Reed and Tromley 1985; Parker 2005). Even the oral aspirator (pooter) used traditionally by entomologists to collect small insects should have a filter capable of stopping 99% of particles with a diameter of 0.3µ (Wolf 1984, 1985).

Due to the risk of producing hazardous dusts, diet ingredients should be handled, mixed, ground, sifted, etc. in well-ventilated areas (Brewer and Lindig 1984).

Moth scales floating in the air are a significant inhalant (respiratory) hazard, especially for persons who are prone to allergic reactions. Allergies tend to develop over time and get worse rather than better. Therefore, it is essential that workers wear face masks at all times when in an area where moth scales are present in the air. Also, filtering of incoming air with dry filters to remove moth scales, with the final filter being a high-efficiency particulate air (HEPA) filter to remove other contaminants, is absolutely essential to minimize the problem (section 22.6).

Several major improvements in protecting workers from moth scales have been made. Toba and Howell (1991) described a system in which scales were removed from the air with filters in an adult collection room, and adult emergence containers were made of cheap fibreboard that were discarded after use (instead of cleaning and sterilizing metal containers). Scales from the pink bollworm are removed from the air by passing it through filters and cyclones (Stewart 1984; Wolf 1984, 1985).

At the OKSIR facility in Canada, the automated adult-collection system operates without the need for personnel to enter the emergence room during moth emergence (section 11.1).

Handling adults, oviposition cages and egg sheets exposes workers to moth scales. Therefore, portable equipment that draws in air (capturing nozzle (Wolf 1984, 1985)), filters and exhausts it, is required.

21.2. CHEMICALS

Material Safety Data Sheets for all chemicals used must be available to all workers. Special precautions are needed if formaldehyde is used as a diet ingredient. This toxic chemical is a contact irritant, poison (Reed and Tromley 1985) and carcinogen (Shapiro 1984; Ashby et al. 1985). Formaldehyde produces volatile fumes so the liquid must be handled inside a fume hood, used glassware must be rinsed in the hood, and the chemical added to the diet in a strongly ventilated area while using a formaldehyde respirator (Reed and Tromley 1985; Wolf 1984, 1985). Protective clothes must be worn when handling — chemical-proof gloves, coveralls or lab coats, safety eyewear and chemical-proof aprons. (Protective clothing protects workers, but also the diet from contamination that may be introduced by personnel.) A chemical spill kit must be kept nearby, and a safety shower and an eyewash station must be situated in the area where hazardous chemicals are handled. Formaldehyde evaporates from the diet over time, so workers should not enter rearing rooms unless necessary and only if a formaldehyde respirator is being worn.

The same kind of precautions should be taken when handling any chemical that is toxic, corrosive or volatile, e.g. acids, bases and disinfectants. Hazardous chemicals must be properly stored and disposed of after use or after the expiry date. Cleaning agents, e.g. NaOCl (bleach) and other disinfectants should be handled and stored with care.

Tests to sample the air for hazardous chemicals, e.g. formaldehyde, are available (Wolf 1984).

Organic solvents, e.g. acetone, ethyl alcohol and diethyl ether should be handled only in a fume hood or in specially ventilated areas. These chemicals are stored in cool and well-ventilated cabinets.

Antimicrobial agents, including bactericides and fungicides, should be stored where they will not come in contact with workers via skin, inhalation or by contamination of foods. Workers handling such chemicals should wear face masks, gloves and other protective clothing to protect their skin.

A fume hood draws air from the laboratory and vents the air outside the building so that fumes cannot enter the room. In contrast, a laminar flow hood passes pre-filtered air over the work surface in the hood and into the room. A fume hood protects the worker from toxic fumes, and a laminar flow hood protects the materials on the work surface from microbial contamination, e.g. plates of sterile medium (see photos in Cohen (2004)).

Chemicals used in eating/drinking and in personal make-up and apparel, e.g. deodorant and hair spray, should be discouraged in the rearing facility.

Smoking cigarettes is prohibited in a rearing facility, not only for the health of workers but also for the insects; nicotine is an insecticide.

21.3. SAFETY

Equipment associated with preparing and dispensing diet can harm the operator, e.g. beaters, mixers, blenders, stirrers with long shafts and propellers, choppers, cutters, grinders, augers, fans, forklift, tractor, etc. The moving parts of such machines must have protective shields, covers or barriers to prevent accidental contact with the operator. To prevent accidents hair should be covered and no loose clothing worn.

Live steam, hot liquids and hot objects, e.g. steam kettle, autoclave, oven and hot plate, can cause severe burns. Therefore, workers must wear a face mask and protective clothing and footwear and use protective equipment, e.g. gloves, eye goggles (possibly full-face shields), as appropriate (Fisher and Leppla 1985).

Ear plugs, or better still ear-muff type protectors, should be worn when working near noisy machines.

Electrical installations and equipment must be properly connected to a power source and grounded to avoid electrical shocks, keeping in mind that washing with water is a very frequent activity in a rearing facility. Wearing rubber boots and gloves will afford protection from electrical hazards.

When using a UV lamp to identify marked adults (section 11.3), wear protective UV eyeglasses. Also, covering the hands with sunscreen can protect them from 'sunburn'.

22. Rearing Facility: Design, Maintenance and Sanitation

22.1. DESIGN AND MAINTENANCE

Rearing lepidopterans requires a much more sophisticated and complicated facility than that required for dipterans, especially regarding the environmental controls (Bloem and Bloem 2000; IAEA 2008). The risks of contaminants destroying the diet and viral diseases infecting the larvae are high. These risks must be mitigated by incorporating multi-faceted environmental controls and design features into the facility which are expensive to purchase and maintain. This situation makes mass-rearing Lepidoptera rather daunting, but enough experience has now been accumulated to provide guidelines on the design and operation of a facility.

In the early stages of the design of the OKSIR facility (Figure 7), a major decision was taken to provide separate environmental controls for each rearing and emergence room. This was an 'expensive' decision, but it permitted clean air to be brought into each room separately and air could not move between rooms. This concept worked well and enabled the facility to produce the planned number of insects on a regular basis. If contamination or infection occurred in one room, the other rooms were isolated.

A related 'expensive' decision was to build many small rearing rooms to hold only one day's diet production. If a room became contaminated or the insects diseased then only one day's production would be lost.

A third major design feature was the creation of three areas based on activities and the type of environment required:

- Clean area (red area of Figure 30) Diet dispensing, rearing rooms, showers, laundry room, lunch room and washrooms; under positive air pressure and restricted entry for designated personnel only;
- Dirty area (blue area of Figure 30) Emergence rooms, cold rooms, oviposition room, egg-sheet handling room, QC room, data storage room, spent-diet handling area, tray washer, cart washer (Figure 31), showers, laundry room, lunch room and washrooms, rearing manager's office, environmental control centre; under negative air pressure and restricted entry for designated personnel only;
- Open area (green area of Figure 30) Diet ingredient storage and diet preparation rooms, mechanical rooms, washrooms, offices, data handling and meeting room, storage rooms, open upper storey for environmental equipment and ducting; visitors area and to the outside of the facility.



A fourth design feature relates to the movement and flow of materials (Fisher 1984a; Fisher and Leppla 1985; Nordlund 1999; Phillimore 2002; IAEA 2008; Taret et al. 2007):

- Carts with trays of maturing larvae could be moved from the rearing rooms (clean area) to the emergence rooms (dirty area) through a special pass-through room (accessible from both sides, but not at the same time);
- Cleaned carts and trays and special carts holding sterilized egg sheets (Figure 27, section 12.6) could be stored in a special sterile room accessible from both the washing area and the diet dispensing area (but not at the same time);
- Materials, e.g. chemicals, tools, etc., could be transferred from the open area into the clean area using a pass-through between the diet preparation area and the diet dispensing area.

A fifth design feature was the unique system of bringing clean and conditioned air into each rearing and emergence room through small holes in the clear plastic walls of the rooms (Figure 9 and 32) (Brinton et al. 1969; Oborny 1998). The air was exhausted through two ducts in the ceiling.

Lastly, adults were collected automatically using UV lights, ducts, moving air and cyclones (section 11.1).



A new codling moth rearing facility (400 m²) was constructed in Argentina in 2006 with an investment of USD 115 000 for infrastructure and equipment; the maximum production capacity is 200 000 moths/week (Taret et al. 2007). A new insect rearing facility in Brazil will produce codling moths as well as other insects (Kovaleski and Mumford 2007; Malavasi et al. 2007).

Information on the design of insect-rearing facilities is available (Kakinohana 1982; Leppla et al. 1982; Fisher 1984a; Griffin 1984a, b; Harrell and Gantt 1984; Owens 1984; Fisher and Leppla 1985; Marroquin 1985; Schwarz et al. 1985; Sikorowski and Goodwin 1985; Singh and Ashby 1985; Mumford and Knight 1996; Leppla and Eden 1999; Wood and Wendel 1999; Fisher 2002; Phillimore 2002; Wyss 2002; IAEA 2004, 2008; Dowell et al. 2005).

Goodenough and Parnell (1985) described the basic engineering design requirements for ventilation, heating, cooling and humidification of insect rearing facilities. Oborny (1998) reviewed the HVAC (heating, ventilation, air conditioning) systems in four rearing facilities. Tween (1987), Dowell et al. (2005), Parker (2005) and IAEA (2008) discussed the merits of a modular facility.

The selection of a location for a rearing facility is discussed by Marroquin (1985), Leppla and Eden (1999), IAEA (2004, 2008), Dowell et al. (2005), Dyck et al. (2005b), Parker (2005) and Taret et al. (2005).

Maintenance is vital to the reliable operation of equipment and to the reliable production of quality insects. Appropriately qualified engineering personnel, and adequate stocks of supplies and replacement parts for filters and equipment, are essential. A regular programme of maintenance activities must be scheduled and adhered to rigorously. It is important that the equipment and building components selected for the facility are easy to maintain using locally available expertise. Computer-controlled and monitored environmental equipment is an advantage if trained personnel are available to maintain such equipment.

22.2. TEMPERATURE

Reliable equipment to maintain the programmed temperature in each room and area is critical. The best system of temperature control is to heat/cool air by passing it over heating or cooling coils (using a refrigeration system) and then forcing it with fans into each room and area. Such a system has the capability to change the temperature rapidly.

22.3. MOISTURE CONTENT OF THE AIR

As the temperature of forced air is being controlled (section 22.2) moisture can be added with steam, or removed. Dehumidification tends to be expensive, but is sometimes essential for incoming air; it is done by passing the air over cold coils and draining the water that collects. In the OKSIR facility, the RH in a rearing room decreases from 75 to 55% during the larval rearing period, and this dehumidification must be regulated by the environmental equipment (Oborny 1998).

22.4. LIGHT AND PHOTOPERIOD

The light intensity is not critical, the main concern is to regulate the photoperiod to prevent (or induce) diapause in developing larvae (sections 9.1 and 13.1). The light source should illuminate all trays of diet in a larval rearing room; vertically positioned fluorescent tubes are appropriate for vertically stacked trays of diet on carts. Since lights produce heat, the tubes can be located within an air plenum behind clear plastic barriers (**Figures 9** and **32**) (section 9.1).

22.5. AIR PRESSURE AND MOVEMENT

Positive and negative air pressures are created by balancing inlet and outlet fans (Sikorowski and Lawrence 1994a; Dowell et al. 2005). Air movement is needed to exchange air at a predetermined rate appropriate to each room (Howell and Clift 1972). A small percentage of inlet air, e.g. 10%, should be fresh air. It is relatively expensive to modify fresh air compared with recirculated air (Oborny 1998).



As discussed in section 9.1, air speed and the number of air exchanges/hour are important to control microbial contaminants (Brinton et al. 1969; Howell 1971; Oborny 1998), especially when the diet dries out slowly during larval development. Horizontal (laminar) air flow between vertically stacked trays is absolutely necessary to control the rate of drying of the diet and to suppress growth of mould. This horizontal air flow is provided by air entering the room from many small holes in the side walls (which contain an air plenum under pressure); each tray receives air from a row of holes just above (**Figures 9** and **32**).

22.6. AIR CLEANLINESS

The air-handling system must contain filters to remove microbial contaminants, virus particles and moth scales (Gast 1968; Sikorowski 1984a). The 1st and 2nd filters should capture larger particles such as scales (Stewart 1984). The last filter must be a HEPA filter for very small particles (300 nm or larger) (sections 15.3, 17.2.4, 17.2.5, 21.1 and 22.7). However, CpGV particles are approximately 314 × 31 nm and the granules 314 × 208 nm, thus quite close to the filtering limits of these filters (Cossentine et al. 2005). HEPA filters are essential for clean areas and rooms and, if CpGV is a threat, are desirable for other parts of the facility. Filters must be changed as needed, and a programme of checking and replacing filters is vital. Air cleanliness must be monitored by periodic use of plated media (section 17.2.5) (Fisher and Leppla 1985).

22.7. SANITATION AND CLEANING EQUIPMENT

Sanitation is the control of microbial contamination. Proper sanitation reduces losses caused by spoilage of an insect diet, increases the efficiency of plant operation, results in easier maintenance of equipment and develops better employee relationships. The purpose of sanitation is to suppress microbial contamination to desired levels (Brewer and Lindig 1984; Roberson and Wright 1984; Sikorowski 1984a, b; Stewart 1984; Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994a, b; Cohen 2004; Bloem et al. 2007).

Sanitation is vital for successful rearing of lepidopterans, especially when artificial diet is used (sections 15, 17.2.5 and 22.6). The diet-dispensing area and rearing rooms (section 22.1) must be very clean. Besides clean air, the exposed surfaces of equipment and the surfaces of the rooms (ceiling, walls and floor) must be kept clean (section 17.2.5) (Fisher 1984a; Reed and Tromley 1985). At the end of the work day, floors must be washed and cleaned with a disinfectant. Walls and floors can be cleaned weekly with household ammonia or detergent (Toba and Howell 1991) or with 5% NaOCl and UV lamps (Bathon et al. 1991) (however UV light has poor penetrability). Prior to diet being dispensed, utensils and work surfaces must be cleaned with disinfectant or autoclaved (Howell and Clift 1972). Ovens can be used to sterilize glassware (180°C for 2 h).

Toba and Howell (1991) described the following practices:

- After each use, moth collection containers and oviposition cages are cleaned in a dishwasher.
- Used diet trays are cleaned and autoclaved at 115.5°C and 18–20 psi for 1 h. Diet tray covers are similarly cleaned and autoclaved for 0.5 h.

Mani et al. (1978) described the following practices:

- Rearing room floors are washed each week with a NaOCl solution, and formaldehyde is vaporized in the room $(1 \text{ cm}^3/\text{m}^3)$.
- Every three months each rearing room is emptied and cleaned. After applying formaldehyde vapour, the room is heated to 45°C for three days.
- Diet trays are washed, disinfected in a cleaning solution and dried at 50°C. Before re-use they are immersed for 18 h in a 5% formaldehyde solution.
- Carts for diet trays are cleaned using steam jets.

Hamilton and Hathaway (1966), rearing on immature apples, described the following practices:

- Rearing rooms are scrubbed with soap and water, and each week sprayed with a 0.5% solution of NaOCl.
- Rearing racks, trays and lids are washed with water and steam cleaned, then immersed in a 1% solution of NaOCl for 1 h.

Stewart (1984) described the sanitation measures followed at the pink bollworm rearing facility:

• Disinfecting work surfaces, floors, walls and ceilings with sanitizing agents such as NaOCl solution, quaternary ammonium compounds, phenolic compounds and stabilized chlorine dioxide solutions. Chlorine dioxide is advantageous because it is relatively stable and non-corrosive, and it can be

rapidly and effectively applied with airless spray guns to almost any surface – particularly walls, ceilings and supplies entering the facility.

- Stringent cleaning of equipment, especially that contaminated with moth scales
- Positive pressurization of diet preparation and egg disinfection areas
- HEPA-filtered air in clean areas
- Restricted movement of workers to prevent travel from dirty to clean areas
- Personal hygiene of workers and sterile clothing
- All glassware, rinse water and clothing used in egg disinfection are autoclaved daily.

Personnel are a major source of contaminants (Sikorowski 1984a; Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994a; Cohen 2004), and all staff must shower before entering a work area and wear clean coveralls or coats, shoe covers and head covers (Figure 27) (Cohen 2004) and in clean areas also face masks. When handling fresh diet, rubber gloves should be worn or hands washed with germicidal soap. Workers must strictly obey rules about restricted access to certain areas (Cohen 2004). A foot wash with a disinfectant may also be located at the entrance to a clean area.

The cost of rearing insects can be greatly reduced using an environmental sanitation programme. Contamination leads to poor insect quality, high mortality, additional workload and loss of confidence in the work. Staff must appreciate the need for sanitation, and regular sanitary measures must be established and maintained. Staff training can reveal the importance of sanitation (Sikorowski 1984a, b; Cohen 2004). The importance of sanitation and personal hygiene must be recognized by and begin with management. Managers must know and understand contamination control principles, furnish a proper work environment and motivate employees to comply with requirements for sanitation and personal hygiene (Sikorowski 1984b; Cohen 2004).

22.8. CLEANING THE REARING FACILITY

At least twice a year, the whole facility should be cleaned and disinfected, i.e. all walls, ceilings and floors, and all hidden areas such as the air plenum in rearing and emergence rooms. Cleaning with steam may be necessary.

22.9. PREVENTING ESCAPE OF FERTILE INSECTS

For the SIT the pest insect itself is being reared, and the escape of fertile insects has to be prevented by stringent containment measures (Leppla and Eden 1999; Parker 2005; IAEA 2008). Besides sealing the building to prevent escape, the OKSIR facility employs four further methods:

- Spent diet is heat treated at 60°C for 3 days (Cossentine et al. 2005) (sections 7.2 and 15.3).
- Negative air pressure in the emergence area discourages moths from flying through an open door against the incoming air flow.

- Doors opening from the emergence area to other areas of the facility have an 'air curtain' (Oborny 1998, photo provided).
- UV light traps kill any flying adults.

23. Management of a Rearing Facility

Few publications have dealt with the issue of management (Fisher 1984b; Schwalbe and Forrester 1984; Singh and Ashby 1985; Leppla and Ashley 1989; Bathon et al. 1991; Bloem and Bloem 1995, 2000; Dyck et al. 2005b). IAEA (2008) is a very helpful publication regarding the management of a rearing facility. In this document, management is referred to in sections 4.8, 4.10, 4.11, 16, 16.1, 17.1, 17.4, 20., 20.1 and 22.7.

23.1. LEADERSHIP

Good leadership is essential for the successful mass-rearing of quality insects. A rearing manager does not have to be an entomologist, although training in entomology and the biological sciences is an advantage. The key characteristics needed are an appreciation of the goals and methods of mass-rearing, and an ability to motivate the workers to follow faithfully the established rearing procedures and sanitation practices. The manager also needs to be well organized, to systematically plan ahead, and to understand the operation of the equipment and the need for building maintenance (Fisher 1984b; Schwalbe and Forrester 1984; Dyck et al. 2005b).

As discussed in sections 16.1 and 17.4, the rearing manager is not the AW-IPM programme manager. Issues of quality of the insects must be dealt with by the programme manager in consultation with the rearing manager and not the other way around. A QC group should be established that reports to the programme manager but works closely with the rearing manager and production groups (Leppla and Ashley 1989).

The need for timely action is not only true for the biological elements of a rearing operation, but managerial actions must also be carried out at the appropriate time.

23.2. PERSONNEL

The key personnel are: rearing manager, maintenance engineer, QC biologist, secretary, and staff to prepare and dispense diet, seed diet, collect adults, set up oviposition cages and collect egg sheets, sterilize egg sheets, dispose of spent diet, wash trays and carts and clean the facility. Singh and Ashby (1985) provided estimates of the number of persons required to rear the codling moth.

As stated in section 17.4, the training, skills and attitudes of workers in a massrearing facility are critical to achieving high-quality production. They must be trained, highly skilled and motivated individuals. Maintaining a complicated and sophisticated rearing facility, with many computer-controlled instruments and different types of machines, requires the full-time input of appropriately qualified and experienced engineering staff (Dyck et al. 2005b).

It is essential that personnel are hired full-time and be well-paid. Job security and opportunities for promotion help to maintain job satisfaction. To make workers feel comfortable and able to concentrate on their tasks, safe practices in the work environment should always be a priority. Various forms of recognition and reward for good performance will encourage employees to improve performance. Another incentive for staff is obtaining specialized training.

Labour-management relations must be kept positive to maintain staff morale. If staff motivation is low, negative personal habits, attitudes, values and even local customs can create significant problems. Rearing and handling live insects is a 24-h/day and 365-days/year job. In ways that do not offend individuals and local customs, the insects must somehow be given the first priority. Some rearing facilities have experienced significant work stoppages due to worker dissatisfaction.

If an employee becomes unproductive or disruptive, and appropriate guidance and encouragement to improve performance is unsuccessful, a rearing manager must have authority to dismiss that employee (Dyck et al. 2005b).

As discussed in section 22.7, workers in a rearing facility must always be conscious of overall cleanliness, and must be willing to take the time and make the effort to make and keep things clean. This involves wearing special clothes and a face mask, which may not be very comfortable. The personal characteristics of workers must be compatible with being clean and making things clean. People who are careless and pay little attention to the guidelines for work procedures are not suitable as employees of a rearing facility.

23.3. OPERATING BUDGET

The initial annual budget for the OKSIR programme (rearing plus field operations) was about USD 1.2 million, but it increased to USD 2.5 million by 1998 and to USD 3.38 million by 2002 (Bloem and Bloem 1995, 2000; K. Bloem et al. 2005). This increase was due to underestimation and miscalculation of costs when the budget plan was originally developed, e.g. a public relations programme had to be started and the cost of diet ingredients increased. Also, mechanical problems developed in the rearing facility, e.g. undersized gear boxes for the diet pumps and insufficient cooling capacity (Bloem and Bloem 1995, 2000).

An operating budget for a codling moth rearing facility has been prepared in Syria (Mumford and Knight 1996) and Argentina (Fugger 2006).

23.4. FINANCES

The usual source of funds to finance a rearing facility is the government. However, in addition to funds from the federal and provincial governments, the OKSIR programme uses operating funds obtained primarily from the local community –

taxation of private property (land used to grow apples or pears and other land as well) (DeBiasio 1988; Bloem and Bloem 1995, 2000; K. Bloem et al. 2005; Dyck et al. 2005b; IAEA 2008). However, this involvement of the community creates an opportunity for uninformed people to influence the programme, and so the role of public relations becomes very important (Bloem and Bloem 2000; Dyck et al. 2005c). IAEA (2008) discusses a financial model that can assist decision-makers with the financial issues related to insect mass-rearing programmes.

23.5. CAPITAL COSTS

The initial investment to construct a rearing facility is substantional (Mumford and Knight 1996; K. Bloem et al. 2005; Fugger 2006; IAEA 2008) (section 22.1). Bloem and Bloem (1995, 2000) cited the cost in 1992–93 of the OKSIR facility at about USD 6 million.

23.6. OPERATING POLICIES

Ordering diet ingredients, supplies and spare parts must be done in good time (section 4.8).

It is essential that rearing staff be trained in and follow safety and sanitation procedures (sections 21 and 22.7).

Each laboratory must develop its own standard operating procedures (SOPs) (section 1.1).

IAEA (2008) discussed issues relating to intellectual property protection.

23.7. OWNERSHIP AND SUPPORT

Usually insect rearing facilities are owned and operated by a government, and there are advantages to such ownership. However, private operations are becoming more common and they can sometimes rear insects at lower costs. Dowell et al. (2005), Dyck et al. (2005b) and IAEA (2008) discussed the pros and cons of private firms operating a rearing facility.

Public support is also important (Patton 1984; Bloem and Bloem 1995, 2000; Dyck et al. 2005b, c). Positive public support for a rearing operation in the community makes workers involved in that operation proud to be there and helps them to produce quality work.

23.8. EVALUATIONS

External evaluations are usually beneficial (Dyck et al. 2005b; Vreysen et al. 2007a).

23.9. RESEARCH ACTIVITIES

It is important that research, or methods development, continues even if a massrearing programme has begun (Leppla et al. 1982; Dyck et al. 2005b, c; Rendón et al. 2005; Vreysen et al. 2007a). Improvements in the rearing technology can and should be made, both to increase insect quality and to decrease the cost (Singh and Ashby 1985). Research leads to methods development, then the new technology is pilot-tested and when all problems are solved it is implemented (Schwalbe and Forrester 1984).

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Glossary

- antimicrobial agent chemical that kills any or all microbial contaminants (Cohen 2004)
- electroantennogram used to measure the electric potential of an insect antenna stimulated by volatile compounds (Gordh and Headrick 2001)
- antioxidant inhibits oxidation; a substance that removes potentially damaging oxidizing agents in a living organism (Oxford Dictionary)
- apyrene sperm spermatozoa that lack a nucleus, and do not fertilize the egg (Gordh and Headrick 2001)
- area-wide integrated pest management (AW-IPM) integrated pest management against an entire pest population within a delimited geographic area, with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area (Klassen 2005; Enkerlin 2007); management of the total pest population within a delimited area (Hendrichs et al. 2007)
- artificial diet food that has been synthesized from one or more ingredients that may be completely defined chemically or that may be partially defined or not defined. An artificial diet and a synthetic diet are essentially synonymous (Cohen 2004). An unfamiliar food which has been formulated, synthesized, processed, and/or concocted by man, on which an insect in captivity can develop through all or part of its life cycle (Singh 1977)
- aseptic rearing using antimicrobial agents in the diet and a sterilized working environment free of harmful contaminating microbes
- attractant a chemical or visual stimulus that results in movement of a pest towards the source (IAEA/FAO 2003)
- bacteriostatic antimicrobial that reduces or inhibits the growth of bacteria (Sikorowski and Lawrence 1994a)
- calling dispensing sex pheromone by a female adult to attract a male (FAO/IAEA/ USDA 2003)
- closed-loop system provides pertinent information on which to base decisions in a quality-control system and regularly provides this information (Webb 1984) [see feedback loop]
- **compatibility (mating)** females of a given strain are able and willing to accept, for mating, the males of another strain; this also includes synchrony and other factors that cause reproductive disconformancy (FAO/IAEA/USDA 2003)
- **competitiveness** ability of an organism to compete with conspecific organisms for a limited environmental resource (FAO/IAEA/USDA 2003)
- control chart a chronological graphical comparison of measured product characteristics with limits reflecting the ability to produce, derived from past

experience (Chambers and Ashley 1984); chronological graphical comparison of the specifications of all quality assessment and control parameters (Moore et al. 1985); to plot a parameter with predetermined limits on a time scale and to present this information in an easy-to-interpret graphical form such as on meanor range-charts that have control limit lines (FAO/IAEA/USDA 2003)

- critical photoperiod that which induces 50% incidence [prevalence] of diapause in a population (Brown 1991)
- data logger an instrument that records temperature and other environmental parameters for a variable length of time (FAO/IAEA/USDA 2003)
- defined diet a diet in which the constituents can be described (ideally consisting of only chemically pure constituents) (Vanderzant 1957, 1966)
- diapause a dynamic state of low metabolic activity, with reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced behavioural activity (Brown 1991); a syndrome of developmental, physiological, biochemical, and behavioural attributes that together serve to enhance survival during seasons of environmental adversity (Denlinger 2003)
- diet the food on which an animal feeds
- dispersal a non-directional movement of insects within or between habitats (Gordh and Headrick 2001)
- ecdysis the process of shedding the integument during moulting (Gordh and Headrick 2001)
- eclosion the act of hatching from the egg shell (Gordh and Headrick 2001)
- emergence the escape of the adult insect from the cuticle of the pupa (FAO/IAEA/ USDA 2003)
- emulsifying agent chemical that forms micelles around each droplet in the dispersed phase of an emulsion to reduce interfacial tension and prevent droplets from coalescing (Cohen 2004)
- epizootic outbreak of an epizootic disease, where a large proportion of an animal population is affected simultaneously (Gordh and Headrick 2001)
- essential nutrient a substance that an insect requires for life but can obtain only from its diet and does not have the metabolic ability to produce (Singh 1977)
- eupyrene sperm spermatozoa with a nucleus which can fertilize eggs (Gordh and Headrick 2001)
- facultative diapause diapause that is induced or terminated by change in photoperiod, temperature, or both (Gordh and Headrick 2001)
- feedback loop returning output information to the beginning of a process for correcting discrepancies between intended and actual performance or for the maintenance of current process standards and procedures (Chambers and Ashley 1984; Moore et al. 1985) [see closed-loop system]

filtrate - liquid remaining after solids are filtered out (Cohen 2004)

flash sterilization – diet is subjected to a temperature >121°C in a heating coil within a steam jacket that allows high temperatures to be reached by compression of the steam that surrounds the coil. Boiling of the diet is prevented by the closed system. The higher temperatures and pressure cook the diet quickly, causing minimal damage to the diet but efficient destruction of microbial contaminants (Cohen 2004)

- flight ability capability to achieve a defined flight performance (FAO/IAEA/ USDA 2003)
- **founder effect** the founders of a new population carry only a random fraction of the genetic diversity found in the parent population (Gordh and Headrick 2001)
- **founder population** insects that are collected from a wild population and used to initiate a laboratory colony
- HEPA filter high-efficiency particulate air filter
- holidic diet artificial diet constituents with known chemical structure (Vanderzant 1966; Chippendale and Beck 1968)
- humectant substance that adjusts water activity (Cohen 2004)
- lux unit of illumination equal to one lumen per square meter (the lumen is about 1/683 watt) (FAO/IAEA/USDA 2003)
- microbial contamination harbouring of, or having contact with, micro-organisms without symbiotic or pathogenic relationships (Sikorowski and Lawrence 1994a)
- micro-organism a protozoan, fungus, bacterium, virus or other microscopic selfreplicating biotic entity (FAO 2007)
- multivoltine having many generations per season or year (Gordh and Headrick 2001) natural diet – natural food of an animal
- natural diet natural 1000 of an anima
- neonate larva newly hatched larva
- non-essential nutrient a substance that an insect requires for life but can be built metabolically by an insect from other substances, e.g. glutamic acid
- nutrient any substance that can serve as part of the metabolism of an organism (Cohen 2004)
- nutrition the study of the food requirements of organisms (Singh 1977)
- nutritional requirements specific, chemically defined components that the insect must have to grow, reproduce, and perform as it should (Singh 1984); the chemical factors of ingested food essential for normal metabolism and development of the insect (Singh 1977)
- olfactometer device for testing the behavioural response of insects to odours (Gordh and Headrick 2001)
- parasitoid an insect that lives on or in another insect (host), and ultimately kills the host (Cohen 2004); an insect parasitic only in its immature stages, killing its host in the process of its development, and free living as an adult (Enkerlin and Quinlan 2004; FAO 2007)
- pass-through a small chamber, with sealable doors on each side, built into the wall between a clean room and a potentially dirty room, and constructed to enable the passing of materials through the chamber from one room to the other room without contaminating the clean room. Only one door is opened at a time. Positive air pressure in the clean room prevents dirty air from entering it when the pass-through is opened
- pathogen micro-organism that is capable of causing a disease under normal conditions of host resistance, and rarely lives in close association with the host

without causing the disease (Sikorowski and Lawrence 1994a); micro-organism causing disease (FAO 2007)

pH – negative log of the hydrogen ion concentration, a measure of the acidic or basic nature of a diet or diet ingredient (Cohen 2004)

phagostimulant – substance that elicits a feeding response (Cohen 2004)

pheromone – a chemical produced by one organism that influences the behaviour of another organism of the same species (FAO/IAEA/USDA 2003)

photoperiod – combination of photophase and scotophase in one day

photophase - light period during one day

preservative - substance to prevent degradation (Cohen 2004)

- process control measuring how things are done, such as diet preparation, seeding of the diet, insect holding and collection, and unfinished product quality such as egg hatch and pupal weight, etc. (Bigler 1992; Calkins and Parker 2005); process control tells how the manufacturing processes are performing, and it controls these processes so that deviations from the product specifications will not occur as a result of variation in the processes (Chambers and Ashley 1984; Moore et al. 1985)
- product control tells how well the product is conforming to specifications and standards of quality, and it gives feedback so that a product's departure from established specifications can be corrected, or it eliminates substandard products (Chambers and Ashley 1984; Moore et al. 1985)
- production control regulates the consistency and reliability of production output, the numbers of items produced, and the timeliness of their production (Chambers and Ashley 1984; Moore et al. 1985)
- propensity an inclination or tendency for an individual insect to carry out an act, or for an individual event to occur (FAO/IAEA/USDA 2003)
- quality the degree to which a product meets the requirements of the objective or of the expected function (FAO/IAEA/USDA 2003); fitness for use (Chambers and Ashley 1984; Moore et al. 1985); the ability of the released insects to perform their function, and to perform relative to some standard (Chambers 1975)
- quality assessment measurement of specific or general traits that indicate fitness, usually against reference standards and tolerances (measuring quality is not the same as controlling quality feedback is required for the latter) (Moore et al. 1985)
- quality control a systematic process whereby management critically evaluates the elements of production, establishes standards and tolerances, obtains, analyses, and interprets data on production and product performance, and provides feedback so as to predict and regulate product quality and quantity (FAO/IAEA/USDA 2003); quality control is a management procedure that develops, maintains, and improves quality (Chambers and Ashley 1984; Moore et al. 1985; Bigler 1994)
- required nutrient nutrient which is required for optimal performance, though not necessarily essential (Singh 1977)

scotophase - period of darkness during one day

SOP – standard operating procedure

- sperm transfer the successful transfer of sperm from a male to a female spermathecae during copula (FAO/IAEA/USDA 2003)
- standard a quality or measure serving as a basis or principle by which others conform or should conform or by the accuracy or quality of others is judged (FAO/IAEA/USDA 2003)
- sterile insect an insect that, as a result of a specific treatment, is unable to reproduce (FAO 2007)
- sterile insect technique (SIT) method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species (FAO 2007)
- strain a breed or stock of insects that have been held in isolated colonies for a period of time (FAO/IAEA/USDA 2003)
- syneresis separation of liquid from a gel (Navon 1968)
- synthetic diet synonym for an artificial diet, often used to connote a defined diet but
 not limited to that sense (Cohen 2004)
- **token feeding stimulant** any substance that triggers a feeding response but does not play a metabolic role in the target species (Cohen 2004)
- total quality control adoption of tools and procedures to regulate the processes of production so that product quality will be insured through control of processes (Chambers and Ashley 1984); total quality control encompasses the entire structure and associated mechanisms for developing and improving product quality and productivity (Leppla and Fisher 1989)
- trap a baited device used for catching (IAEA/FAO 2003)
- triturated ingredients ingredients ground to a fine powder using for example a mortar and pestle; often used in diets as a means of mixing two or more solids such as a vitamin present in a low concentration with a sugar present in a much higher concentration
- wild insect an insect that has never been domesticated or held in a rearing colony (FAO/IAEA/USDA 2003)

Annex 1 List of Primary Equipment

Adult collection system Air compressor Augers (transporting large quantities of a diet ingredient, e.g. sawdust, within a rearing facility) Autoclave Balances Blender Boiler (steam) Cages (oviposition) Cage-turning equipment Carts (diet) Carts (egg sheet) Carts (hand) Carts (moth disposal) Chiller (sexing adults) Chopper (heavy duty, to cut up paper pulp) Cold-storage facility (storing some diet ingredients) Colony counter Computers and accessories Dispenser (paraffin wax) Dissecting tools Dryers (laundry) Egg-sheet wire-mesh 'books' Environmental control equipment for all rooms and areas of facility, including filters Food processor Forklift Freezer Fume hood Furniture for QC laboratory and lunch rooms Generator (electricity) Glassware, Petri dishes, etc. Graduated cylinders Hammer mill (paper pulp) Hood (portable for collecting moth scales) Hot plate

Kettle (steam-jacketed) with counter-rotating paddles (or flash sterilizer or extruder) Laminar flow hood Light meter Lockers (clothes, etc. for workers) Magnetic stirrer Magnifiers (illuminated) Microscopes (stereo, compound) and accessories Mixer Mixer, cooler (vat mixer and cooler) and dispenser (diet) Mortar and pestle (to pulverize Calco Red dye) Office equipment and furniture Oven (drying) Oven (microwave) Paper cutter (egg sheets) pH meter Pressure cooker (to heat and dispense paraffin wax) Pumps (diet) Pumps (water) Refrigerator Scarifier (steam) (for diet) Sensors (temperature, relative humidity, air speed) Shaker/sifter (sawdust) Sieves Tanks (hoppers) (diet) Tanks (egg sheets) Tubs (soak trays before scrubbing and washing) Temperature and relative humidity recorder, e.g. Hobo[™] data logger Thermometer (digital) Timer Tools for equipment servicing and repair Tractor and trailer Travs (diet) UV lamps UV traps Vacuum cleaner Vacuum/pressure pump Washer (carts) Washers (laundry) Washer (trays) and tray-scrubbing machine and disinfection rinse Water distiller Water softener or demineralizer In this document, equipment for preparing diets is discussed in section 6.5. Butt (1975) provided a list of mixers used to prepare codling moth diets.

Griffin (1984b) listed equipment used at a boll weevil production facility.

Papers in Singh and Moore (1985), e.g. Ashby et al. (1985) and Reed and Tromley (1985), listed equipment items used to rear insects in a laboratory.

Wolf (1985) listed sources of equipment used to protect workers from health hazards in an insectary.

FAO/IAEA/USDA (2003) included a list of known sources of key equipment and supplies.

Cohen (2004) provided a chapter on equipment used for processing insect diets.

Annex 2 List of Companies that Provide Dietary Ingredients

Becton, Dickinson and Company (DIFCO products) http://www.bd.com/products/

Bio-Serv http://www.bio-serv.com/

Nutritional Biochemicals Corporation, Cleveland, Ohio, USA

Southland Products Inc. http://www.tecinfo.com/~southland/

Ward's Natural Science http://www.wardsci.com/

Brewer and Lindig (1984) provided a list of diet ingredients and some of their sources.

Ashby et al. (1985) and Reed and Tromley (1985) provided lists of diet ingredients and some of their sources.

Rearing codling moth for the sterile insect technique

The codling moth *Cydia pomonella* is amongst the most severe pests of pome fruit in the temperate regions of the world. Broad-spectrum insecticides have mainly been used to control this pest resulting in several negative environmental consequences. The demand for alternative control techniques is therefore increasing worldwide, and includes synthetic growth regulators, mating disruption, attract and kill, microbiological control agents, and the sterile insect technique (SIT). The integration of sterile insects with these control practices within the context of area-wide integrated pest management offers great potential. However, efficient and effective mass-rearing of the target insect is a fundamental component of the SIT but its complexity for Lepidopteran pests is very often underestimated.

There has been an increasing interest to develop codling moth SIT for integration with other control tactics over the past years. This document compiles and summarizes available information on the rearing of the codling moth in relation to the SIT. Aspects such as colonization, adult and larval diet, sexing, quality control, shipment, disease control, data recording and management are described. It is not a text book but is developed so that individual sections can be consulted by the reader when necessary. The document therefore, does not provide guidelines *per se*, nor is it a compendium of standard operating procedures, as these will need to be developed for each rearing facility based upon local needs and availability of materials and ingredients. The document is an attempt to bring together all existing information on the rearing of codling moth.

