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Rearing codling moth for the sterile insect technique

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Rearing codling moth for the sterile insect technique

by
V.A. Dyck

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Rearing Codling Moth for the Sterile Insect Technique

V.A. Dyck

Preface

The codling moth ***Cydia pomonella*** (Linnaeus) (Lepidoptera: Tortricidae) is amongst the most severe pests of pome fruit in the temperate regions of the world. Control of this pest has relied mostly on the use of broad-spectrum insecticides with all their negative environmental consequences, but also increasing resistance to a growing list of insecticides. Worldwide, farmers have been demanding alternative control techniques which are not only efficient but also friendly to the environment. These additional control techniques include synthetic growth regulators, mating disruption, attract and kill, microbiological control agents, and the sterile insect technique. The integration of sterile insects with other control practices within the context of area-wide integrated pest management (AW-IPM) offers great potential, as has been demonstrated with great success in the past 15 years in the Okanagan Valley of British Columbia, Canada.

Efficient and effective mass-rearing of the target insect is a fundamental component of the sterile insect technique (Sterile Insect Technique). Mass-rearing knowledge is also needed for other control methods, such as the production of codling moth virus and other microbials, and will also be needed for other genetic control methods that are anticipated in the future. It is a very challenging activity, especially for Lepidopteran pests, and its complexity is very often underestimated. Many years of research and methods development are usually required before all elements of the rearing process have been sufficiently mastered to deliver an end product (the sterile male) that can successfully compete with wild males following sterilization and release.

In the last years, in view of the above described problems, there has been an increasing interest by Member States to develop codling moth SIT for integration with other control tactics. The development of this document is a response to this increased interest, and it compiles and summarizes available information on the rearing of the codling moth, be it in the laboratory or on a larger scale. The information in this document deals with aspects such as colonization, adult and larval diet, sexing, quality control, shipment, disease control, data recording and management. It is not intended to be read from cover to cover, but the information is presented so that individual sections can be consulted by the reader when necessary. Hence, the document 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 list of references in this document is exhaustive, and an attempt has been made to be complete. The document is unique as, for the first time, it brings together all existing information on the rearing of the codling moth.

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1. Introduction

1.1. SCOPE

The emphasis in this document is on mass-rearing systems for the codling moth *Cydia pomonella* (L.) (Horak and Brown 1991; Brown 2006) intended for use in the sterile insect technique (SIT) in area-wide integrated pest management (AW-IPM) programmes (Knipling 1966; Butt 1991; K. Bloem et al. 2005; Dyck et al. 2005a; Vreysen and Hendrichs 2005; Hendrichs et al. 2007; Vreysen et al. 2007b). However, it also covers some aspects of laboratory-scale rearing.

This document covers virtually all aspects, both theoretical and practical, of laboratory and mass-rearing of the codling moth, and also includes a major overview on quality control. Since in future international programmes live codling moths are likely to be shipped long distances, current shipping practices are summarized. This document also discusses management issues. Source details on a large number of cited references are provided as is a glossary and list of primary equipment used in a rearing facility.

Standard operating procedures (SOPs) help to standardize and reduce variation in the tasks done in a rearing facility (Schwalbe and Forrester 1984; Bruzzone et al. 1993; IAEA 2008) but they are not provided in this document. As Parker (2005) comments, “Each rearing facility should develop standard operating procedures (SOPs) for rearing operations, quality control operations, and finally responses to adverse quality control findings.” The Okanagan-Kootenay Sterile Insect Release Program (OKSIR) facility in Osoyoos, Canada created a series of SOPs for mass-rearing the codling moth (Moore 2003).

1.2. RATIONALE

The information in this document is intended to assist both those who are relatively new at rearing this insect and those who wish to improve existing rearing systems. To date, most experience in rearing the codling moth has been in North America and Europe, and also some in Australia and New Zealand (IAEA 2008). Recently, there has been a considerable increase in interest in the temperate-climate countries of the southern hemisphere where apples and pears are grown commercially (Addison and Henrico 2005; Botto 2006; Kovaleski 2006; Taret et al. 2006).

1.3. BACKGROUND

Most publications deal with laboratory rearing which was initially done using apples, especially immature green (thinning) apples (Dickson et al. 1952; Hamilton and Hathaway 1966; Pristavko and Boreyko 1971; Hathaway et al. 1973). This

method is usually used on a relatively small scale, but large numbers of codling moth were reared on thinning apples at the Yakima Agricultural Research Laboratory in Washington State, USA (Hathaway et al. 1971; White and Hutt 1972).

In the 1960s and 1970s, much work was done in North America and Europe on developing artificial larval diets, in some cases using natural food materials but in most cases developing a completely artificial diet from nutritional ingredients (Bathon et al. 1991). However, some ingredients were and still are chemically undefined (House 1961).

Considerable research on rearing was conducted by J.F. Howell and colleagues at the Yakima Laboratory (Howell 1967, 1970, 1972a, 1972c, 1981; Hathaway et al. 1971; Howell and Clift 1972; Toba and Howell 1991; Howell and Neven 2000) and by others in the USA (Redfern 1964; Rock 1967). Diets were also being developed in Europe (Coutin 1952; Navon 1968; Sender 1969, 1970; Navon and Moore 1971; Huber et al. 1972; Pristavko and Yanishevskaya 1972; Shumakov et al. 1974; Mani et al. 1978; Bathon 1981; Guennelon et al. 1981; Huber 1981). Summaries of codling moth diets can be found in Hamilton and Hathaway (1966), Sender (1969, 1970), Navon and Moore (1971), Huber et al. (1972), Shumakov et al. (1974), Butt (1975), Singh (1977), Ashby et al. (1985) and Reed and Tromley (1985).

Initially, artificial diets were used on a small scale, e.g. in small compartments in plastic trays or in plastic cups, test tubes, etc. (Hamilton and Hathaway 1966; Navon 1968; Howell 1967, 1970; Huber et al. 1972; Bathon 1981; Burton and Perkins 1984; Bathon et al. 1991; Reiser et al. 1993; Keil et al. 2001; Gu et al. 2006) as this avoids possible cannibalism. Later, as individual rearing techniques were changed to mass-rearing procedures, trays (Brinton et al. 1969; Howell 1971; Howell and Clift 1972; Batiste and Olson 1973; Hathaway et al. 1973; Mani et al. 1978; Reiser et al. 1993; Bloem et al. 2000) or boxes (Guennelon et al. 1981) were used. Larger production capability (and at a lower cost) was achieved when Brinton et al. (1969) developed a diet that substituted agar with other ingredients. This diet has been modified by other workers (Wildbolz and Mani 1971). The diet dried out as the larvae grew, matured and formed cocoons, and at adult emergence it was dry and hard. Nevertheless, as only adults were needed, handling larvae and pupae was avoided, reducing rearing costs.

A searchable database of worldwide codling moth literature from 1700–1997 is available at the Codling Moth Index (CMI) from the Codling Moth Information Support System (CMISS 2007). A good source of information on methods of rearing insects, primarily parasitoids and predators for biological control, is the IOBC Working Group on Quality Control of Mass-Reared Arthropods (AMRQC). The proceedings of previous workshops are available (AMRQC 2007).

There are many references on insect rearing, both in general and those dealing with Lepidoptera (Knipling 1966; Martin 1966; Smith 1966; Vanderzant 1966, 1974; Beck and Chippendale 1968; Chippendale and Beck 1968; Gast 1968; Walker 1968; Poitout and Bues 1970, 1972; Dadd 1973; Ivaldi-Sender 1974; Singh 1977; Kakinohana 1982; Leppla et al. 1982; Bartlett 1984, 1985; Collins 1984; Fisher 1984b; Joslyn 1984; King and Leppla 1984; Owens 1984; Schwalbe and Forrester

1984; Sikorowski 1984b; Stewart 1984; Fisher and Leppla 1985; Goodenough and Parnell 1985; Marroquin 1985; Moore 1985; Singh and Ashby 1985; Singh and Moore 1985; Tween 1987; Anderson and Leppla 1992; Mangan 1992; Mastro 1993; Reiser et al. 1993; Ochieng'-Odero 1994; Gooding et al. 1997; Leppla and Eden 1999; Nordlund 1999; Smith 1999; Wood and Wendel 1999; Leopold 2000, 2007; Cohen 2001, 2004; Hagler and Jackson 2001; Fisher 2002; Wyss 2002; Enkerlin and Quinlan 2004; Dowell et al. 2005; Dyck et al. 2005b, c; Parker 2005; Rendón et al. 2005; Taret et al. 2005; IAEA 2008).

2. History of Rearing the Codling Moth

2.1. REARING AND MASS-REARING

Singh (1977) distinguished laboratory and mass-rearing on the basis of scale and economics — in mass-rearing the objective is to produce large numbers of 'acceptable' insects at the lowest possible cost. Chambers (1977) defined mass-rearing as "the production of insects competent to achieve program goals with an acceptable cost/benefit ratio and in numbers per generation exceeding ten thousand to one million times the mean productivity of the native population female." Leppla et al. (1982) defined it as "a systematic enterprise accomplished with machinery in integrated facilities for the purpose of producing a relatively large surplus of insects for distribution".

The key concept is that insects are handled in groups and not as individuals. The various steps in rearing — egg collection, diet infestation and collection of larvae, pupae and adults — are all done by handling insects *en masse*. Mass-rearing usually involves large-scale rearing, but 'large scale' is a relative term and difficult to define. Large-scale rearing is not necessarily mass-rearing. Very large numbers of codling moths could be reared individually in millions of plastic cups using an 'army' of workers; this would certainly be large-scale rearing, but would not be mass-rearing.

An artificial diet is necessary for mass-rearing because it yields more uniform insects than would be obtained from a natural diet. It also provides predictable quality and reliable production (Leppla and Ashley 1989). Sanitation is also more easily achieved when using an artificial diet.

However, a small 'mother colony', kept separate from the main colony, may be reared differently to produce a virus-free colony or a new strain (Marroquin 1985). It may even be essential in these situations to rear insects individually. Similar to this concept is 'fractional colony propagation' (Hoffman et al. 1984).

2.2. IMMATURE APPLES

Rearing codling moth larvae on mature apples is problematic (Howell 1991) because relatively few newly hatched larvae penetrate and feed in mature apples, and at room temperature they decay rapidly. However, green immature apples (thinning apples) are penetrated much more readily (70% of neonate larvae enter these apples, acoording to Howell (1972a)) and remain as a suitable food for larvae for several weeks; they have been used in many laboratories (Dickson et al. 1952; Proverbs and Newton 1962a, b, c; Hamilton and Hathaway 1966; Hathaway

1966, 1967; Proverbs et al. 1966, 1967, 1969; Rock 1967; Jermy and Nagy 1969, 1971; White et al. 1969, 1970, 1972, 1973; Wildbolz and Riggenbach 1969; Butt et al. 1970, 1973; White and Hutt 1970, 1972; Hathaway et al. 1971, 1972, 1973; Pristavko and Boreyko 1971; Proverbs 1971, 1972; Howell 1972b; Moffitt and Albano 1972b; Moffitt et al. 1972; Butt et al. 1973; Ferro and Harwood 1973; Robinson 1973, 1974, 1975; Robinson and Proverbs 1973; White 1975; White and Mantey 1977; White et al. 1977; Toba and Howell 1991; Howell and Neven 2000).

Before use, apples are washed in water and strong detergent to remove insecticide residues (Hamilton and Hathaway 1966). However, water does not adequately remove residues (Toba and Howell 1991) and a residue analysis is required. Apples are surface-sterilized by dipping in a 0.5% sodium hypochlorite (NaOCl) solution for 5–10 min, washing in water for 5–10 min and then air-drying (Hamilton and Hathaway 1966; Jermy and Nagy 1971).

Thinning apples are collected during manual apple thinning in early summer. These apples, 2.5–4 cm in diameter, keep well in cold storage (0–5°C) for up to one year (Dickson et al. 1952). Many varieties are suitable, e.g. Jonathan, Winesap, Rome Beauty and Golden Delicious (Hamilton and Hathaway 1966; Dickson et al. 1969; Jermy and Nagy 1971).

To infest apples, egg sheets are placed on the apples in trays (Dickson et al. 1952; Hathaway 1967; Jermy and Nagy 1971; Pristavko and Boreyko 1971). Hamilton and Hathaway (1966) used 4.6 eggs for each apple, but later used newly hatched larvae. Moths can also be caged on the thinning apples and eggs laid directly. The tray's inner surfaces must be covered with rough screen because codling moth females lay eggs on virtually any smooth surface. Hatched larvae burrow directly into the apples. During larval development, the infested apples are held in metal or cardboard trays and covered with a tight-fitting screen/mesh/cloth lid to prevent larvae from escaping. The carrying capacity of a 4 cm diameter apple is approximately three larvae (Ferro and Harwood 1973). When larvae are mature (prepupal stage) they emerge from the apples to form cocoons, usually in corrugated cardboard (or fluted fibreboard) strips (Dickson et al. 1952; Hathaway 1967; White and Hutt 1970, 1972; Jermy and Nagy 1971; Pristavko and Boreyko 1971; Toba and Howell 1991; Howell and Neven 2000). Larvae pupate in the cocoons. Provided that a long daylength has been maintained the insects do not enter diapause and adults will emerge. Low light intensities are sufficient for the photoperiod reaction (Wildbolz and Riggenbach 1969). Light intensity should be at least 161 lux at the surface of the apples (Proverbs and Newton 1962a), but Dickson et al. (1952) used only 32 lux and Pristavko and Boreyko (1971) used 500–700 lux. Infested apples can be held at 22–30°C in the laboratory or in a greenhouse under daily fluctuating temperatures. Relative humidity (RH) was maintained at 35% (Dickson et al. 1952; Hamilton and Hathaway 1966), 60% (White and Hutt 1970; Pristavko and Boreyko 1971; Toba and Howell 1991) or 60–70% (White and Hutt 1972). In these conditions the life cycle is completed in about one month. Dickson et al. (1952) obtained a yield of 60 moths from 600

eggs and 130 apples, but Hathaway (1967) and Jermy and Nagy (1971) obtained about one moth/apple.

Adults are obtained by placing cardboard strips with cocoons in emergence cages. The cages are held at about 20-29ºC and 35–70% RH (Dickson et al. 1952; Proverbs and Newton 1962a; Hamilton and Hathaway 1966; Jermy and Nagy 1971; Butt et al. 1973; Toba and Howell 1991).

Problems associated with rearing on immature apples include granulosis virus (Jermy and Nagy 1971), *Drosophila*, fungus-feeding insects and mould (Hamilton and Hathaway 1966).

2.3. DIETS THAT STAY MOIST AND SOFT

Many laboratories rear the codling moth in order to collect larvae for virus production or postharvest research, and they can be easily extracted from soft diets (Howell 1970; Brassel 1978; Huber 1981; Reiser et al. 1993). Also, mature larvae tend to leave a soft diet by themselves, 'negative hygrotactic' behaviour (Huber et al. 1972), to seek out a dry cocooning site. This situation mimics nature where a mature larva exits an apple to find a dry crevice on a tree in which to spin a cocoon. In the laboratory corrugated cardboard strips provide cocooning sites for mature larvae. Pupae are obtained by opening the strips. If larvae in diapause (section 13) are required, then the same type of diet is used so that the larvae will exit the diet for pupation (Singh and Ashby 1986; Bloem et al. 1997, 2000).

Moist and soft diets are produced by incorporating agar or another gelling agent. Agar is rather expensive and cheaper substitutes have been sought (Vanderzant and Davich 1958; Navon and Moore 1971; Howell 1972c; Leppla 1976; Spencer et al. 1976; Moore 1985; Giret and Couilloud 1986; Honda et al. 1996; Chaudhury and Alvarez 1999).

2.4. DIETS THAT DRY OUT AND HARDEN

For the SIT, large numbers of adults are required for sterilization and release, and there is no need to incorporate a gelling agent to facilitate the extraction of larvae or pupae. Omitting agar in the diet reduces rearing costs and also handling costs associated with larvae and pupae. The Brinton et al. (1969) diet slowly dries out as larvae grow and mature, and when larvae have spun cocoons in the upper portion of the drying diet it has become rather hard but adults can still emerge (Howell 1971; Wildbolz and Mani 1971; Batiste and Olson 1973, Brassel 1978; Mani et al. 1978; Bloem et al. 1997; Mohammed et al. 1997; Botto 2006; OKSIR 2007). However, if pupae are required, Carpenter et al. (2004) tested procedures for their extraction using a de-silking chemical and pressure washing.

A requirement of using this type of diet is to maintain very close control of RH and air movement. If it dries too quickly, young larvae will die; if it dries too slowly, larvae leave the diet when they reach maturity. If trays of diet are stacked one above the other on carts, horizontal air flow at all heights above the floor must be carefully regulated so that all trays of diet dry out at the same rate.

3. Colonization from Field-Collected Material

For the SIT, it is advisable to initiate a colony using locally collected insects (Proverbs 1982; Proverbs et al. 1982) to ensure compatibility with wild insects after release. However, it is often difficult to establish a laboratory colony from field-collected material (Jermy and Nagy 1971; Bloem et al. 1997; Parker 2005); wild insects do not adapt easily to laboratory rearing, and adaptation takes several generations.

3.1. COLLECTING LARVAE IN DIAPAUSE

Corrugated cardboard bands, wrapped around the trunks of apple trees in infested orchards 1–2 months before harvest, provide cocooning sites for mature diapausing larvae (Dickson et al. 1952; Hamilton and Hathaway 1966; Jermy 1967; Judd et al. 1997; Giliomee and Riedl 1998; Judd and Gardiner 2005; Jumean et al. 2007; Taret et al. 2007). At harvest, the bands with cocooned larvae are collected (Blomefield et al. 2006) and stored in black polyethylene bags. Care must be taken to eliminate parasitoids and predators (Dickson et al. 1952). Section 13. describes the environmental conditions for storing larvae in diapause and later to obtain adults to initiate a new colony.

3.2. COLLECTING LARVAE IN SPRING

A colony can be established most easily by collecting field-infested immature apples (from unsprayed trees) in the spring. Virtually all of the larvae found in host fruits at this time of year will be non-diapausing larvae. Infested fruit is held in a laboratory until larvae emerge (section 2.2). Larvae are then captured in corrugated cardboard strips to complete the pupal stage. The strips are then placed in emergence cages and emerging adults are used to initiate a new colony.

3.3. COLLECTING ADULTS

This is not commonly done because it is difficult to capture live adults in the field that are still of good quality. However, to a limited extent, catching living already-mated females is possible using ultraviolet (black) light traps that use a screen cage to hold trapped live insects (Proverbs et al. 1966, 1967, 1973; Butt et al. 1970; Batiste et al. 1973; White et al. 1973; Hagley 1974; Howell 1988; Weissling and Knight 1994; Riedl et al. 1998; Keil et al. 2001).

Bait traps, using molasses/yeast or other odorous bait, may attract adults (Weissling and Knight 1994) but it is difficult to collect and keep the adults alive.

Passive interception traps (Knight 2000; Weissling and Knight 2004) are not suitable for collecting live adults.

Males can be caught in virgin female-baited (Butt et al. 1973) or sex-pheromone traps with some kind of a ‘holding cage’ for the trapped insects. A Multipher trap (Sanders 1986; Gastier 2007; Sunion 2007) may also be appropriate; this is a non-sticky bucket trap using a lure to attract males (Vincent et al. 1986, 1990; Spear-O’Mara and Allen 2007). It is also possible to attract female adults (Reed and Landolt 2002; Hughes et al. 2003; Hern and Dorn 2004), and there is also a bisexual lure available (Blomefield and Knight 2000; Stelljes 2001). Trécé (2007b) markets the “Pherocon® CM-DA COMBO™ multigender attractant”.

3.4. PREVENTING INTRODUCTION OF VIRUS

Even though natural outbreaks of the codling moth granulosis virus (CpGV) in the field appear to be very rare (Zimmermann and Weiser 1991), the virus is present in field populations, and there is always a risk of introducing this virus when field-collected insects are used to initiate a colony (Gast 1968; Reed and Tromley 1985; Singh and Ashby 1985; Bathon et al. 1991). It is important to minimize the risk by following these guidelines:

- Initiate a colony with adults and not larvae. Even if larvae are collected from the field, it is adults that should be used to initiate a colony.
- Egg sheets should be surface-sterilized using formaldehyde (vapour or solution) (Bathon et al. 1991) or a fumigant such as methyl bromide (section 15.3.1).
- Treated egg sheets should be stored in plastic bags with a moistened wick for high RH (Ashby et al. 1985). When eggs hatch, individual F_1 larvae are transferred with a fine brush onto fresh sterile diet in individual plastic cups with covers. The F_1 larvae are reared individually until pupation and adult emergence; virus-infected F_1 larvae will die. The adults can be used to initiate the colony.

3.5. USING AN ESTABLISHED COLONY

Importing insects from an ongoing colony elswhere has the advantage that the imported insects may be virus-free or at least have a very low level of infection. However, for the SIT, mating compatibility should first be checked, but recent compatibility tests among populations of codling moth from different geographical origins show no evidence of incompatibility (Blomefield et al. 2005, 2006; Taret et al. 2006, 2009). There is also no evidence of hybrid inviability or hybrid sterility (Robinson and Proverbs 1973).

Codling moths can be shipped relatively easily (Ashby et al. 1985) (section 14.). The easiest to ship are eggs, larvae and pupae, but eggs or pupae are preferred because they can and should be surface-sterilized to minimize virus infection (Bathon et al. 1991). Chilled adults can be shipped long distances (Blomefield et al. 2005, 2006). If adults are shipped, their eggs should be surface-sterilized at the receiving laboratory to destroy virus.

3.6. NUMBER OF INSECTS REQUIRED

It is important to maintain genetic diversity in a strain (Bartlett 1984, 1985; Leppla and Ashley 1989; Wajnberg 1991; Mangan 1992), and it should be initiated with a sufficient number of individuals from a wide genetic background (Moore et al. 1985). Unfortunately, it is not possible to know exactly how many are ‘sufficient’. Calkins and Parker (2005) and Parker (2005) provided excellent reviews of strain management.

Singh (1977) recommends that “as many individuals as possible should be used to start a colony.” However, it appears that 500–1000 mated female adults should be sufficient. Proverbs et al. (1982) founded a colony with about 2000 insects but some colonies are established with as few as 100 females and 100 males (Leppla 1993).

Chambers (1977) stated that one should colonize with “maximum possible numbers, selecting from a central portion of the population and from the biotype appropriate to target interaction, and other procedures that maximize genetic diversity and suitability.”

Parker (2005) stated that “Insects newly collected from the field rarely thrive in the laboratory, and the first few generations usually suffer high mortality, with the colony stabilizing after about five generations (Bartlett 1984, Leppla 1993). This process involves the rapid selection of individuals better adapted to the laboratory rearing conditions, resulting in a rapid decline in heterozygosity. There is considerable concern that these changes will result in insects significantly different from the wild population, and therefore non-competitive, although as yet it has not been possible to show unequivocally that a reduction in heterozygosity per se leads to a reduction in competitiveness.”

3.7. INTRODUCTION OF WILD GENOTYPES

Periodically, once a year in winter, some wild adults, e.g. 500–1000 males, should be incorporated into the colony (Shorey and Hale 1965; Singh 1977; Guennelon et al. 1981; Proverbs 1982; Joslyn 1984; Wajnberg 1991; Rogers and Winks 1993; Bloem et al. 1999b, 2004). Using only males will minimize the risk of introducing CpGV (egg-laying wild females can introduce CpGV into the colony) (Moore 2003).

To maximize the introduction of new genetic material from wild males, they should be put into oviposition cages at the time of year when the colony is the smallest (Pashley and Proverbs 1981). The wild males are put into cages with colony females and no colony males; this will ensure that the wild genotypes are incorporated into the colony. Eggs produced from these colony females should be surface-sterilized before being used.

In the OKSIR facility, a special room was constructed next to the cold room (Dyck et al. 1993; Dyck 1999). Wild adults obtained from infested apples were reared in this room and then brought into the oviposition room through the cold room to avoid introducing pest insects from the apples.

3.8. BACK-UP STRAIN/COLONY

Ashby et al. (1985) recommended that a back-up colony be maintained, away from the main laboratory colony, to avoid total loss in case of equipment failure or a severe epizootic of a disease (Rogers and Winks 1993), or to replace the main colony should it become ineffective in the field (section 2.1).

4. Diet Ingredients

4.1. ARTIFICIAL DIETS FOR INSECTS

An artificial diet "confers one main advantage over natural foods: the seasonal unavailability of the natural diet allows continuous laboratory rearing of insects of known 'quality' which are available throughout the year for various laboratory tests" (Singh 1983). The ability to regularly produce insects of consistent quality is a vital feature for the SIT. Normal nutrition requires that nutrients are available, metabolically suitable, and chemically and physically acceptable, and that supplementary sources of nutrients are provided as needed. Standardized diets and rearing methods produce quality insects (Howell 1970; Singh 1984). Ingredients for artificial diets are available from commercial sources throughout the year, and they are generally more economical than natural food materials (Rock et al. 1964; Howell 1967) (Annex 2).

An ideal diet for mass-rearing programmes should have the following qualities (Singh 1977, 1983):

a. Provide all nutrients needed to produce acceptable insects — nutritionally efficient and meet the insect's behavioural requirements.
b. Inexpensive and economical.
c. Innocuous to use and easily prepared from locally and readily available ingredients.
d. Long storage life.
e. Produce an average yield of adults of at least 75% from initial viable eggs. Size and rate of development should be similar to that in nature. The adults should mate, lay viable eggs and continuously reproduce without loss of vigour or fecundity. The behaviour of the insects should be 'normal' and the quality 'acceptable'.

Where an artificial diet was unsatisfactory, the addition of natural food materials sometimes provided the needed ingredients, e.g. 'corn leaf factor', rice stalks, mulberry-leaf extracts (Chippendale and Beck 1968).

4.2. DIETS FOR LEPIDOPTERA

Several general-purpose diets have been created that are suitable for many species of Tortricidae and some other Lepidopteran species (Singh 1977, Bathon et al. 1991). The Ivaldi-Sender diet, originally developed for the oriental fruit moth *Grapholita molesta* (Busck) (Ivaldi-Sender 1974), has also been used to rear the codling moth. The ingredients and methodology of this soft diet (described in detail by Bathon et al. (1991)) are shown in Table 1.

TABLE 1
The Ivaldi-Sender General-Purpose Diet[1]

Composition		**Preparation**
Ingredients	*Amount*	Add the agar powder to boiling water, and stir until the agar is dissolved. Without further heating add the corn semolina and mix thoroughly. After boiling again for a few seconds mix in the yeast powder followed by the wheat germ, without further heating. Let mixture cool down to about 60°C and then add ascorbic acid, dissolved in a little amount of distilled water, and the mould inhibitors, dissolved in a few mL of alcohol (96%). Mix the diet thoroughly with a hand mixer until it is homogeneous and then pour it into plastic cups or — to obtain diet plates about 2-cm thick that can be cut into pieces — into trays. Excessive water evaporates within a few hours. When working in the presence of specific pathogens an additional surface sterilization is recommended. For surface sterilization, the diet in the cups or trays is exposed to UV light for about 15 min. Now the diet is ready for use. It can be stored at 4°C for about one month.
Agar powder	20 g	
Corn semolina	50 g	
Wheat germ	50 g	
Yeast powder (brewer's or torula yeast)	50 g	
Ascorbic acid	4.5 g	
Benzoic acid	1.8 g	
Methyl *p*-hydroxybenzoate	1.8 g	
Water	780 mL	
Total diet (approximately)	958 g	

[1] From Bathon et al. (1991), and derived from Ivaldi-Sender (1974).

Table 2 shows details of a diet developed by Singh (1983, 1985) for insects from several orders, including the codling moth. This diet was described in detail by Bathon et al. (1991), and it can be freeze-dried and stored for later use.

4.3. DIETS FOR CODLING MOTH

Insect diets are not usually developed by combining chemically defined ingredients (Vanderzant 1957; House 1961; Cohen 2004) but are developed over time from natural to semi-synthetic to synthetic ingredients. Most current artificial diets for codling moth are synthetic and do not contain any natural ingredients, e.g. apple pulp or seeds. Hamilton and Hathaway (1966), Howell (1970) and Reed and Tromley (1985) summarized the initial attempts to create an artificial diet for the codling moth. The first attempt to rear the codling moth on an artificial diet was by Theron (1947).

The numerous diets used today are quite similar. Differences are due to local attempts to simplify or reduce the cost of the diet, or to utilize locally available ingredients, and not due to real differences in the nutritional requirements of the strain being reared. As for many lepidopteran species, diets for the codling moth tend to be complex and therefore rather expensive.

Semi-synthetic diets for the codling moth have often been developed from diets for other lepidopterans. Brinton et al. (1969) and Howell (1970) used a diet developed by Ignoffo (1963) for the cabbage looper *Trichoplusia ni* (Hübner). Sender (1970) and Guennelon et al. (1981) tested a diet based on work by Poitout and Bues (1970, 1972) on noctuids. Modifications to the wheat germ diet of the bollworm *Helicoverpa zea* (Boddie) (Vanderzant et al. 1962) proved to be a good diet for the codling moth (Rock 1967). Redfern (1964) and Hamilton

TABLE 2
The Singh General-Purpose Diet[1]

Composition		
Ingredients	*Amount*	*%*
Dry mix to prepare 6.5 kg finished diet		
Agar	150 g	12.32
Casein	210 g	17.24
Cellulose powder	600 g	49.25
Wesson's salt mix	60 g	4.93
Wheat germ	180 g	14.78
Cholesterol	3 g	0.25
Linoleic acid	15 g	1.23
Dichloromethane[2] (evaporates)	50 mL	
Finished diet		
Dry mix (see above)	1218 g	18.56
Distilled water	4290 mL	65.33
4*N* KOH	30 mL	0.46
Vanderzant vitamin mix	120 g	1.83
Sucrose	180 g	2.74
Glucose	30 g	0.46
Streptomycin sulphate BP[2]	900 mg	
Penicillin[2]	900 mg	
Prochloraz[2] (fungicide)	260 mg	
Distilled water	600 mL	9.15
Mould inhibitor	90 mL	1.37
Total diet (approximately)	6558 g	100

Preparation

Composition of the stock mould inhibitor solution: Methyl *p*-hydroxybenzoate, 37.5 g; sorbic acid, 50.0 g; 95% ethyl alcohol, 425 mL.
Preparation of stock 4*N* KOH solution: 56 g KOH is added carefully to 250 mL distilled water.
Preparation of dry mix: Mix the agar, casein, cellulose powder, Wesson's salt mixture and the finely ground wheat germ together. Dissolve cholesterol and linoleic acid in dichloromethane, then mix thoroughly with the dry ingredients. Leave in fume cupboard for 2–3 days, stirring periodically to allow solvent to evaporate.
Preparation of finished diet: Mix the dry mix, distilled water and KOH together, cover and sterilize in autoclave at 121°C for 20 min. Combine 600 mL distilled water, vitamin mixture, sucrose, glucose, streptomycin, penicillin and prochloraz together. Mix thoroughly and add to autoclaved diet (cooled to 60°C) along with mould inhibitor. Adjust the pH of the diet between 4.5 and 5.0. The diet is dispensed into test tubes with a diet dispensing machine or is manually dispensed into waxed Lily[3] cups or plastic containers.

[1] From Bathon et al. (1991), and derived from Singh (1983, 1985). The cost of the diet was about USD 2.05/kg.

[2] Not included in total weight.

[3] Plastic disposable cup (http://www.solocup.com/solocanada/index.html).

and Hathaway (1966) tested a larval diet of the boll weevil *Anthonomus grandis grandis* Boheman, and after several changes to the diet, including using ascorbic acid, were able to rear the codling moth.

Some diets have relatively few ingredients, with no specific vitamins or minerals added, e.g. Sender (1969, 1970), but instead include some natural ingredients from yeast and grains such as maize semolina and wheat germ. However, insect quality parameters, especially behavioural parameters, should be carefully measured and monitored over a long period of time before concluding that a simple diet is satisfactory for mass-rearing (sections 16, 17, 18, 19).

TABLE 3
Ingredients in Artificial Diets that Stay Moist and Soft

Ingredient[1]	Publication										
	Redfern 1964	Hamilton and Hathaway 1966 (Nr 2)	Rock 1967 (g/100 g)		Information from Butt (1975) and Hatmosoewarno and Butt (1975) (g/kg)						
			Casein diet	Wheat germ diet	Shorey and Hale 1965	Navon and Moore 1971	Bulytginskaya (Russia)	Falcon (USA)	Anderson (Australia)	Howell (USA)	Pristavko and Yanishevskaya 1972 (Nr 16)
Distilled H_2O (mL)	220	1100	Make 100 g	Make 100 g	706.2	560	Make 1 kg	837.8	847	861	Make 1 kg
Agar (g)	4	20	3	3	14.1		15–20	24.3	18.6	10.7	24
Sodium alginate						11.6					
Calcium phosphate (dibasic)						2.9					
Casein (g)		60	4	3.5		46.6	40	34.1	34.5	27.1	34
Yeast (g)	2.5	12.5				46.6[2]	15				
Brewer's yeast					35.3						20
Sucrose (g)	12	60	5	3		46.6	40	25.9	26.4	27.1	32
Apple pulp (g)	12	60									100
Apple seeds (g)	6										3
Beans/soybeans (g)	12				235.4	116.6					
Walnuts (g)	3										
Peanut shells						153.2					
Wheat germ (g)		45		3			60–80	29.5	29.5	46.5	36
Linseed oil (mL)	2	10									10
Safflower oil			0.4	0.3							
Cellulose (g)		30					5		4.9		6
Cellulose powder (g) e.g. Alphacel	6							4.9			
Carboxy methyl cellulose										4.3	

Ascorbic acid (g)		2	10			3.5	0.4	8	8.2	4.2	3.2	7
Acetic acid (glacial)							14.2					
Potassium hydroxide		7.5 mL	37.5 mL	3.5 mL	3 mL				5.4	4.9		
Wesson's or mineral salts[3] (g)		2.5	12.5	0.471	1.2				9.7	9.8	7.7	12
Mould inhibitor[4] (mL)		4.5	22.5		2.2							
Sorbic acid						1.1	0.4	0.9	7.7	0.65	1	
Methyl *p*-hydroxybenzoate						2.2	0.9		1.5	1.5	1.1	2
Ethyl alcohol												20
Tween 80®[5]				0.3								
Cysteine (g)		0.125	0.625	0.2	0.2							1
Glycine (g)		0.25	1.25									
Cholesterol (g)		0.125	0.625	0.2								1
Antibiotic										0.14[6]		
Sodium hypochlorite											0.4	
Formaldehyde						2.2[7]			1.5		0.3	
Vitamin mixture[8]		2.5 mL	12.5 mL	0.952	0.95			FN[9]	9.7	9.8	9.7	FN[9]
PI[10] Weight (mg)	**Female pupa**	**38.3[11]**				**28–30**					**44.5**	
	Female adult	**25[11]**						**21–23**				
Yield	**Survival (%)**	**60** **33[11]**	**60**	**79** **90[15]**	**55–65**	**83**	**50**					
	Nr pupae/ kg diet					**206**	**250**	**230–250**	**150**	**238**	**100 (larvae)**	
Cost[20]	**USD/1000 pupae**					**15[16]**			**11[16]** **21[17]**	**8[16]** **98[17]**	**2[16]** **2[17]**	

TABLE 3 (continued)
Ingredients in Artificial Diets that Stay Moist and Soft

Ingredient[1]	Publication										
	Coutin 1952 (see Singh 1977)	Navon 1968	Sender 1969	Sender 1970	Edelman 1970 (see Singh 1977)	Huber et al. 1972 (diet A)	Huber et al. 1972 (diet B)	Huber 1981	Shumakov et al. 1974 (see Singh 1977)	Bathon 1981	Guennelon et al. 1981 (per kg of diet)
Distilled H_2O (mL)	100	480	170	170	Make 100	1000	1100	1000	Make 100	780	755.5
Agar (g)	2.5	24	5	5	2	18	18	10	2	20	20
Casein (g)					5				4		
Milk powder (g)						54	54				
Yeast (g)					8 mL			41	1.5	50[2]	
Brewer's yeast (g)		18	10	7.5		45	45				37.8
Sucrose (g)		48			4	54	54		4		
Glucose (g)	10										
Apple pulp (g)		100	9		4						
Apple seeds (g)					1.5	90		20			
Pear seeds (g)	20										
Beans/soybeans (g)		24					120	120			
Maize semolina (g)			10	28						50	141
Wheat germ (g)			12	7	4			53	4.5	50	35.3
Linseed oil (mL)						10	10				
Maize oil (mL)			0.4								
Cellulose (g)					2	27			2		
Cellulose powder (g) e.g. Alphacel		60									
Ascorbic acid (g)		0.3	1	1	0.7	180 mL	180 mL	5.8	0.8	4.5	5
L-malic acid (g)		4									
Benzoic acid (g)			0.3	0.25				2.6		1.8	2.3
Citrate buffer (mL) (pH 3.4)		120									

Potassium hydroxide (mL)							14					
Wesson's or mineral salts[3] (g)						0.84	13.5	13.5				
Mould inhibitor[4] (mL)							24	24				
Methyl *p*-hydroxybenzoate (g)		0.2	1.5	0.3	0.2	0.15			2.6	0.15	1.8	1.8
Ethyl alcohol (mL)									30			
Cysteine (g)						0.1						
Glycine (g)						0.1						
Cholesterol (g)						0.2	0.55	0.55				
Antibiotic[6] (mL)				1								
Formaldehyde (mL)						0.5 g		2		0.5 g		1.3
Choline chloride (g)							1.2	1.2		0.05		
PI[10] Weight (mg)	**Female pupa**		**28.8[11]**								**47**	
	Female adult		**20.1[11]**							**21.7**		**37[21]**
Yield	**Survival (%)**		**20–30 28[11]**	**88**	**83**		**70–80**	**70–80**				**77 44 17 40**
	Nr adults/ kg diet									**230**	**57.2[22]**	**333 250 156 510**
	Nr adults/L diet											**375[21]**
Cost[20]	**USD/1000 adults**											**2.2[16] 46**
	USD/kg diet											**1.1[16]**

TABLE 3 (continued)
Ingredients in Artificial Diets that Stay Moist and Soft

Ingredient[1]	Publication								
	Hatmosoewarno and Butt 1975 (CW) (Vanderzant 1966)	Hatmosoewarno and Butt 1975 (MB) (Burton 1969)	Reed and Tromley 1985	Howell 1970	Howell 1971	Howell and Clift 1972	L. Neven (USA) (pers. comm.)	Brinton et al. (1969) modified by Dyck in 1993	International Minerals and Chemical Corp. (Information from Hathaway et al. 1971 and Butt 1975)
Distilled H_2O (mL)	1000	1000	1280	3400	3600	160000	3500	6500	
Agar (g)	28.5		25.69	90	45–60	2000	18	105	
Carageenan (g)		15.3							
Sawdust (sifted) (g)								624	
Casein (g)	49.9			126	126	5040			
Brewer's yeast (g)	42.8	41.7	64.09						
Sucrose (g)	57.6			96	96	5040	87	156	
Apple pulp (g)				108					
Beans/soybeans (g)		91.7	457.09				410	780	
Wheat starch (g)							81		
Wheat germ (g)	42.8	66.7		108	108	8640	180	51	
Whole wheat flour (g)								402	
Gluten (g)								30	
Linseed oil (mL)	6.4								
Cellulose powder (g) e.g. Alphacel	14.3			18					
Carboxy methyl cellulose (g)						800			
Ascorbic acid (g)		4.3	6.49	15	15	600	12.6		
Propionic acid (mL)							8.1		
Citric acid (g)	11.6							46.5	
Potassium hydroxide (mL)	3.5			18					
Sodium benzoate (g)	2.6								

Wesson's or mineral salts[3] (g)		12.8			36	36	1440	5.4	33	
Fungicide[12] (g)								0.45		
Sorbic acid (g)			1.3	2.09		5	200	2.7		
Methyl *p*-hydroxybenzoate (g)		1.9	2.7	4.09	5.4	5.4	216	3	9	
Ethyl alcohol								FN[13]		
Propylene glycol[14] (mL)						30		30		
Cholesterol (g)		1.4								
Antibiotic[6] (g)		0.0578	0.0833		0.250			3.2		
Sodium hypochlorite (mL)							800			
Formaldehyde (mL)			4.3	4	15	15	60			
Choline chloride (g)									12	
Vitamin mixture[8] (g)		12.8	5		36	45 mL	1800 mL	33	33	
Total volume (L)								**4.3**		
PI[10] Weight (mg)	**Female pupa**	**39**	**38**	**35.8**	**44.5** **36.6[11]**					**41.2**
	Female adult				**25.5** **24[11]**					**27.8**
PI[15] Weight (mg)	**Female pupa**				**42.2**					**43.4**
	Female adult				**28.6**					**29.1**
Yield	**Survival (%)**	**76**	**65**	**97**	**88** **47[11]**	**92** **21**				**47[11]** **48[15]**
	Nr pupae/kg diet									**100–150**
	Nr adults/L diet				**250[18]**	**80[19]**	**81** **39**			
Cost[20]	**USD/1000 pupae**									**24[16]** **18[17]**

TABLE 3 (continued)

Ingredients in Artificial Diets that Stay Moist and Soft

[1] Ingredients are listed in no particular order.

[2] Torula yeast.

[3] Mineral salts vary among diets. See section 5.5. for details on procedures for making mineral salt mixtures.

[4] Mould inhibitor prepared as a pre-mixture: Methyl *p*-hydroxybenzoate 15 g, sorbic acid 20 g, and ethyl alcohol 170 mL (Redfern 1963; Hamilton and Hathaway 1966; Rock 1967).

[5] Tween 80® (polyoxyethylene sorbitan mono-oleate) is an emulsifier.

[6] Example of antibiotic – Aureomycin® (chlortetracycline HCl).

[7] Formaldehyde (40%) (Formalin®).

[8] Vitamin mixture varies among diets. See section 5.4. for details on procedures for mixing vitamins.

[9] Vitamins used by Bulyginskaya, and Pristavko and Yanishevskaya, are not included in this table. See section 5.4.

[10] Performance Indicator (PI) – selected as a crude measure of the quality and suitability of the diet for rearing the codling moth. (It is assumed that a heavier pupa results from a better diet. (Butt (1975) noted that pupal weight declines with age.))

[11] Data obtained by Hathaway et al. (1971) when the diet of the listed publication was tested.

[12] Fungicide used is Benlate® (benomyl).

[13] Enough ethyl alcohol used to dissolve sorbic acid and methyl *p*-hydroxybenzoate.

[14] Propylene glycol is a humectant which slows dehydration.

[15] For comparison, insects reared on immature apples could be considered as ideal; Hathaway et al. (1971) observed that the weight of female pupae was 43.4 mg, and of female adults 29.1 mg; Rock (1967) observed 90% survival on immature apples.

[16] Cost of ingredients only.

[17] Cost of labour only. (Guennelon et al. (1981) estimated that two-thirds of rearing costs are labour costs.)

[18] Howell (1967) reported that about 250 larvae needed 1 L diet (in trays).

[19] If reared on immature apples, 1 L apples yielded 42 adults.

[20] Cost estimates are approximate only. USD=United States Dollars. Information on IMC diet (Hathaway et al. 1971) provided by Butt (1975).

[21] Data obtained by D. Stenekamp (pers. comm.) of South Africa when the diet of the listed publication was used.

[22] Data obtained by Reiser et al. (1993) when the diet of the listed publication was tested.

The selection of a particular diet or modifying a diet is a local decision based on the availability of ingredients, cost, equipment and facilities available and the purpose of rearing. However, the major criterion in choosing a diet should be the quality of the end product. To provide an approximate measure of quality, Tables 3 and 4 include weights of female pupae and adults. Some diets are probably more complex than necessary, but it is difficult to determine clearly the essential ingredients and those that during the historical development of the diet have been included somewhat accidentally, i.e. without specific knowledge of their nutritional role.

4.4. DIETS THAT STAY MOIST AND SOFT

Most diets used today to rear the codling moth stay moist and soft throughout the period of larval development. A moist diet enables mature larvae to leave the diet and find a dry crevice in which to spin a cocoon (Howell 1971).

To keep a diet moist and soft, a gelling agent, usually agar, is added. Agar is rather expensive, and cheaper substitutes have been tested (section 2.3). In the diet of Navon and Moore (1971), a binding gel is formed by reaction between sodium alginate and calcium ions under acid conditions. Sodium alginate is about one-fifth the cost of agar, and Guennelon et al. (1981) noted that its use would reduce the cost of the diet by 40%. Syneresis (separation of liquid from a gel) must be prevented because small larvae drown in free water (not bound to the diet).

Diet comparisons have been made (Hathaway et al. 1971; Shumakov et al. 1974; Butt 1975; Singh 1977). An updated comparison is shown in Table 3 which provides diet details and references, and allows individual rearing centres to select a diet, or to modify an existing diet, that is suitable for local conditions. Original publications should be consulted for details, especially for vitamins and minerals (sections 5.4 and 5.5). However, the pupal and adult weights in Table 3 permit a comparative assessment of the diets. Those diets producing female pupae weighing less than 39–43 mg, and female adults weighing less than 28–30 mg, should be regarded with caution.

Simple diets with no specific inclusion of mineral or vitamin mixtures (except for ascorbic acid) have been used widely (Coutin 1952; Shorey and Hale 1965; Navon 1968; Sender 1969, 1970; Navon and Moore 1971; Shumakov et al. 1974; Bathon 1981; Guennelon et al. 1981; Huber 1981; Reed and Tromley 1985). The required vitamins and minerals are probably provided through the inclusion of semi-synthetic ingredients, e.g. yeast, natural food such as apple pulp or seeds, beans, maize, wheat, wheat germ. These diets tend to be used for laboratory rearing.

The development of moist/soft diets appears to have stopped by 1985, but further work is probably continuing in the private sector in relation to producing CpGV. Fisher (1984a) describes an insect production programme at the Dow Chemical Company (including production of the codling moth) but, in general, procedures used in commercial rearing of insects are often not published or accessible.

TABLE 4
Ingredients in Artificial Diets that Dry Out and Harden

Ingredient[1]	Publication												
					Information from Butt (1975) (g/kg)					Mani et al. 1978 (g/kg)			
	Brinton et al. 1969 (%)	Brinton et al. (1969) diet tested by Hathaway et al. (1971)	Brinton et al. (1969) diet used by Dyck in 1993 (Bloem et al. 2007; OKSIR 2007)[23]	Howell 1972c	Howell 1972c	Anderson (Australia)	Brinton et al. 1969	Mani/Charmillot (Switzerland)	Wearing (New Zealand)	Nr 1 (Brinton et al. 1969)	Nr 2	Ashby et al. 1985	M. Mansour (pers. comm.) and Mohammad et al. 1997
Distilled H_2O (mL)	71.7	2500	605[2]	6500	781	626	717	660	644.9	660	650	8400	4000
Sawdust/wood chips[3] (g)	6.89	275	280 mL			80	68.9	80	82.9	80	80	1084	
Paper pulp (long fibre) (g)	1.24	50	8			16.3	12.4	15		15	15		
Straw (legume) (g)													261
Casein (g)	2.69	108				32.5	26.9	30	33	30		432	
Yeast (g)						37[4]					50		
Sucrose (g)	2.69	108	17.2	150[5]	18[5]	32.5	26.9	30	33	30	10	432	103.2
Apple pulp (g)				100	12								
Soybean meal (g)			86[6]	900	108								655
Wheat bran (g)	1.8	72				21	18	20	22	20	30	288	
Whole wheat flour (g)	9.86	395	44			120	98.6	120	121.1	120	60	1584	
Wheat starch (g)				150	18								
Maize semolina											60		
Wheat germ (g)	0.9	36	5.8	300	36	10.8	9	16	11	16	30	144	
Gluten (g)			3.3										
Flour mixture (g) (wheat/corn/barley)													306[7]
Barley germ (g)													750

Sunflower oil									4		4			
Cellulose fibre (g)					15	1.8				13.9			181.2	
Ascorbic acid (g)			15		16	1.9	4.4	3.58	5.5	8.8		5.5	57.6	
Propionic acid (mL)											3.2	3.2		
Citric acid[8] (g)		0.9	36	5.1[9]			10.8	9	10	11	10	10	144	30.6
Linoleic acid (mL)										0.6			7.2	
Wesson's or mineral salts[10] (g)		0.62	25	3.5	10	1.2	7.6	6.2	6.8	7.6	6.8		100	21
Sorbic acid (g)			3.75		50	6	1.1	0.9	0.9	0.9		0.9	12	
Methyl *p*-hydroxybenzoate (g)				1	6	0.7				0.5			6	8
Ethyl alcohol										7.4				
Propylene glycol (mL)					60	7.2								
Cholesterol (g)										0.2			2.8	
α-tocopherol											0.15			
Antibiotic[11] (g)							0.11	0.14	0.088			0.09		
Formaldehyde				0.3[12]	15[13]	0.3								1.8 g
Choline chloride		0.1	3 mL	1.3 g				1	1	1	1			7.8 g
Triturated ingredients		0.61		3.7 g										
Vitamin mixture[14] (g)			45		50[15]	6					6.55		144 mL	22.2
Calco Red dye[16]				2.2 mL							0.04	0.04		13.2 mL
Paraffin wax (g)				0.5										
Total volume (L)				**1**									**13 kg**	**6**
PI[17] Weight (mg)	**Female pupa**	**30–50**	**29.1**		**52.2**	**52.2**			**40.4** **32.2**	**37.2**	**35.6**	**35.6**	**36.5**	
	Female adult	**26.7** **23.7[18]**	**19.1**					**28**		**24.7**				
PI[19] Weight (mg)	**Female pupa**	**28–53**	**43.4**		**43.9**									
	Female adult	**32.2**	**29.1**											

TABLE 4 (continued)
Ingredients in Artificial Diets that Dry Out and Harden

Ingredient[1]		Publication												
						Information from Butt (1975) (g/kg)					Mani et al. 1978 (g/kg)			
		Brinton et al. 1969 (%)	Brinton et al. (1969) diet tested by Hathaway et al. (1971)	Brinton et al. (1969) diet used by Dyck in 1993 (Bloem et al. 2007; OKSIR 2007)[23]	Howell 1972c	Howell 1972c	Anderson (Australia)	Brinton et al. 1969	Mani/Charmillot (Switzerland)	Wearing (New Zealand)	Nr 1 (Brinton et al. 1969)	Nr 2	Ashby et al. 1985	M. Mansour (pers. comm.) and Mohammad et al. 1997
Yield	Survival (%)	52[20] 16[18]	30[21]	39[30] 52[30]	41						30[20]	30[20]	60–70	
	Nr pupae/kg diet						81		100–200					
	Nr adults/kg diet							121		77	170	170	178	
	Nr adults/L diet	200[22] 201[18] 380[18]		263[23] 441[23]	18[24] (larv.)									
Cost[25]	USD/1000 pupae					1.35[26]	5.23[26] 113[27]	1.58[26] 0.52[27]	1.06–2.12[26] 4.35–5.80[27]	15.36[26]				
	USD/1000 adults	0.93[26] 1.41[26] 2.02[27]		2.86[29] 1.80[29] 1.57[29] 5.62[23] 2.94[23]							17.86	16.63		
	USD/L diet				0.15[28]									

1 Ingredients are listed in no particular order.

2 Ordinary tap water is acceptable since the diet is cooked in a steam kettle. Up to 10% more water may be needed to create the correct consistency of the diet. If possible,

use hot water to reduce the diet preparation time.

[3] Softwood sawdust or chips were obtained from local sawmills; fir wood was used by Brinton et al. (1969). Particles of sawdust were about 3 mm in diameter, and those of wood chips up to about 2 × 2 × 20 mm. Sawdust weighs about 200–250 g/L, depending on compaction and water content. The wood helps retain moisture, slowing the drying process. Also, the wood roughens the surface of the diet, increasing acceptance by neonate larvae and making it more suitable for larval entry into the diet. Note that care must be taken to ensure that the sawdust does not contain insecticides arising from spraying of trees (Wildbolz and Mani 1971).

[4] Torula yeast.

[5] Howell (1972c) used invert sugar (a liquid), which is a mixture of glucose and fructose obtained by a hydrolysis of sucrose.

[6] Canola (soybean) meal.

[7] Flour mixture at 1:1:1.

[8] Citric acid was used to reduce the pH of the diet to 3.5. A low pH reduced the development of micro-organisms in the diet.

[9] Fumaric acid used instead of citric acid.

[10] Mineral salts vary among diets. See section 5.5. for details on procedures for making mineral salt mixtures.

[11] Examples of antibiotic – Aureomycin®, chlortetracycline HCl 5.5%.

[12] Diluted 0.3 mL formaldehyde (37%) in 7.2 mL H_2O before adding to the diet. Formalin® is a commercial product (37–40% formaldehyde solution in water). Note that formaldehyde is an animal carcinogen, so great care must be taken to protect workers from inhaling the fumes.

[13] Howell (1972c) diluted the Formalin® stock: 1 mL stock/10 mL water.

[14] Vitamin mixture varies among diets. See section 5.4. for details on procedures for mixing vitamins.

[15] Howell (1972c) used the vitamin mixture described in Howell (1971) — see section 5.4.

[16] Calco Red vegetable dye is included to mark the internal organs to permit later identification of captured adults in the field. Dyck prepared a mixture of dye and oil as follows: Heat 80 mL vegetable oil, e.g. soybean oil, to 130°C, mix in 1.2 g powdered Calco Red dye and stir. M. Mansour prepared a mixture of 15 g dye per litre of vegetable oil.

[17] Performance Indicator (PI) — selected as a crude measure of the quality and suitability of the diet for rearing the codling moth. (It is assumed that a heavier pupa results from a better diet. (Butt (1975) noted that pupal weight declines with age.))

[18] Data obtained by Batiste and Olson (1973) when the diet of the listed publication was tested. 380 adults/L diet is calculated from 0.38 adults/mL diet.

[19] For comparison, insects reared on immature apples. The weights attained could be considered as ideal.

[20] Brinton et al. (1969) reported a recovery from eggs to adults of 52%, and Mani et al. (1978) reported 30%.

[21] High pupal mortality occurred in this experiment.

[22] If reared on immature apples, 1 L apples yielded 50 adults.

[23] It is assumed that the diet used in the OKSIR Program is the same as, or similar to, that used by Dyck in 1993 (Proverbs et al. 1982; Bloem et al. 1997; Bloem and Bloem 2000; K. Bloem et al. 2005). Values of yield and cost (Bloem et al. 2007) are for 1994 and 2004.

[24] Howell (1972c) estimated larval production at 18/L diet, and also at 42/L apples.

[25] Cost estimates are approximate only. USD=United States Dollars.

[26] Cost of ingredients only. USD 1.41 in the column for Brinton et al. (1969) was obtained from BCFGA (1972).

[27] Cost of labour only. USD 2.02 in the column for Brinton et al. (1969) was obtained from BCFGA (1972).

[28] Cost of diet/tray about one-half of apples/tray.

[29] In 1993 the cost (USD 2.86) to rear 1000 adults was obtained by Bloem et al. (1997) using a modified version of the Brinton et al. (1969) diet at the codling moth rearing facility (OKSIR) in Osoyoos, Canada. This cost in 1997 was USD 1.80 (Bloem et al. 1997) or USD 1.57 (Bloem et al. 2000).

[30] In 1993, 39%, and in 1997, 52% (Bloem et al. 1997).

Diets that are moist and soft tend to dry out over time, and the division in this document between two categories, moist/soft diets (with agar) and dry/hard diets (without agar), is thus somewhat arbitrary. The diet developed by Guennelon et al. (1981), which includes agar and maize semolina and is listed here as a moist/soft diet, tends to dry out and mature larvae usually spin cocoons in the diet (Guennelon et al. 1981; T. L. Blomefield, pers. comm.). Also, Hatmosoewarno and Butt (1975) showed that most of the larvae reared on the modified bean diet (Burton 1969) pupated inside the diet. Larvae pupate in the sawdust, bean and maize-meal diets because of dryness, but in wheat germ diets the larvae leave the diet (Reed and Tromley 1985).

4.5. DIETS THAT DRY OUT AND HARDEN

The omission of agar allows diet to dry out rather quickly. If drying is too fast, the diet cracks and shrinks and larvae inside the diet will die before they are mature. Therefore, other ingredients (inexpensive and locally available, e.g. whole wheat flour, wheat bran, paper pulp, wood chips) must be added to prevent rapid drying. These ingredients act as a binder to hold the diet components together and delay drying. If these materials are bulky and inedible, they also act as a cheap bulking agent to provide the physical space for burrowing larvae. Particles of wood chips can also act as barriers between feeding larvae to minimize cannibalism (Dickson et al. 1952; Hamilton and Hathaway 1966; Navon 1968; Brinton et al. 1969; Howell 1970, 1971; Ferro and Harwood 1973; Hathaway et al. 1973; Brassel 1978; Guennelon et al. 1981; Reiser et al. 1993). Immature larvae are strongly solitary, but aggressive larvae do bite and inhibit the growth of submissive larvae (Howell 1971). Mature larvae are not cannibalistic and tend to form aggregations.

Brinton et al. (1969) initiated a major departure from agar-based diet in order to produce codling moth adults for sterilization and release (Proverbs et al. 1982; Dyck and Gardiner 1992; Dyck et al. 1993; Bloem and Bloem 1995, 2000; Bloem et al. 2007; OKSIR 2007). To reduce production costs and simplify rearing operations, a diet and rearing system were developed that yielded adults that emerged directly from diet held in trays. The diet dried out slowly as the larvae matured, permitting larvae to spin cocoons and pupate in the diet; good control of moisture in the air and of air movement was required to regulate the drying process. This was the first artificial codling moth diet created without agar or a gelling agent, and is regarded as the pioneer diet of the dry/hard type of diet; it became known as the sawdust diet.

Table 4 provides details and references of diets that become dry and hard. Many workers have used the Brinton et al. (1969) diet (Wildbolz and Mani 1971; Batiste and Olson 1973; Butt 1975; Brassel 1978; Mani et al. 1978; Ashby et al. 1985; Mohammad et al. 1997). Botto (2006) modified the agar diet developed by Guennelon et al. (1981) by substituting agar with paper pulp and sawdust; the end result will probably be similar to the Brinton et al. (1969) diet. However, Guennelon's diet is being used for mass-rearing in South Africa (D. Stenekamp, pers. comm.). Howell (1972c) did not use sawdust, and found that production

on a soya/wheat germ/starch medium was 2–3 times better than on the standard agar diet and equal to that obtained with immature apples. Larval acceptance was exceptionally good (90%), and the pupae were significantly heavier than those grown on apples.

Brinton et al. (1969) noted that the addition of peptone (0.4 g to 100 g diet) increased the average weight of female adults from 26.7 to 31.9 mg. Wildbolz and Mani (1971) also tested the addition of peptone (and also safflor-oil) and concluded that the adults produced were larger. Nevertheless, peptone has apparently not been used on a regular basis in codling moth diets.

Brinton et al. (1969) called their vitamin mixture triturated (i.e. ground to a fine powder) (details in section 5.4). Several diets shown in Table 4 do not include a vitamin mixture and diet Nr. 2 (Mani et al. 1978) also omits minerals. As mentioned in section 4.4, the inclusion of semi-synthetic ingredients, e.g. yeast and maize, permits the production of quality insects without the specific addition of vitamins or minerals.

Several modifications to the Brinton et al. (1969) diet have been made:

- Costs were reduced by replacing most of the casein with a mixture of soyflour and gluten, by substituting acetic acid for citric acid and by replacing Wesson's salts with a simplified mineral mixture (Proverbs 1974). Wheat bran [originally added to improve the physical consistency of the diet] was replaced by 0.5% gluten [to help bind the ingredients together], casein (milk protein) replaced by 13% soybean meal (about 38% protein) and citric acid replaced by 0.8% fumaric acid [to lower diet pH] (Proverbs et al. 1982).
- To reduce drowning of young larvae, water content was reduced by about 20% (Wildbolz and Mani 1971).
- Batiste and Olson (1973) used a commercial vitamin mixture.
- M. Mansour (pers. comm.) and Mohammad et al. (1997) (in Syria) replaced sawdust and paper pulp by legume straw, replaced wheat germ by barley germ, replaced gluten by a 1:1:1 mixture of maize, barley and wheat flour, and the amount of water was adjusted.
- In Argentina, sawdust was replaced by soya bran (Botto 2006; Taret et al. 2007).

Some of the diets shown in Table 4 performed well in terms of the weight of females produced, but others did not achieve the weights suggested in section 4.4.

4.6. OBTAINING DIET INGREDIENTS

Raw materials should be generally available, economical, uniform in nutrient density and of stable quality (Singh 1984). Ingredients are usually purchased separately and then mixed at the time of preparing the diet (Singh 1977; Brewer and Lindig 1984; Ashby et al. 1985; Reed and Tromley 1985; Singh and Moore 1985) (Annex 2). However, there may be economies of scale or added convenience by purchasing ready-made mixtures from commercial companies (Hathaway et al. 1971). Reuveny and Cohen (2004) used an artificial diet called Manduca Premix–Heliothis Premix.

Vitamins and minerals can be purchased from chemical supply houses or general laboratory supply companies.

Sawdust and wood chips can be obtained from a local sawmill, and paper pulp from paper manufacturers.

Animal feed suppliers can provide ground grains and feed additives that include antibiotics. Food materials in a feed store probably are cheaper than in a laboratory supply house.

Some diet ingredients, e.g. sucrose, flour, wheat germ, apples, beans, maize, starch, nuts, sodium chloride, NaOCl, oils, milk powder, paraffin wax, etc. can be purchased in food stores, and Brewer's yeast purchased from beer producers. Some chemicals, e.g. potassium hydroxide, potassium chloride, calcium carbonate, ascorbic acid and alcohol can be purchased at a pharmacy.

Information on availability of ingredients may be obtained from persons experienced in using artificial diets, the website of the IOBC Working Group on Quality Control of Mass-Reared Arthropods (AMRQC 2007) and issues of the former newsletter Frass.

The training course on Principles and Procedures for Rearing Quality Insects offered by the Department of Entomology and Plant Pathology, Mississippi State University, USA, provides an excellent opportunity to learn about rearing insects, including sources of dietary ingredients and insect rearing equipment and facilities.

4.7. LOCALLY OBTAINED INGREDIENTS

Reducing the cost of a diet is a major challenge, and one way is to avoid importing expensive diet ingredients and substitute with locally obtained materials (section 4.5). Creativity and an understanding of the purpose of each dietary ingredient are needed to be able to substitute ingredients effectively without lowering the quality of the produced insects. Long-term experiments on insect quality are required to ensure that a cheaper diet produces quality insects.

4.8. STORING DIET INGREDIENTS

The degradation of diet ingredients over time can be minimized through appropriate storage (**Figure 1**). Heat, light, microbes and stored-product insect pests are a risk to the quality of ingredients. Goodenough (1984) summarized storage procedures, and pointed out that a storage facility needs proper environmental control and the provision of adequate records and inventory control.

Cohen (2004) states that "the most common causes of deterioration or other ways that an ingredient can become unacceptable is through contamination, uptake of moisture, oxidation (especially of fatty acids and vitamins), or substitution of sources or processing methods of materials."

Managers of a rearing facility should adopt the concept of the ingredient cycle (Cohen 2004). "This system prescribes that all ingredients be assigned a replacement date that takes into account the shelf life of the ingredient and allows for procurement of its replacement prior to the expiration of the ingredient's shelf life. It should be noted that a 'lead time' of at least one generation of the target

FIGURE 1
Storage of diet ingredients, bags handled using a fork lift. OKSIR facility, Osoyoos, Canada

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insect is needed, with multiple-generation lead time the better choice. This assures adequate time for each ingredient to be tested, preferably by the bioassay technique, prior to the complete depletion of the ingredient in question. For example, the current batch of vitamin mixture would be used as a control that is tested side-by-side with the new, replacement batch of vitamins. If there were a problem in the newly purchased replacement vitamin mixture, there would be a high likelihood that the problem could be detected with enough time left for replacement with a satisfactory vitamin mixture."

"Ingredient cycling requires development of an organized schedule of ordering and testing each material in the diet or at least the components that are most likely to present problems. The ingredients that are most likely to cause problems are those that are perishable and those that are processed by rather elaborate procedures. This includes such substances as the gelling agents (e.g. agar and carrageenan), vitamins (especially vitamin C), flours or meals (e.g. wheat germ or soy flour) and antimicrobial substances."

If feasible, the long-term storage of perishable diet ingredients should be avoided; it is better to have a well-organized system of ordering materials that arrive a little in advance of the time needed. When any material is received, the date of receipt and the lot number must be written on the containers.

In general, storage at a low temperature, e.g. 2–4°C, lowers the growth rates of microbes and the rates of degrading chemical reactions. Most ingredients should be stored in a cold, dry, dark place that is low in oxygen. Ascorbic and linoleic acids must be stored in dark bottles (Ashby et al. 1985). Brewer and Lindig (1984) suggest, for prolonged storage, a cool dry place (e.g. 15.6°C and 40% RH) should provide maximum shelf life for the ingredients. Refrigeration and dry conditions extend the shelf life of vitamins. Moisture-proof containers are recommended for wheat germ and also many other ingredients such as casein, sucrose, agar, mould inhibitors, minerals, etc.

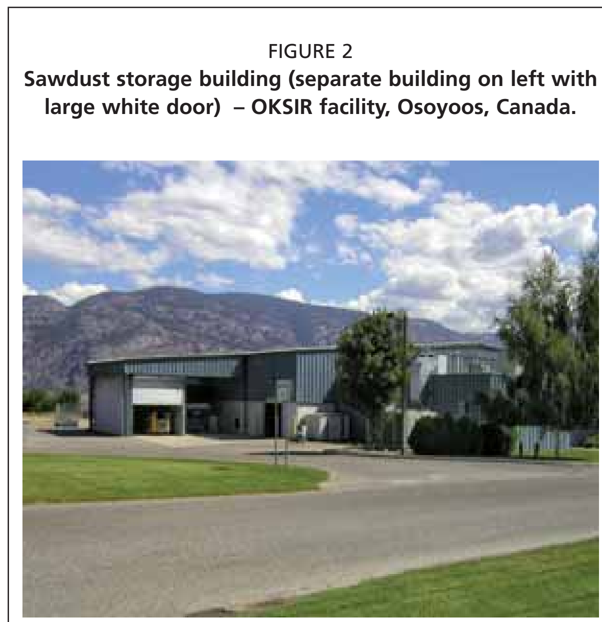
It is prudent to heed the manufacturer's storage instructions. Finished diets are more susceptible to degradation that diet ingredients.

Frozen storage may be appropriate for some ingredients that contain little or no water. It is important in both frozen and cold storage that the refrigeration equipment functions properly and a maintenance system is in place to detect malfunctions. Guennelon et al. (1981) used both freezing (–18°C) and cold (4°C) conditions for storing diet ingredients. Wheat germ quality degrades rapidly through hydrolytic action of enzymes and thus should be stored in a frozen condition (Moore et al. 1985).

Sawdust and paper pulp must be stored in a dry place, usually in a special building constructed for this purpose (**Figure 2**).

FIGURE 2

Sawdust storage building (separate building on left with large white door) – OKSIR facility, Osoyoos, Canada.



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4.9. DIET YIELD

Artificial diets should produce an average yield of 75% adults from the initial viable eggs (Singh 1985). The yield of codling moths from the various diets is highly variable (**Tables 3** and **4**) (section 18.1.5). However, a yield of 200 pupae or adults per kilogram or litre of diet would be a good objective for a mass-rearing programme. Note the high yield at the OKSIR rearing facility, more than 400 adults per litre of diet.

Pristavko et al. (1978) found that, when larvae were fed individually on artificial diet, only 2–4 g of diet were needed to rear one insect. However, when larvae were fed in groups, the yield was lower — 8–12 g of diet were needed for each insect. They also found that the optimum rearing density was 40–80 larvae per 100 ml of diet.

When considering yield, it is important to consider the type of diet used (some diets have more bulking agent and non-nutritive ingredients than others), the quality of insects produced and the cost of production (Gast 1968). It is more important to reduce the cost per insect than increase percentage yield from egg to adult (Gast 1968).

4.10. COST OF DIET

Quality insects should be produced at the lowest possible cost per insect (Knipling 1966; Gast 1968) by using cheaper diet ingredients or using a different type of diet. However, there is not an infinite amount of time and resources to keep searching for cheaper diet ingredients or seeking to reduce the complexity of the diet. At some point in time a decision has to be made to use a particular diet, and then look for cheaper alternatives as a side issue later. Tables 3 and 4 give some approximate figures for the cost of ingredients in diets.

Early in the Canadian research work, the British Columbia Fruit Growers' Association (BCFGA 1972) reported that the diet to produce 1000 adults cost USD 1.41. Later, using a modified version of the Brinton et al. (1969) diet, Bloem et al. (1997) noted that the diet cost for rearing 1000 adults was USD 1.80. Fugger (2006) cites the cost of diet at USD 1.29/1000 moths.

4.11. COST OF PRODUCTION

There is a great variation among laboratories in the cost of production, and this is related to the number of insects reared and the different uses to which they are put (Butt 1975) (**Tables 3** and **4**). Other major factors are the type and constituents of the diet used, and the local operational cost of rearing. Mechanization and automation can, in the long run, reduce production costs (Gast 1968; Proverbs 1974, 1982; Goodenough 1984; Harrell and Gantt 1984; Smith 1999; Wood and Wendel 1999; Parker 2005).

Mumford and Knight (1996) used a production cost of USD 2.18/1000 adults for a proposed mass-rearing programme for the codling moth in Syria. Early in the Canadian research work, BCFGA (1972) reported that the cost of diet and labour to produce 1000 adults was USD 3.43.

The cost of production in 2004 in the OKSIR Program in Canada was USD 2.94 per 1000 adults (**Table 4**). Due to gradual refinements in process control between 1994 and 2004 (Bloem and Bloem 2000), the cost of producing 1000 adults was reduced from USD 5.62 to 2.94 (Bloem et al. 2007). Fugger (2006) cites a value of USD 3.67/1000 adults. Hendrichs et al. (2005) listed the cost at USD 1.9 for 1000 male adults.

4.12. MARKING USING A DYE IN THE DIET

Mark/release/recapture experiments with codling moth adults require a method of marking the moths that does not affect their behaviour, especially flight and orientation to a sex pheromone source. Methods of marking insects have been reviewed by Hagler and Jackson (2001), Hagler and Miller (2002), Parker (2005) and Hood-Nowotny and Knols (2007).

When a fat-soluble dye, e.g. Calco Red, is added to the diet, the colour accumulates in the fat body of the larvae and is retained in the adults (Hagler and Jackson 2001). When the abdomen of a marked adult is squashed, the colour is easily visible; sometimes it is even visible through the abdominal integument. Calco Red is not harmful to the marked insects (Proverbs 1982, Keil et al. 2001) and requires less labour and is easier than for externally applied fluorescent dyes (section 11.3).

Calco Red was used to mark the boll weevil (Gast and Landin 1966), the tobacco budworm *Heliothis virescens* (F.) (Hendricks and Graham 1970), the pink bollworm *Pectinophora gossypiella* (Saunders) (Graham and Mangum 1971; Stewart 1984; Henneberry 1994; Tabashnik et al. 1999) and the codling moth (Mani et al. 1978; Proverbs 1982; Proverbs et al. 1982; Bloem et al. 2001). A . Barrington (pers. comm.) and Mediouni and Dhouibi (2007) used Calco Red to mark the painted apple moth *Teia anartoides* Walker and the carob moth *Ectomyelois ceratoniae* Zeller, respectively.

The procedure for mixing Calco Red into the diet is as follows:

a. Pulverize blocks of Calco Red dye in a mortar and pestle to make a fine powder.
b. Heat vegetable oil, e.g. soybean oil, to 130°C and add the powdered dye; stir until the dye has dissolved in the oil; allow mixture to cool.
c. Prepare the mixture at a ratio of 300 g dye to 20 L oil (Proverbs et al. 1982).
d. Add 2.2 mL of the Calco Red mixture/litre of diet during the cooking process. (Proverbs et al. (1982) used 4 mL of the mixture/L of diet.)

Calco Oil Red N-1700® dye was used by Graham and Mangum (1971) for the pink bollworm at a concentration of 0.015–0.010% dye (wt/v) in the diet. At this concentration no effects on the rate of development, adult longevity, fecundity or mating were found. Graham and Mangum (1971) and Henneberry (1994) found that marked females laid dyed eggs and contained dyed spermatophores after mating with marked males, but the results were inconsistent. In tobacco budworm adults, the spermatophores in unmarked females mated with marked

males were a distinct red (Hendricks and Graham 1970). Tests on codling moth were inconclusive (V.A . Dyck, unpublished data).

A. Barrington (pers. comm.) reported that Calco Red was used at a rate of 0.5 g/kg diet for the painted apple moth. The Calco Red powder was mixed with enough 95% ethyl alcohol to form a thick paste, and this paste (using a little water to rinse out the container) was added to the autoclaved diet ingredients as they were mixed and cooled. The diet changes from a dull purple colour to a brilliant red. The red dye in an adult male was easy to recognize when its abdomen was squashed, and dyed females laid pink eggs.

A sensitive 'dye test' was developed in the USA to help determine if a field-trapped pink bollworm adult was marked with the dye (Unpublished, California Department of Food and Agriculture, Pink Bollworm Control Program; D. Keaveny and G.S. Simmons, pers. comm.):

- Remove a moth from a trap and place into a vial containing 0.5 mL xylene. Swish the moth around to remove any adhering sticky material from the trap, empty the vial's contents onto absorbent paper to remove excess xylene and allow the moth to dry.
- Place a small amount (0.5 mL) of acetone into another vial and transfer the dried moth. Crush the moth with a clean glass rod so that any dye in the moth will be dissolved in the acetone. Insert a cut-out paper point (Whatman #4 filter paper), about 4.5 cm long, into the vial (with tip at the top) and allow the acetone to evaporate by flowing up the paper point.
- Any dye in the moth will be carried up the paper and deposited at the tip (which serves to concentrate it). A dye-marked moth will yield a point showing a red or pink colour. If the moth was not marked, the point will be yellow.

5. Nutritional Aspects of Diets

Much of the information in this section is from Chapter 3 of Cohen (2004). Other publications to consult are House (1961), Dadd (1973), Vanderzant (1974), Singh (1977) and Moore (1985).

An essential nutrient is a substance that an insect requires for life but can obtain only from its diet and does not have the metabolic ability to produce. Valine, an amino acid, is an example of an essential nutrient, and it must be obtained from the diet for protein synthesis (an exception may be the case of insects that have symbionts living within them). Glutamic acid, another amino acid, is a non-essential nutrient, and can be synthesized by an insect using a carbon source such as a sugar or lipid.

True nutrients in diets provide nutrition, i.e. serve as energy sources, building blocks for synthesis, or co-factors for enzymatic pathways. They are the raw materials of the metabolic pathways, the structural components, or the minerals that function in insect physiology. However, other ingredients serve other functions: feeding stimulants, token stimulants, stabilizers, preservatives and bulking agents, etc.

The criteria used to determine diet adequacy are usually body weight, body size, survival rate, adult longevity and reproduction; duration of development is not necessarily a good indicator of diet adequacy (Rock et al. 1964). Percentage yield of adults from eggs or neonate larvae is another criterion (Navon 1968), but yield must be considered in the context of the cost of rearing (Gast 1968).

5.1. PROTEINS

Proteins are a vital part of an insect diet, providing nitrogen, and insects use whole proteins (polypeptides) that are then broken down into their amino acid components. However, they require 8–10 essential amino acids (methionine, threonine, tryptophan, valine, isoleucine, leucine, lysine, phenylalanine, arginine and histidine) in the diet. An easily measured diet constituent, lysine, is generally used as an indicator of protein quality (Singh 1984). Other amino acids are not essential because they can be synthesized, e.g. serine, asparagine, aspartic acid, glutamine, glutamic acid, alanine, cysteine, glycine, tyrosine, proline. Cysteine was also used as a stabilizer to prevent excessive loss of ascorbic acid due to heating (Beck and Chippendale 1968) (section 5.4).

Rock and King (1967), by carcass analysis, estimated the growth requirements for amino acids in the codling moth.

Animal proteins, e.g. egg yolk vitellin, milk proteins (caseins), contain all the essential amino acids in high quantities. Casein is very rich in amino acids (Moore 1985); Moore lists the amino acid content of several protein sources.

When hydrolyzed food substitutes are used, e.g. soy or yeast hydrolyzate, the insects must use an unnatural form of its nitrogen source (in which there are many free amino acids). Free amino acids may not be as palatable as the protein form of the nitrogen component.

In hydrolyzed foods, proteins and polysaccharides that may be toxic or in some other way disagreeable to the insect are destroyed by the hydrolysis process. Many toxins are destroyed by processing, e.g. heating, the diet ingredients. Raw soy flour, wheat germ and meals made from legumes contain a large number of lectins and digestive enzyme inhibitors that are made edible by heating.

Common sources of protein are casein, wheat germ and soy flour (Brewer and Lindig 1984). Wheat germ provides an excellent source of nutrition: high protein and lipid contents, abundant trace minerals and fairly high vitamin content (except for vitamins A and C), and it has been used in many insect diets (Singh 1984). In the simplified diet of Sender (1969), the inclusion of wheat germ and maize semolina eliminated the need for added vitamins and minerals. Soybean meal has a very similar profile to wheat germ, except that it has a higher protein and lipid content and lower carbohydrate content. Navon (1968) used full fat soy meal because it contained high-quality proteins, phytosterols, saponifiable fats and phospholipids, minerals, carbohydrates and part of the vitamin-B complex. However, the diet was autoclaved to destroy the antitrypsin factor of the soy.

5.2. CARBOHYDRATES

Insects use carbohydrates as building materials and as fuels (energy), and the insect cuticle (chitin) is made of amino sugars. Common sources of carbohydrate are sucrose, wheat germ, starch and cereal grains (Brewer and Lindig 1984). Some carbohydrate sources, e.g. cellulose, cannot be digested, but they may be used as bulking agents.

5.3. LIPIDS

Lipids include sterols, oils, fats and phospholipids, and their importance in insect nutrition has been underestimated. All insects require a source of dietary sterols as essential nutrients. Lipids function as building-blocks of cell membranes, hormones, nutrient transporters, sources of energy and as structural material. Cholesterol is a precursor of ecdysone, the moulting hormone (Singh 1984). Lipids are insoluble and immiscible in water. Since sterols dissolve with difficulty they are often not provided correctly.

Common sources of lipids are wheat germ, lecithin, cholesterol and other sterols, grain oils and glycerol, but some diets use purified essential fatty acids such as linoleic and linolenic acids (Brewer and Lindig 1984); deficiencies of these fatty acids cause deformed wings in adult Lepidoptera (Rock 1967, Singh 1984). Linseed oil is a source of linolenic acid (Chippendale and Beck 1968). The peroxide value of oil is an excellent method of determining its rancidity (Brewer and Lindig 1984).

TABLE 5
Vitamins in Artificial Diets (Part 1)

Vitamin[1]	Publication										
	Redfern 1964[2]	Hamilton & Hathaway 1966 (Nr 2)	Rock 1967 (mg/100 g)		Information taken from Butt (1975) and Hatmosoewarno and Butt (1975) (g/kg)					Edelman 1970 (see Singh 1977)	Shumakov et al. 1974 (see Singh 1977)
			Casein diet	Wheat germ diet	Bulytginskaya (Russia)	Falcon (USA)	Anderson (Australia)	Howell (USA)	Pristavko and Yanishevskaya 1972 (Nr 16)		
Vitamin mixture						9.7	9.8	9.7			
Distilled H_2O (mL)	447.4	427.4									
Choline chloride (g)	50	25	100	100	0.005				1	0.04	0.05
Nicotinic acid (g)			12	12	0.01				0.12	0.025	0.01
Niacinamide (nicatinamide) (g)	0.5	0.5									
Calcium pantothenate (g)	0.5	0.5	4	4	0.012				0.004	0.012	0.012
Pyridoxine hydrochloride (g)	0.125	0.125	6	6	0.0018				0.006		
Biotin (g)	0.01		0.025	0.025	0.0005					0.00005	0.00005
Citric acid					9						
Folic acid (g)	0.125	0.125	2	2	0.001				0.02	0.001	0.001
Riboflavin (g)	0.25	0.25	2	2					0.012		
α-tocopherol			15	15					0.15		
Cyanoco balamin									0.00005		
Thiamine									0.012		
Thiamine hydrochloride (g)	0.125	0.125	1.2	1.2							
B_1 (g)										0.0012	
B_2 (g)										0.0018	
B_6 (g)										0.0018	0.0018
B_{12} (g)			0.004	0.004						0.0004	
B_{12} (in mannitol) (g)	1	1									
Inositol (g)			10	10						0.008	
Ascorbic acid			800	800							

TABLE 5 (continued)
Vitamins in Artificial Diets (Part 1)

Vitamin[1]	Publication						
	Hatmosoewarno and Butt 1975 (CW) (Vanderzant 1966)	Hatmosoewarno and Butt 1975 (MB) (Burton 1969)	Howell 1970	Howell 1971[3]	Howell and Clift 1972	L. Neven (USA) (pers. comm.)	Singh 1977
Vitamin mixture (g)	12.8	5	36[4]	45 mL[5]	1800 mL[6]	33	
Howell (1971) Vitamin Mixture:							
Distilled H_2O (mL)				Adjusted to 12 000 with H_2O			
Choline chloride[7] (70%) (mL)				750			
Nicotinic acid (g)				12			
Calcium pantothenate (g)				12			
Pyridoxine hydrochloride (g)				3			
Biotin (g)				0.24			
Folic acid (g)				3			
Riboflavin (g)				6			
α-tocopherol (g)				96			
Thiamine hydrochloride (g)				3			
B_{12} (0.1%) (mL)				24			
Inositol (g)				240			
Tween 80®[8] (g)				200			
Vanderzant's Fortification Mixture (Composition/1000 g)							
α-tocopherol (g)							8
Ascorbic acid (g)							270
Biotin (mg)							20
Calcium pantothenate (g)							1
Choline chloride (g)							50
Folic acid (crystalline) (mg)							250
Inositol (g)							20
Niacinamide (g)							1
Pyridoxine hydrochloride (mg)							250
Riboflavin (mg)							500
Thiamine hydrochloride (mg)							250
B_{12} (in mannitol) (g)							2

[1] Vitamins are listed in no particular order.

[2] Details described in Vanderzant (1957), Vanderzant and Davich (1958) and Redfern (1963).

[3] The pH of the vitamin mixture solution was adjusted to 6 with 0.1 N NaOH. The pH of the finished product should be 5.7–6.

TABLE 5 (continued)
Vitamins in Artificial Diets (Part 1)

[4] Vitamin mixture purchased from Nutritional Biochemical Company (includes choline chloride and vitamins A and D).

[5] 45 mL taken from the vitamin solution (12 000 mL) prepared using the vitamins listed.

[6] Vitamin solution used according to description in Howell (1970).

[7] Product is 60.69% wt/v.

[8] Tween 80® (polyoxyethylene sorbitan mono-oleate) is an emulsifier.

5.4. VITAMINS

There is little specific knowledge about the functions of vitamins in insects and the effects of vitamin deficiency, and thus there are no clear recommended minimum daily requirements. However, most authors have suggested the addition of certain vitamins (**Tables 5** and **6**). A popular mixture of vitamins is called Vanderzant's Vitamin Mixture (details in Table 5, Singh (1977) and Cohen (2004)). Some diets do not specifically add vitamins because other diet ingredients, especially the semi-synthetic ones, provide the needed vitamins.

Vitamins are divided into two groups, water-soluble and lipid-soluble. The water-soluble group includes B vitamins, vitamin C (ascorbic acid) and some other compounds such as choline. The B vitamins function as co-factors in many metabolic pathways, e.g. energy utilization (thiamine, riboflavin, niacin), or as growth factors (biotin, folic acid).

Ascorbic acid is essential for many phytophagous insects, serving as a phagostimulant, an antioxidant and in other ways, including cuticle sclerotization. Ascorbic acid is very susceptible to degradation, especially when in solution, or exposed to heat, light, oxygen or free radicals (hence the late addition during diet preparation (section 6)). During diet preparation, there is about a 46% loss of ascorbic acid (Brinton et al. 1969; Proverbs 1982). Ascorbic acid is commonly present in its L-ascorbic acid form in many fresh fruits and green tissues of plants. Thus, if grains are used as main diet components, they must usually be supplemented with ascorbic acid (Vanderzant et al. 1962; Redfern 1964; Chippendale and Beck 1968; Navon and Moore 1971). The minimum dietary requirement for ascorbic acid was between 0.4 and 0.8 g/100 g diet (Rock 1967), and it has a pronounced effect on growth and development in the codling moth.

Thiamine (vitamin B_1) is a co-factor in biochemical pathways of energy transduction from the chemical bonds of carbohydrates and lipids to those of high-energy phosphates, especially ATP.

Riboflavin (vitamin B_2), probably essential to most insects, functions as a co-factor for the flavoproteins and is crucial in the energy metabolism pathways involved in ATP production.

Niacin (and its derivative nicotinamide) is involved in energy transduction pathways.

Pyridoxine and its phosphate derivatives (vitamin B_6) are involved in several pathways of amino acid metabolism (not all insects require this vitamin).

TABLE 6
Vitamins in Artificial Diets (Part 2)

Vitamin[1]	Publication								
				Information from Butt (1975) (g/kg)					
	Brinton et al. 1969 (%)	Brinton et al. (1969) diet tested by Hathaway et al. (1971)	Brinton et al. (1969) diet used by Dyck in 1993	Brinton et al. 1969	Mani/Charmillot (Switzerland)	Wearing (New Zealand)	Mani et al. 1978 (Nr 1) (mg/kg)	Ashby et al. 1985 (to prepare 1 L of stock vitamin mixture)	M. Mansour (Syria) (pers. comm.) and Mohammad et al. 1997
Vitamin mixture (g)		45[2]						144 mL	22.2
Triturated ingredient mixture	0.61		3.7 g						
Distilled H_2O (mL)								200	
Ethyl alcohol (95%) (mL)								700	
Choline chloride[7] (70%) (mL)								100	
Nicotinic acid (g)							10	1	
Niacinamide				0.01	0.01	0.01			
Calcium pantothenate (g)				0.01	0.01	0.01	10	1	
Pyridoxine hydrochloride (g)				0.0025	0.0025	0.0025		0.25	
Pyridoxine							2.5		
Biotin (2%) (g)				0.001	0.01	0.01	10		
d-biotin (g)								0.02	
Folic acid (g)				0.0025	0.0025	0.0025	2.5	0.25	
Riboflavin (g)				0.005	0.005	0.005	5	0.5	
α-tocopherol (g)					0.15				
Thiamine hydrochloride (g)				0.0025	0.0025	0.0025		0.25	
Thiamine							2.5		

B_{12} (0.1%) (mL)			0.002	0.02	0.002	20	
Ascorbic acid						5500	
Sorbic acid						900	
Antibiotic (aureomycin)						90	
Cyanocobalamin (g)							0.2
Brinton et al. (1969) triturated ingredients							
Niacinamide (g)	5						
Calcium pantothenate (g)	5						
Riboflavin (g)	2.5						
Thiamine HCl (g)	1.25						
Pyridoxin (g)	1.25						
Folic acid (g)	1.25						
B_{12} (0.1% in mannitol) (g)	1						
Biotin (g)	0.1						
Aureomycin (5.5%) (g)	810						
Ascorbic acid (g)	1804						
Sorbic acid (g)	449						
Brinton et al. (1969) triturated ingredients used by Dyck *PREMIX*							
Niacinamide (g)		0.289					
Calcium pantothenate (g)		0.289					
Riboflavin (g)		0.144					
Thiamine HCl (g)		0.072					
Pyridoxine HCl (g)		0.072					
Folic acid (g)		0.072					
Mannitol (g)		0.058					

TABLE 6 (continued)

Vitamins in Artificial Diets (Part 2)

Vitamin[1]	Publication								
				Information from Butt (1975) (g/kg)					
	Brinton et al. 1969 (%)	Brinton et al. (1969) diet tested by Hathaway et al. (1971)	Brinton et al. (1969) diet used by Dyck in 1993	Brinton et al. 1969	Mani/Charmillot (Switzerland)	Wearing (New Zealand)	Mani et al. 1978 (Nr 1) (mg/kg)	Ashby et al. 1985 (to prepare 1 L of stock vitamin mixture)	M. Mansour (Syria) (pers. comm.) and Mohammad et al. 1997
B_{12} (mg)			0.058						
d-biotin (g)			0.006						
Ascorbic acid[3] (g)			1.378						
FINAL MIX									
Premix (see above) (g)			2.38						
Chlorachel-50[4] [aureomycin] (g)			19.34						
Sorbic acid (g)			22.62						
Ascorbic acid (g)			89.66						

[1] Vitamins are listed in no particular order.

[2] Commercially available vitamin mixture from Hoffman-LaRoche, Inc.

[3] Ascorbic acid used in premix as a bulking agent.

[4] There is 50% aureomycin in an animal feed additive called Chlorachel-50.

Inositol, part of the vitamin B complex, has been shown to be essential for the boll weevil and at least a beneficial nutrient in several species of insects. However, diets with soybean protein or yeast extract or brewer's yeast do not require inositol (Vanderzant 1959).

Pantothenic acid is essential to all insects (except if microbial symbionts supplement this vitamin). It is a co-factor of coenzyme A .

Biotin and folic acid are carriers for one-carbon groups in intermediate metabolism pathways. Biotin is found in many foods, and deficiencies are rare. Biotin deficiency slows larval growth and decreases the fertility of adults. Folic acid is also an essential factor in nucleic acid synthesis and functions as a pigment precursor.

Choline, carnitine, cyanocobalamin (vitamin B_{12}) and lipoic acid are not universally required by insects, but may improve growth or fertility. Choline is involved in the production of cell membranes and carnitine is also involved in lipid metabolism.

The second group of vitamins, lipid-soluble, includes the vitamin A complex (β-carotenes and other carotenoids) which are essential for vision (Singh 1984) and for normal growth. Also the carotenoids are potent antioxidants. Vitamin E (α-tocopherol) is a fertility/fecundity factor and also an antioxidant. These lipid-soluble factors are very sensitive to oxidation by light, free radicals, excessive heat, or aging. They can become stale, rancid, or degraded from long storage, lack of refrigeration, exposure to light or pro-oxidants, or microbial contamination.

Vanderzant (1957), Vanderzant and Davich (1958), Howell (1971) and Ashby et al. (1985) describe the preparation of vitamin mixtures. The procedures are not simple and these papers should be consulted. Some chemicals must first be dissolved in an appropriate solvent, the solvent evaporated and then mixed with other chemicals in a particular sequence. Some vitamins are heat stable and others are heat labile (Howell 1971). An emulsifier, e.g. Tween 80, may be needed. The mixture must be stored in a way that permits it to have a reasonably long shelf-life.

5.5. MINERALS

There is also a lack of specific knowledge about the functions of minerals in insects and the effects of mineral deficiency, and there are no clear recommended minimum daily requirements. However, most authors have suggested the addition of certain mineral salts at certain dosages (**Tables 7** and **8**). A popular mixture of mineral salts is called Wesson's Salt Mixture (details in Table 7, Singh (1977) and Cohen (2004)). This mixture was originally developed for vertebrate nutritional research and so may not necessarily be optimal for insects (Chippendale and Beck 1968). In fact, some diets do not have specific minerals because other diet ingredients, especially the semi-synthetic ones such as wheat germ, provide some minerals. The majority of ingredients contain some minerals, and therefore the overall mineral composition of a diet is not identical to the salt mixture added to the diet.

TABLE 7
Minerals in Artificial Diets (Part 1)

Mineral[1]	Publication									
			Rock 1967		Information taken from Butt (1975) and Hatmosoewarno and Butt (1975) (g/kg)					
	Redfern 1964	Hamilton and Hathaway 1966 (Nr 2)	Casein diet (mg/100 g)	Wheat germ diet (g/100 g)	Falcon (USA)	Anderson (Australia)	Howell (USA)	Pristavko and Yanishevskaya 1972 (Nr 16)	Edelman 1970 (see Singh 1977)	Huber et al. 1972
Wesson's salts (g)	2.5	12.5		1.2						
Mineral salt mixture (g)					9.7	9.8	7.7	12	0.84	
Rock's salt mixture:										
K_2HPO_4			160							
NaH_2PO_4			80							
NaCl			20							
$MgSO_4 \cdot 7H_2O$			100							
$FeSO_4 \cdot 7H_2O$			40							
$CuSO_4 \cdot 5H_2O$			2							
$MnSO_4 \cdot H_2O$			5							
$ZnCl_2$			5							
KI			1							
$CaCO_3$			60							
Huber's salt mixture:										
KH_2PO_4 (g)										11
$NaHCO_3$ (g)										9
$MgSO_4 \cdot 7H_2O$ (g)										3.7
$Ca_3(PO_4)_2$ (g)										12

TABLE 7 (continued)
Minerals in Artificial Diets (Part 1)

Mineral[1]	Publication					
	Hatmosoewarno and Butt 1975 (CW) (Vanderzant 1966)	Howell 1970	Howell 1971	Howell and Clift 1972	L. Neven (USA) (pers. comm.)	Singh 1977 (%)
Wesson's salts (g)	12.8	36	36	1440		
Mineral salt mixture (g)					5.4	
Wesson's salt mixture						
Calcium carbonate						21
Copper sulphate ($5H_2O$)						0.039
Ferric phosphate						1.47
Manganous sulphate (anhydrous)						0.02
Magnesium sulphate (anhydrous)						9
Potassium aluminium sulphate						0.009
Potassium chloride						12
Potassium dihydrogen phosphate						31
Potassium iodide						0.005
Sodium chloride						10.5
Sodium fluoride						0.057
Tricalcium phosphate						14.9

[1] Minerals are listed in no particular order.

Each mineral salt contains a cation (positively charged) and an anion (negatively charged). Also, some salts are hydrated, e.g. copper sulphate, $CuSO_4 \cdot 5H_2O$ (meaning that it is hydrated with five water molecules). The hydration state is considered when calculating the amount of a given mineral such as copper in a given weight of a hydrated salt. The hydration state influences solubility of the salt.

Some salts have three kinds of ions, e.g. potassium dihydrogen phosphate. Compounds may exist in three forms — monobasic, dibasic or tribasic, each with different characteristics, and it is important to know which one is being used.

All animals require minerals in their diets, including phosphorus, chloride, calcium, potassium, sodium, manganese, magnesium, iron, copper and zinc (Singh 1984). Minerals cannot be biosynthesized; if an insect requires a mineral, it must be present in the diet in adequate amounts and appropriate form.

Potassium is involved in numerous chemical reactions and is a component in the structure of many substances, e.g. phospholipids, nucleic acids. Phosphate is

TABLE 8
Minerals in Artificial Diets (Part 2)

Mineral[1]	Publication										
					Information from Butt (1975) (g/kg)						
	Brinton et al. 1969 (%)	Brinton et al. (1969) diet tested by Hathaway et al. (1971)	Brinton et al. (1969) diet used by Dyck in 1993	Howell 1972c	Anderson (Australia)	Brinton et al. 1969	Mani/Charmillot (Switzerland)	Wearing (New Zealand)	Mani et al. 1978 (Nr 1) (g/kg)	Ashby et al. 1985	M. Mansour (Syria) (pers. comm.) and Mohammad et al. 1997
Wesson's salts (g)	0.62	25		10	7.6	6.2	6.8	7.6	6.8	100	21
Mineral salt mixture (g)			3.5								
Brinton et al. (1969) salt mixture used by Dyck											
Tricalcium phosphate (g)			55								
Monopotassium phosphate (g)			47.6								
Potassium chloride (g)			21.2								
Ferric ammonium sulphate (g)			3.4								

[1] Minerals are listed in no particular order.

absolutely essential to bioenergetic activity. Appropriate ratios of potassium to sodium, or magnesium to sodium, stimulate insect feeding responses.

Chloride is universally required by all organisms, being involved in the maintenance of membrane potential and as a factor in several enzymatic reactions. Potassium and sodium are essential components in actions of excitable tissues and involved with regulation of pH. All three minerals are involved in water regulation. Calcium is involved with muscle activity. Magnesium, manganese, zinc and copper are involved in enzyme processes.

Many essential metabolic activities are dependent on iron (Cohen 2004), e.g. enzyme reactions, antioxidant activities, production of an ecdysis hormone, cuticle formation, nitrogenous waste product synthesis and the cytochrome system.

Fluoride and iodide have not been shown to be important in insect nutrition, but they are present in Wesson's Salt Mixture.

5.6. OTHERS

Many nutrients are also phagostimulants, e.g. sugars, some amino acids, lipids, ascorbic acid, and potassium and magnesium compounds stimulate biting, chewing and swallowing. However, some substances serve only as biting incitants and feeding stimulants, i.e. token stimuli, e.g. sinigrin, some waxes, wheat germ oil, several plant secondary compounds (Beck and Chippendale 1968). Moore (1985) provided a long list of compounds that show phagostimulatory activity in insects. Landolt et al. (1999) found several plant essential oils that acted as an arrestant for neonate larvae of the codling moth. Also, neonate larvae are attracted and orient to the odours of apples, particularly to α-farnesene and especially if the apples are already infested with larvae (Sutherland 1972; Landolt et al. 1998, 2000; Bradley and Suckling 1995).

Preservatives are added to prevent microbial contamination or oxidation:

- Antibacterial agents, e.g. antibiotics and antiprotozoan agents.
- Antifungal agents, e.g. sorbic acid, methyl *p*-hydroxybenzoate (methyl paraben), propionic acid (propanoic acid), formaldehyde.
- Antioxidants, e.g. ascorbic acid, tocopherols (α-tocopherol) (Vanderzant 1957), carotenes, butylated hydroxytoluene (BHT).

Many of these substances are very unstable if overheated, maintained in solution too long, or exposed to light or pro-oxidants. Certain kinds of antioxidants are useful, maybe even essential, to many insects.

It is important to determine the pH of a diet since pH influences palatability and stability, the activity of preservatives and the solubility of nutrients. Most antifungal agents work only in acidic pH, and even without antibiotics bacterial growth is suppressed at a lower pH (Navon 1968). Diets for the codling moth are acidic (as are apples). The pH is lowered by adding acids, e.g. hydrochloric, acetic or phosphoric. Sorbic and propionic acids are usually used as antifungal agents, but they also lower the pH of diets. Some acids are commonly used in human foods, e.g. citric acid, benzoic acid. Raising pH is achieved by adding bases, e.g. sodium hydroxide, potassium hydroxide, sodium carbonate, sodium bicarbonate.

Some diet ingredients act as buffers to stabilize pH, e.g. the phosphates and sulphates of sodium, potassium, magnesium and calcium.

Water is a key component of an insect diet. Using distilled water removes risks of introducing micro-organisms or chemicals. The water concentration of the diet is critical – too much may encourage microbial growth or drown the insects and too little may render the diet unsuitable (Moore 1985).

Emulsifying agents are stabilizers and cause lipid phase and aqueous phase materials to mix. Natural agents include nutrients that also are emulsifiers, e.g. milk proteins, soy proteins, phospholipids. Artificial agents include polyoxyethylene sorbitan mono-oleate, e.g. Tween 80 (Vanderzant 1957).

Diet texture is modified by using gelling agents, e.g. agar, and non-nutritive fillers, e.g. cellulose. Carboxy methyl cellulose prevents the particulate components from settling to the bottom of the preparation container before the diet gels (Howell and Clift 1972). Some nutritionally inert components are added as carriers of other substances or as bulking agents.

Gelling agents are expensive but they improve diets by:

- Making a high water-content mixture into a gel so that the diet will not collapse on tunnelling insects.
- Preserving the mixed state of the diet components.
- Preserving the non-equilibrium conditions that help prevent reactions between ingredients.
- Acting as nutrients, e.g. proteins, pectins, starches.

Gelling is caused by hydration of the gelling agent; liquid water becomes bound to the agent, restricting its movement. Common carbohydrate gelling agents are agar (also an adhesive), starch, gelcarin, glutin, carrageenan (suspends diet components), carboxy methyl cellulose and pectin; a protein gelling agent is gelatine (Brewer and Lindig 1984, Moore 1985).

6. Diet Preparation

6.1. INTRODUCTION

The procedures for preparing codling moth diets are not uniform from diet to diet, partly because the diets have different ingredients and partly because each laboratory has developed its own unique procedures including the exact sequence of adding the ingredients. The detailed preparation procedures used for diets listed in Tables 3 and 4 can be found in the published papers. Even though there may be some flexibility in exactly how a diet is prepared, there are some common practices in preparation. This section summarizes these common procedural practices.

Singh (1977) reviewed the subject of insect diets — content and preparation, and summarized the preparation procedures of 15 diets for the codling moth.

According to Moore (1985):

- "Good laboratory and chemical techniques should be used including accurate weighing of ingredients and complete mixing, especially of the smaller quantities. These small quantities may be incorporated by trituration (grinding to a fine powder) with sucrose or an inert material such as cellulose, or by dissolving in an organic solvent such as alcohol or ether, applying to the solid components and evaporating the solvent.
- Most diets require heating to dissolve the agar, and the diet may be sterilized as a part of the process. Sterilization may cause the breakdown of some ingredients, but Vanderzant (1969) suggests that there may be some desirable effects also — heating stops enzyme action in plant products, ruptures cells and affects solubility of ingredients. Heating should be at the lowest practical temperature and the shortest time to minimize any detrimental changes in the diet. Flash sterilization (Sikorowski and Goodwin 1985) heats the diet to an elevated temperature for a short time, and produces a better quality diet than the conventional autoclave (Sikorowski and Lawrence 1994a).
- Care should be taken to insure that condensation of moisture from the diet does not occur and drown the young larvae or induce contamination in the diet."

Some common practices in diet preparation:

- Follow aseptic procedures in the laboratory. The diet preparation room should be scrupulously clean (Reed and Tromley 1985). Working areas and equipment must be cleaned regularly with a disinfectant, e.g. NaOCl, or anti-microbial agent. Glassware should be washed with hot soapy water, rinsed with distilled water and covered until used (section 22.7).
- Some ingredients require overnight soaking prior to preparing the diet, e.g. whole beans.

- Diet trays must be washed with strong detergent (e.g. trisodium phosphate) and water, sterilized with steam and dipped in a NaOCl (bleach) solution.
- Ingredients should be weighed and measured ahead of the actual diet preparation (**Figure 3**).
- Some ingredients are needed in very small quantities and therefore these are often mixed with other larger-volume ingredients before diet preparation. This premix permits a thorough distribution of each ingredient, ensuring a good mixing of the ingredients with other materials in the final diet. A premix can be stored and used for several batches of diet over time.

FIGURE 3
Augers, hopper and balance to deliver high-volume diet ingredients from storage room to cooking room and kettles below. (OKSIR facility, Osoyoos, Canada)

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The same principle applies to liquid ingredients that can be diluted and bulked in water (e.g. ascorbic acid) or other solvent.

- Some ingredients should be dissolved in water before being added to the diet.
- Cholesterol and lipids should be dissolved in acetone, hot ethyl alcohol or methylene chloride (dichloromethane). In a fume hood allow the solvent to evaporate; then add the fatty ingredients to the diet (Moore 1985).
- Carboxy methyl cellulose (CMC), a thickening agent, must be wetted with alcohol or other wetting agent to prevent lumping (Howell 1972a, Howell and Clift 1972).
- Tween 80 may be added to act as an emulsifier for fat-soluble ingredients.

FIGURE 4
Steam-jacketed kettles for cooking the diet, each with two counter-rotating paddles to mix and stir the diet

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Note blue ducts from ceiling to each kettle which automatically deliver via augers weighed amounts of high-volume ingredients. (OKSIR facility, Osoyoos, Canada)

FIGURE 5
Diet is dispensed on to trays and then smoothed with a wide spatula. (OKSIR facility, Osoyoos, Canada)

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- Acetone or ethyl alcohol may be used as solvents for mould inhibitors, e.g. methyl *p*-hydroxybenzoate, potassium sorbate (Moore 1985).
- Mix ingredients thoroughly in a blender, or for large volumes in a steam-jacketed kettle that has mixing paddles (**Figure 4**). Heat according to the type of diet being prepared. In some laboratories the diet is autoclaved (L. Neven, pers. comm.). Heating sterilizes the diet, and some ingredients may need to be cooked to make them edible.
- Some ingredients can be sterilized without heating, e.g. liquids passed through a membrane filter, e.g. Millipore (Rock 1967).
- Adjust the pH of the diet by adding an acid or a base.
- Add ascorbic acid and other ingredients that are heat sensitive after the diet has cooled to 60°C.
- During diet preparation, workers must wear face masks to protect them from hazardous dusts and fumes, and rubber gloves to protect them from corrosive liquids. Diet handling and preparation rooms must be well-ventilated to remove hazardous fumes and dusts (section 21).

FIGURE 6
Two parallel diet-dispensing lines with belts showing enclosed aluminium portions (with blue units) that include paraffin wax sprayers and steam scarifiers. (OKSIR facility, Osoyoos, Canada)

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- Mechanizing and automating procedures (Harrell and Gantt 1984) can reduce labour costs and also accelerate the processes, e.g. an auger and hopper connected to a balance so as to deliver a predetermined amount of an ingredient, water pump to deliver a predetermined amount of water, diet pump, automated tray-filling system and line, automated paraffin wax sprayer and scarifier (**Figures 5** and **6**).
- Hot paraffin wax can be sprayed onto the surface of freshly dispensed diet in a tray using a hot pressure system (Howell 1967; Mani et al. 1978), spread onto the diet with a brush (Ashby et al. 1985), or sprinkled onto the diet using a modified 'salt shaker'. The film of wax must be somewhat porous and be about 0.75 mm thick (Ashby et al. 1985; Howell 1967). The wax layer slows dehydration and discourages mould growth (Howell 1967). Immediately after applying wax, scarify or roughen the surface with hot steam jets (preferable) (**Figure 6**) or with a sterile fork (depth about 0.4 cm) (Brinton et al. 1969). A rough surface encourages neonate larvae to enter the diet (Beck and Chippendale 1968, Bathon et al. 1991).

- To avoid infection by microbes (section 15), Batiste and Olson (1973) encased newly prepared trays of diet in polyethylene bags which were blown up, each having an air inlet and exhaust. Regulated clean air with a constant RH was blown into the bag throughout the growth period of the larvae. In South Africa, brown paper bags are used to cover trays of diet to minimize contamination and keep the RH high (Addison and Henrico 2005; D. Stenekamp, pers. comm.).

Navon and Moore (1971) minimized desiccation by putting the diet into polyethylene bags and then punching holes in the bags to permit larval penetration. These authors also tested polyethylene film instead of the bags.

6.2. DIETS FOR LEPIDOPTERA

The preparation of the Ivaldi-Sender general-purpose diet is described in Table 1 and that of the Singh general-purpose diet in Table 2. Consult Ivaldi-Sender (1974), Singh (1983, 1985) and Bathon et al. (1991) for details.

6.3. DIETS THAT STAY MOIST AND SOFT

At an early stage of diet preparation, dissolve agar powder in hot water and boil until the agar solution thickens and, when removed from the container, forms a gel. When the agar solution cools to about 60ºC, add other diet ingredients.

Note items discussed in section 6.1.

6.4. DIETS THAT DRY OUT AND HARDEN

On the day before diet preparation, shred the sheets of paper pulp and soak in water overnight (this encourages the rapid disintegration of the pulp during diet preparation). Also sift and weigh (or measure) dry sawdust.

The exact amount of water added to the diet during preparation can be determined only through experience. Brinton et al. (1969) stated that "It is most important to get the correct consistency, and this can be achieved only through experience." Ashby et al. (1985) noted that "sterile water can be added if the finished diet is too dry".

Note items discussed in section 6.1.

6.5. EQUIPMENT FOR PREPARING DIETS

Standard laboratory equipment includes: glassware, Petri dishes, pipettes, holding and mixing containers, plastic bottles, mixing tools, fume hood, laminar-flow hood, balances (several kinds), magnetic-stirring hot plate, oven, microwave oven, thermometers, pH meter, timer, graduated cylinders, refrigerator, freezer and water distiller or water-filtration system. Equipment (Annex 1) that is somewhat unique to preparing insect diets includes: sawdust sifter, paper-pulp chopper (hammer mill), grinder, shaker/mixer (dry diet ingredients), blender/mixer, steam-jacketed kettle with counter-rotating paddles or autoclave or flash sterilizer, vat mixer, heated pressurized sprayer (paraffin wax), steam-jet system (scarify diet in tray), augers, holding/cooling tanks, diet dispensing system with tray-handling

equipment, trays, carts (hold trays), diet pumps, water pump, tray washer and cart washer.

Papers in the book edited by Singh and Moore (1985) included lists of relevant equipment. Chapter 12 in Cohen (2004) provided information on equipment used for processing insect diets. In Annex 2, Butt (1975) listed the mixers used to prepare various diets. Edwards et al. (1996) and Miller et al. (1996) described the extrusion technique used in processing the diet of the pink bollworm.

7. Disposing of Spent Diet and Liquid Wastes

7.1. ENVIRONMENTAL CONCERNS

Mass-rearing facilities (**Figure 7**) using an artificial diet produce a large quantity of solid and liquid waste, i.e. spent diet (**Figure 8**) and cleaning and washing liquids. When such a facility is constructed, arrangements must be made for the disposal of these wastes in an environmentally acceptable way. If this is not done properly, then environmental pollution will occur followed by community problems (IAEA 2008).

Liquid wastes must be treated before being released into nearby bodies of water. Solid wastes after treatment may be buried in a sanitary landfill, burned, or sold as fertilizer.

If Calco Red dye is present in the spent diet, the dye might remain non-degraded in a landfill for many years; this matter must be considered before such diet is buried.

7.2. TEMPERATURE TREATMENT

Treatment by heating is a common method of disposing of spent diet; heat destroys any living organisms in the diet. In the OKSIR facility, spent diet is placed into

FIGURE 7
OKSIR codling moth mass-rearing facility at Osoyoos, Canada,

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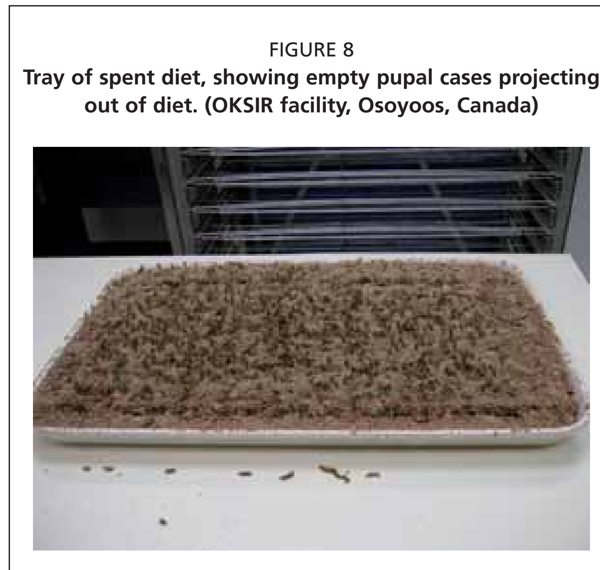
FIGURE 8
Tray of spent diet, showing empty pupal cases projecting out of diet. (OKSIR facility, Osoyoos, Canada)



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a special 'heat treatment room', held at 60°C for three days (Cossentine et al. 2005) (section 15.3) and then taken to the community landfill.

Toba and Howell (1991) suggested that paper trays and cardboard strips be held in a room at about 49°C for 12 h to kill any insects present before discarding them. They also suggested that spent diet be frozen at about –18°C for two days to kill any insects present; the same treatment is applied to used diet trays before cleaning and reuse.

Adults from oviposition cages are killed by freezing for several hours.

7.3. GRINDING

Grinding the spent diet to a powder destroys any living insects in the diet, and then this ground diet could be buried in a landfill. However, Cossentine et al. (2005) pointed out that grinding the spent diet creates dust in the immediate vicinity of the rearing facility. This dust could contain the CpGV (section 15.3), and it could be drawn into the facility through air-intake ducts (sections 22.6 and 22.7) (Gast 1968). This disposal method is no longer recommended.

7.4. LANDFILL

It is relatively easy and inexpensive to dispose of solid wastes in a sanitary landfill providing the environmental and legal issues have been addressed. As already noted (section 7.1), if Calco Red dye is present in the spent diet, the dye might remain non-degraded in a landfill for many years.

Moths that have been killed by freezing should be placed into degradable containers and buried in a landfill.

Workers handling spent diet and moths must wear face masks to protect themselves from dust and moth scales.

7.5. LIQUID WASTES

Liquid wastes must be treated before being released into nearby bodies of water. If a facility can not use the local sewer system and local treatment plant for the disposal of liquids, then an independent treatment operation must be built and operated by the facility (Wyss 2002). Liquid waste must not be dumped in an untreated form into a nearby body of water. Special arrangements may be needed to dispose of chemicals.

8. Seeding the Diet with Eggs or Larvae

The next sections on rearing provide information from Singh (1977), Reed and Tromley (1985) and Ashby et al. (1985), but also from personal experience in rearing the codling moth using the Brinton et al. (1969) diet, modified as per Proverbs et al. (1982), at the Pacific Agri-food Research Centre (PARC 2007) in Canada during 1982–1993.

8.1. EGGS

8.1.1. Eggs on Waxed Paper Sheets

Codling moth females lay circular, thin, flat eggs on any smooth surface. Waxed paper has been used to collect eggs since 1952 (Dickson et al. 1952) and it is certainly the most common oviposition substrate (**Figure 9**). Paper waxed on both sides is more sturdy and durable (especially after surface sterilization) than paper waxed on only one side. Other surfaces tested include plastic sheets (White and

FIGURE 9

Trays of freshly prepared diet on a cart

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Egg sheets are placed on wire mesh racks above the diet. Wall of rearing room (behind cart) has small holes at many levels through which regulated/conditioned air enters the room from an air plenum (vertical fluorescent lights inside air plenum) to pass over the trays (OKSIR facility, Osoyoos, Canada).

Hutt 1971; Mani et al. 1978; Bathon et al. 1991) and plastic pellets (Hathaway et al. 1972, 1973; Toba and Howell 1991).

Following microbial de-contamination (section 12.6), sheets with eggs are laid onto pieces of wire mesh over freshly prepared diet in trays (Bloem et al. 2000). Sheets laid directly onto diet encourage the growth of mould (Howell and Clift 1972). The exact size of the egg sheet will depend on the dimensions of the roll of waxed paper being used and how the paper has been cut, but it should cover almost the entire surface of the diet so that it becomes seeded uniformly throughout.

The number of eggs per sheet placed on a tray is important for rearing efficiency — too few eggs result in wastage of the diet and too many eggs result in wastage of insects due to competition for food (Howell 1970, 1971). Brinton et al. (1969) used an egg density of about 5 eggs/cm^3 of diet. Bloem et al. (1997, 2000) used 2500–3000 eggs on each tray of diet (about 3 L diet) as did Taret et al. (2007). Howell and Clift (1972) infested each tray with 1500–3000 eggs. In South Africa, 625 eggs/L diet are placed on freshly prepared diet (D. Stenekamp, pers. comm.). However, the appropriate number of eggs for each mL of diet is dependent on many factors and should be determined for each rearing situation.

Eggs on the egg sheets should be at the black-head stage and will begin hatching within the next day or two. When eggs hatch, the neonate larvae spin silken threads and lower themselves to the diet surface to enter the diet. The light intensity in the rearing room should be subdued (Brinton et al. 1969); bright light discourages larvae from entering the diet and they wander off (Batiste and Olson 1973; Reed and Tromley 1985).

Egg sheets should be removed from the trays when all viable eggs have hatched (2–7 days) (Brinton et al. 1969; Howell 1971; Howell and Clift 1972; Ashby et al. 1985; Toba and Howell 1991; Bloem et al. 2000). Removing the used egg sheets permits better air circulation over the diet surface and thus helps prevent the growth of mould.

8.1.2. Eggs in a Slurry

The pink bollworm produces eggs that are round, and this permits their easy removal from the oviposition substrate and accumulation in a liquid carrier, forming a slurry (Stewart 1984). The number of eggs per mL of slurry can easily be determined, and it enables the eggs to be delivered onto the diet surface volumetrically and mechanically and in known numbers. This system is used for the pink bollworm (Stewart 1984), cabbage looper (Leppla et al. 1974), boll weevil (Goodenough 1984, Roberson and Wright 1984, Smith 1999, Wood and Wendel 1999) and Mediterranean fruit fly (Schwarz et al. 1985). According to Roberson and Wright (1984), a pump and sprayer "deliver 4 mL of eggs (2100 eggs) in a furcellaran solution (0.5%) to the diet surface. The furcellaran solution is used to suspend the eggs, thus enabling uniform distribution with spraying."

8.2. LARVAE

For rearing on agar-based diets in small cups (Howell 1970) and on individual immature apples, the standard procedure is to place neonate larvae, one by one, onto the diet (Bathon et al. 1991). However, Howell (1971, 1972c) also placed neonate larvae individually onto diet in trays.

The procedure is as follows:

- Sterilize a small fine-tipped brush with alcohol, then with acetone and finally rinse in distilled water before picking up a larva with the brush (Howell 1971) or dip the brush in a 0.5% solution of NaOCl, then in distilled water (Hathaway et al. 1971).
- Pick up a larva from the egg sheet with the fine point of the wet brush and transfer it to the diet.
- Sterilize the brush **each** time a larva is transferred from the egg sheet to the diet.

This procedure is very time consuming and laborious, but in experimental situations and to prevent contamination with microbes, it is necessary to follow the procedure.

9. Rearing, Sexing and Collecting Larvae

9.1. ENVIRONMENTAL CONDITIONS

- **Temperature:** 25–28ºC

  Hutt (1979), Proverbs (1982), Proverbs et al. (1982) and Dyck et al. (1993) suggested that the field competitiveness of mass-reared adults during cool weather might be improved if they were reared under fluctuating temperature simulating that occurring in the spring. Guennelon et al. (1981) used 25ºC during the day, 20ºC during twilight and 15ºC during the night. Bloem and Bloem (1995, 2000) and Bloem et al. (1998a) used 21ºC for 12 h and 33ºC for 12 h, and found that dispersal activity in the field increased slightly. However, with this regime, moth production was reduced, the operational costs of making the temperature changes were high and the small benefits were not regarded as cost-efficient. However, in a non-peer reviewed document, Jallow and Judd (2007) concluded that "moths reared under fluctuating temperatures were significantly more competitive than moths reared under either constant temperature or through diapause. These effects were observed regardless of the sampling method (i.e. capture in pheromone-baited traps or in mating tables)". These results were unexpected (Bloem et al. 1998a) and indicate that an optimal rearing strategy has not yet been identified (sections 13, 14 and 19.4).

- **Relative Humidity:** 50–80%, usually 60–70%

  It is important to control the RH when mass-rearing in trays of diet, but the ambient RH is relatively unimportant when rearing in closed containers. For diets that dry out and harden, RH is critical. A reduction in RH as the diet dries out is required, e.g. from 75 to 55 or 50% within three weeks (Bloem et al. 2000; Taret et al. 2007).

- **Light:** Photophase:scotophase 16L:8D–18L:6D

  The main purpose in controlling light is to establish the photophase period to avoid diapause (long photophase, short scotophase) or to induce diapause (short photophase, long scotophase) in larvae (section 13). Also, dim or subdued light is required when neonatal larvae are entering diet in trays (section 8.1.1). The photophase that produces non-diapause larvae is not the same in all geographic locations and for all strains, therefore, local considerations are important. However, a photophase:scotophase of

18L:6D will avoid diapause development at all locations. Since in nature larvae develop inside fruit with low light intensity, they are quite sensitive to daylength, and light intensity in a rearing room can be quite low but still achieve daylength control for the larvae.

- **Air Movement**

  Air movement, both the direction and speed, must be regulated for diets held in open trays and especially diet that dries out and hardens. Horizontal (laminar) air flow between vertically stacked trays on carts is absolutely necessary to control the rate of drying of the diet and to suppress the growth of mould. Howell (1971) used two complete air exchanges per minute, but Brinton et al. (1969) found that three exchanges per minute were required. In the OKSIR facility, horizontal air flow is provided by air entering the room from many small holes in the side walls; each tray receives air from a row of holes just above it (**Figure 9**). Mani et al. (1978) described a similar system in which air was blown over trays of diet at a speed of 5–10 cm/sec.

9.2. MORTALITY

The highest mortality during the larval stage is for neonatal larvae between the time of eclosion and entry into and satisfactory feeding on diet in trays.

9.3. SEXING MALE AND FEMALE LARVAE

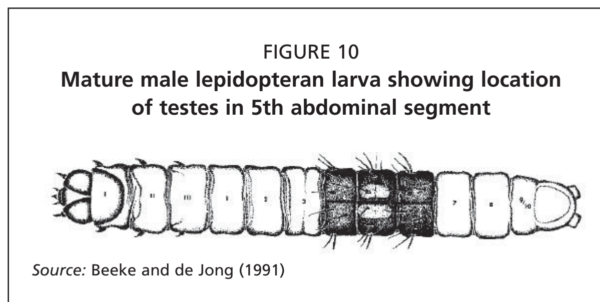
The identification of sex of mature (5th instar) larvae relies on the appearance of the testes as two dark elliptical bodies in the 5th abdominal segment, one on either side of the midline in male larvae (Proverbs and Newton 1962a; Hamilton and Hathaway 1966; Hansen and Harwood 1968; Reed and Tromley 1985; Beeke and de Jong 1991) (**Figures 10 and 11**).

9.4. COLLECTING LARVAE

Collecting larvae is relatively easy if a soft diet is used, but it is still a laborious manual task when working with a large number of insects. However, the task is much easier if heat is used to drive the larvae out of the diet (Brassel 1978).

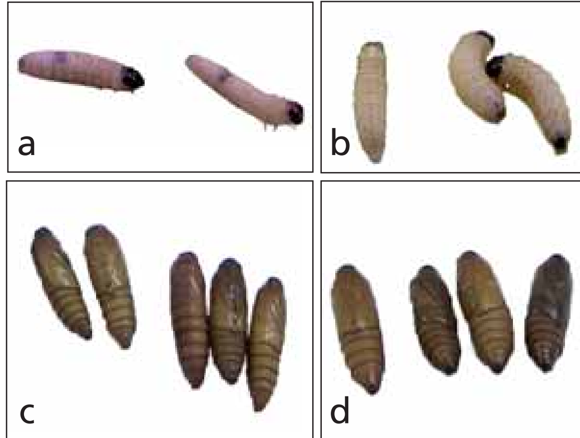
FIGURE 10

Mature male lepidopteran larva showing location of testes in 5th abdominal segment



Source: Beeke and de Jong (1991)

FIGURE 11
Codling moth larvae and pupae



A – male larvae, showing dark testes in abdomen,
B – female larvae,
C – male pupae,
D – female pupae

Source: Taret et al. 2006

10. Rearing, Sexing and Collecting Pupae

10.1. ENVIRONMENTAL CONDITIONS

- **Temperature:** 24–28°C

- **Relative Humidity:** 20–70%, usually 50–60%
  It is very important to control RH when mass-rearing in trays, but the ambient RH is relatively unimportant when rearing in closed containers. For diets that dry out and harden, the RH may be quite low during pupal development and adult emergence (Bloem et al. 1998a, 2000).

- **Light:** Photophase:scotophase 16L:8D–18L:6D
  The main purpose in controlling light is to establish the photophase period, particularly for developing larvae. However, many mass-rearing systems keep pupae in constant darkness so that emerging adults are attracted to and move towards a UV light source. The adults are then trapped in a container which may be located in a cold room (0–2°C) to inactivate them.

- **Air Movement**
  Air movement, both direction and speed, must be regulated for diets held in open trays and especially diet that dries out and hardens. Horizontal air flow between vertically stacked trays controls the rate of drying of the diet and suppresses fungal growth. Howell (1971) used two air exchanges/min, but Brinton et al. (1969) found that three exchanges/min were required. In the OKSIR facility, horizontal air flow is provided by air entering the room from many small holes in the side walls; each tray receives air from a row of holes just above it (**Figure 9**). Mani et al. (1978) described a similar system in which air was blown over trays of diet at a speed of 5–10 cm/sec.

10.2. MORTALITY

Pupal mortality is usually low.

10.3. SEXING MALE AND FEMALE PUPAE

Peterson (1965) described four dark segmental bands beyond the wing pad tips on the ventral surface of male pupae; female pupae have only three bands (Reed and Tromley 1985). Males have four freely articulated abdominal segments caudal

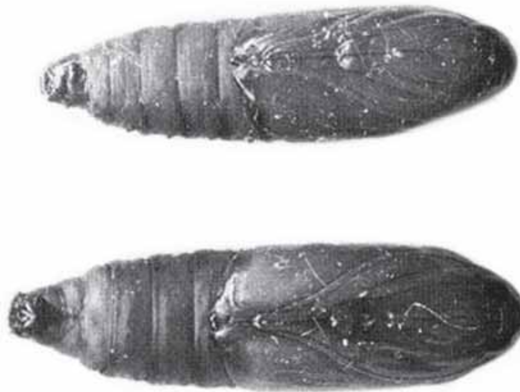
FIGURE 12
Ventral view of abdomen of female pupa of the codling moth

Source: Howell (1991)

FIGURE 13
Ventral view of abdomen of male pupa of the codling moth

Source: Howell (1991)

FIGURE 14
Ventral view of male (upper) and female (lower) pupae of the codling moth.



Source: Bathon et al. (1991)

to the wing pads, but females have only three. Bathon et al. (1991) describe this difference as five abdominal segments ventrally in the male and four in the female (**Figures 12–14**). Female pupae tend to be larger in diameter and heavier than male pupae (Reed and Tromley 1985, **Figure 14**). However, probably the easiest morphological feature is the location and appearance of the genital aperture (pore)

on the ventral side of the abdomen. The ostium bursae of the female pupa is found on the 8th and 9th abdominal segments (**Figure 12**), but the genital ostium of the male pupa is entirely within the 9th segment (**Figure 13**) (Bathon et al. 1991; Beeke and de Jong 1991; Howell 1991).

The sexes cannot be satisfactorily separated by machine. However, a sizing machine (Schoenleber et al. 1970) used rollers to divide pupae into 10 groups based on their diameter (Goodenough 1984). There is considerable overlap in size between the sexes; only in class 1 were there 100% males (10% of males, the smallest), and only in classes 9 and 10 (21% of females, the largest) were there 100% females (Schoenleber et al. 1970).

10.4. COLLECTING PUPAE

Collecting pupae from soft diet is done by allowing mature larvae to spin cocoons in corrugated cardboard strips placed on top of the diet (Dickson et al. 1952; Hamilton and Hathaway 1966; Hathaway et al. 1972; Howell and Clift 1972; Toba and Howell 1991). After pupation, the cardboard strips are torn open to break the cocoons and then the pupae are collected. Bathon et al. (1991) used rolls of polyethylene strips, and Proverbs and Newton (1962a) used slotted wooden strips held together with rubber bands.

Larvae tend to remain in diets which dry out and harden, and they form cocoons and pupate inside the diet; adults emerge directly from the diet. Collection of pupae is quite difficult, and involves breaking up the diet and removing the pupae individually. Carpenter et al. (2004) attempted to find a method of extracting pupae efficiently. This included dissolving the silk cocoons by agitation in various concentrations of NaOCl and pressure washing the dry diet with water and NaOCl. Extracted mature pupae tolerated the treatment but only about 50% of adults emerged, and larvae and newly formed pupae were killed. Harvesting pupae of some lepidopteran species has been mechanized (Stewart 1984; Nordlund 1999). Pupae can be surface-sterilized by dipping in a 3% NaOCl solution (Bathon 1981; Taret et al. 2007). Sikorowski and Goodwin (1985) provided a method of surface sterilization of cabbage looper pupae.

11. Collecting, Holding, Marking and Sexing Adults

11.1. COLLECTING EMERGED MOTHS

Collecting adult moths is difficult due to the respiratory health hazard to workers from moth scales in the air (section 21.1). In the laboratory, emerging adults are attracted by light into transparent plastic bottles or screened cages (Hamilton and Hathaway 1966; Jermy and Nagy 1971; Ivaldi-Sender 1974; Mani et al. 1978; Guennelon et al. 1981).

At the Pacific Agri-food Research Centre (PARC 2007), trays of diet (from which adults were about to emerge) were placed on racks inside a large wooden box. Metal funnels were fixed onto one side of the box and a transparent plastic/screen cage placed over each funnel. Light in the room attracted the emerging adults into these cages (Brinton et al. 1969). Later, the adult-collection system was changed to reduce labour costs. The lights of a rearing room with open trays of diet on carts were turned off and UV lights below boxes (with screen bottoms) on the floor were turned on. Adults were attracted into the boxes by the light, and after 10–15 min a lid was placed on the box.

FIGURE 15

UV light inside opening of vacuum trap or suction device in ceiling of an emergence room. Duct leading away from this device channels adults to a cyclone. (OKSIR facility, Osoyoos, Canada)

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FIGURE 16
Upper part of cyclones where moths enter via flexible grey-coloured ducts. The cyclones extend down into the cold room. (OKSIR facility, Osoyoos, Canada)

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FIGURE 17
Lower part of a cyclone in a cold room in which adults collect (OKSIR facility, Osoyoos, Canada)

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Another small-scale system was used at the Yakima, USA, laboratory (Howell and Clift 1972). Corrugated cardboard strips with pupae were placed into an emergence box. A waxed paper bag was fixed over an opening on the top of the box to trap emerging moths.

To avoid the health hazard from moth scales, scientists at the Yakima Laboratory (Hutt et al. 1972; Moffitt and Hathaway 1973; Toba and Howell 1991) developed a system of collecting moths in a cold room. Subsequently Dyck et al. (1993) developed a similar system in which, with no human intervention (and no human exposure to scales in the air), flying adults in an emergence room were attracted to a UV light (Bloem and Bloem 1995, 2000) which was turned on for 50 min out of every hour (Bloem et al. 1998a, 2000). Emerged adults flew to the UV light (**Figure 15**) in the ceiling housed in a suction device or vacuum trap that transported adults through large ducts (**Figure 16**) into cyclones in a cold room (0–2°C) (**Figure 17**). Due to the sudden decrease in air speed in a cyclone, the insects fell to the bottom of the cyclone, were inactivated by the cold and were collected (Wolf and Stimmann 1971; Stewart 1984; Bloem et al. 1997, 1998a, 2000, 2004). This adult collection system has an advantage in that only adults that can fly are collected, helping to ensure that good flight ability is continuously selected for and maintained (Bloem and Bloem 2000) (sections 18.5.2 and 19.5). Mediouni and

Dhouibi (2007) used a similar adult collection system for mass-rearing the carob moth.

Batiste and Olson (1973) developed an adult emergence-collection device in which trays of diet were held in aerated darkened compartments at 28°C. Emerged adults were attracted through stainless-steel tubes into collection tubes within a refrigerator (10°C) by continuous UV light.

Moffitt and Hathaway (1973) showed that collecting adults using UV light and cold storage might have some negative effects on the response of males to a sex-attractant trap in the field.

11.2. HOLDING ADULTS IN THE COLD

Chilled adults are easily handled in a cold room (0–2°C) for weighing, counting, sexing and irradiating. Chilling is superior to CO_2 as an immobilizing agent (White et al. 1970). However, Bloem et al. (1998a) discovered that chilling for too long can be detrimental to adults intended for field release; moths that had been held in the cold for 12 h performed better than those held for 36 h (Bloem and Bloem 2000). (Note sections 14.5 and 18.5.5 regarding the cold storage of adults.)

11.3. MARKING ADULTS USING FLUORESCENT DYES

In release and recapture studies, adults are marked with a fluorescent dye prior to release; usually the dye is a powder or dust (zinc 8-hydroxyquinoline) (Logan and Proverbs 1975). The powders are available in several colours (DayGlo 2007).

Moffitt and Albano (1972a, b), Proverbs et al. (1969, 1975, 1978) and Logan and Proverbs (1975) described how to apply and use such dyes. DayGlo dusts were applied by Bloem et al. (1998a, 2004) at the rate of 5 mg per 23–25 g of adults. Subsequent to the release, insects captured in traps are observed with long-wave UV light to detect the presence of dye.

The over-application of these dyes has been shown to have some negative effects on the behaviour of adults, e.g. olfactory response of males to calling females (Proverbs 1971, 1972; Logan and Proverbs 1975). Moffitt and Albano (1972b) reported that Blaze Orange and Rocket Red reduced fecundity, and Arc Yellow, Fire Orange and Neon Red reduced egg hatch. Helecon® 2200 and Rocket Red produced a reduced response of marked males to females, and these two markers were not used in field work on insect behaviour. Eosin-Y had no apparent effects on adult moths, but was harder to apply and more difficult to detect than were fluorescent powders. (Eosin-Y is a non-fluorescent red tissue stain).

Powder on marked insects may also contaminate non-marked wild insects that are trapped along with them in the field, causing misidentification (Proverbs 1982).

Hagler and Jackson (2001) and Parker (2005) reviewed the use of dyes for marking insects (also section 4.12).

11.4. SEXING MALE AND FEMALE ADULTS

When viewed from the ventral side, the sex of a codling moth adult is easily identified by overall size (female is usually larger in diameter and heavier than

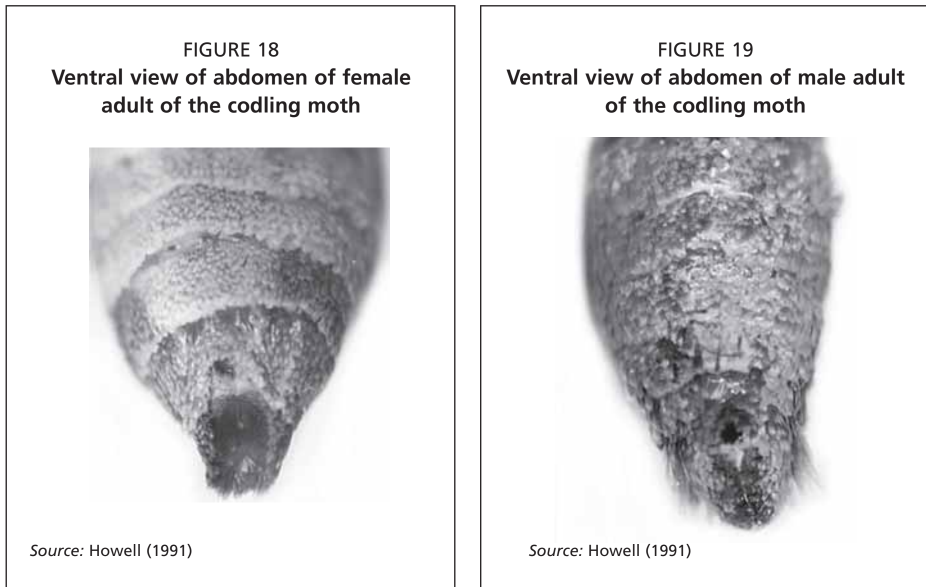


FIGURE 18
Ventral view of abdomen of female adult of the codling moth

Source: Howell (1991)

FIGURE 19
Ventral view of abdomen of male adult of the codling moth

Source: Howell (1991)

the male) and structure of the external genitalia. Females have a large anal papilla (**Figure 18**) (a circular brown spot at the tip of the abdomen) and a dark ostium bursae in front of the papilla and males have a pair of claspers (**Figure 19**) (Howell 1991, Trécé 2007a, Wunderlich 2007). Also, males have a black spot in the centre of the underside of the forewing (Howell 1991).

Manual separation of the sexes can be done, at room temperature, by laboriously sucking up adults individually with a mouth aspirator (pooter) or low-suction portable vacuum aspirator (to protect the worker from breathing in moth scales) (Reed and Tromley 1985). Adults can be immobilized by a draft from a fan, enabling a worker to sex them visually and then suck them up individually (Hamilton and Hathaway 1966).

Moths can be sexed with a magnifier when immobilized in a cold room, e.g. 2–4°C. However, working in a cold room is uncomfortable for staff, and a 'cold table' can be made in which a metal surface is chilled. Adults placed on the cold surface are chilled and immobilized, permitting the sorting to be done in comfort. Adults can also be sexed using a stream of cold air at 9°C (air passed over ice) (Batiste and Olson 1973).

No satisfactory automated system to separate the sexes has been developed. However, it may be possible to separate the sexes by (1) collecting early-emerging adults (mainly one sex) and then collecting late-emerging adults (mainly the other sex) (Howell 1991), or (2) attracting emerging male adults to a sex-pheromone source and then trapping them.

Schoenleber et al. (1970) described five different mechanical devices designed to separate chilled (near 0°C) adults:

- Perforated round-hole screen – uses differences in insect thickness

- Perforated slotted-hole screen – uses differences in insect breadth and length
- Air-gravity separator – uses differences in insect density
- Electrostatic separator – uses differences in charge of insects
- Vibrating inclined table separator – uses differences in insect surface texture and shape

The results showed that none was very good at separating the sexes, with the best being the slotted screen (about two-thirds of the moths in each of two 'separated groups' were of the same sex).

11.5. GENETIC SEXING

Research on a genetic sexing system is on-going (Marec et al. 2005, 2007; Vreysen and Hendrichs 2005; Vreysen et al. 2006; Makee and Tafesh 2007). However, this work has not yet produced a genetic sexing strain that can be used for field programmes (Franz 2005).

12. Mating and Feeding Adults, and Collecting, Incubating and Treating Eggs

12.1. ENVIRONMENTAL CONDITIONS

The following conditions are common for mating, oviposition and incubating eggs. (These activities are combined here since they all occur in an oviposition room.)

- **Temperature:** 24–28°C
- **Relative Humidity:** 50–70%
  The RH must be between 50-70% during oviposition (Wildbolz and Mani 1971) to prevent the desiccation of eggs. However, once eggs have been laid and are being incubated in an airtight container, the RH can be maintained by enclosing a wet wick.
- **Light:** Photophase: scotophase 16L:8D–18L:6D
  Since mating and oviposition tend to occur at dusk (Bathon et al. 1991), lights should be dim or subdued; light intensies reported include 32–54, <150 and <538 lux. Some laboratories use natural daylight rather than artificial lighting, others constant light.
- **Air Movement**
  The presence of scales (from adults in cages) makes air circulation in the mating and oviposition room problematic, and filters in the air-handling system must be used to remove these scales. When in a mating and oviposition room, workers should wear face masks.

12.2. MATING ADULTS

Sexual activity peaks around dusk. In nature, females call by releasing a sex pheromone and males respond by finding the females, and then mating takes place. A successful mating lasts about one hour; the first mating tends to be shorter in duration than later matings (White et al. 1975; Howell et al. 1978; Howell 1991). After pushing the spermatophore into the bursa copulatrix, the male ejaculates into it a milky seminal fluid of mostly apyrene sperm followed by a more compact substance containing eupyrene sperm (Ferro and Akre 1975; White et al. 1975; Howell 1991). The spermatophore hardens in the female bursa copulatrix; it can even be felt when squeezing the abdomen of a mated female between two fingers. A spermatophore is shaped like a bilobed pear (Howell 1991; Trécé 2007a). Ferro

and Akre (1975) provided drawings of the female and male reproductive systems, and a drawing of the position of female and male genitalia during mating.

Males may copulate several times, but the size of the spermatophore transferred tends to decline after the first mating (Howell 1988). Males can produce a spermatophore about once a day, for 3–5 days. If the sex ratio favours females, males will mate more often than if the ratio is 1:1 (Howell et al. 1978).

On average female codling moths copulate once or twice. Mated females usually discontinue calling and do not remate, especially if the spermatophore received is large (Howell 1988). Howell et al. (1978) found that 37% of females mated more than once in laboratory cages. Increasing the sex ratio in favour of males increased the number of times a female mated (but decreased female longevity and fecundity), but increasing the sex ratio in favour of females decreased the number of times a female mated (Pristavko and Boreyko 1971).

No special mating cage is required; mating takes place within the oviposition cage. Nearly all males and females are sexually active on the first day following emergence (Howell 1988), and mating is virtually complete within 12–48 h (Howell et al. 1978) and females soon begin to lay fertile eggs.

12.3. FEEDING ADULTS

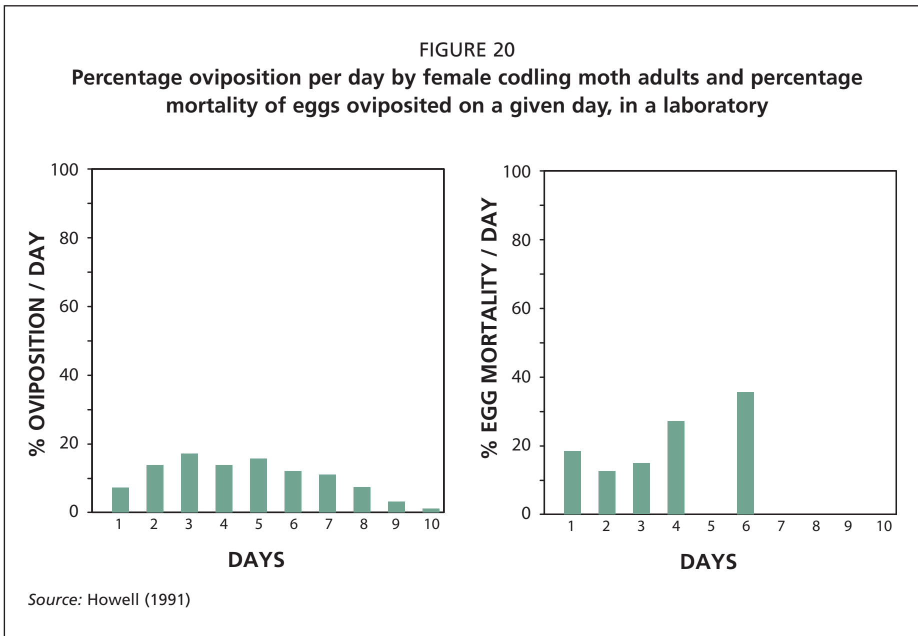
Feeding adults increases longevity. Some authors reported that post-emergence öogenesis was possible only when water was given to the moths (Howell 1981). Water is provided to adults during mating and oviposition, usually by wetting a cotton wick, cotton wool, sponge or piece of filter paper, or by providing a feeding bottle (Dickson et al. 1952; Hamilton and Hathaway 1966; Navon 1968; Proverbs and Logan 1970; Jermy and Nagy 1971; Navon and Moore 1971; Pristavko and Boreyko 1971; Wildbolz and Mani 1971; Huber et al. 1972; Reed and Tromley 1985; Bathon et al. 1991; Dyck et al. 1993). Sometimes sucrose or honey solution (3–10%) is provided (Jermy 1967; Sender 1969; Wildbolz and Mani 1971; Hatmosoewarno and Butt 1975; Guennelon et al. 1981; Ashby et al. 1985; Bathon et al. 1991; Neven et al. 2000). Nevertheless, feeding adults is time consuming and causes some mortality since adults may get trapped under wet pads (Howell 1981).

Fecundity can be increased by feeding a solution of 10% sucrose and 0.3% sorbic acid (Huber et al. 1972). Navon and Moore (1971) provided a complex diet (water, honey, ascorbic acid, thiamine, riboflavin, nicotinamide and choline chloride) to adults and found that this more than doubled fecundity when compared with water only (females lived up to 15 days). However, since the majority of the eggs were oviposited during the first half of a female's life, it would not be rational to keep cages longer than about 6 days.

Feeding adults with water, a carbohydrate or a carbohydrate-protein solution increases longevity (Howell 1981; Ashby et al 1985) but this does not significantly increase mating, oviposition or egg viability (Howell 1981).

Females lay 80–94% of their eggs within the first six days of life, regardless of the availability of water or food (Howell 1981, 1991; Gu et al. 2006) (**Figure**

FIGURE 20
Percentage oviposition per day by female codling moth adults and percentage mortality of eggs oviposited on a given day, in a laboratory



Source: Howell (1991)

20). The highest number of eggs laid is on days two and three (Bloem et al. 1998b) and 38% of the eggs oviposited on day six or later are sterile (Howell 1991) (**Figure 20**). Therefore, rearing programmes tend to maintain adults for only about 5 days, and feeding adults may not be worthwhile (Hamilton and Hathaway 1966; Howell 1970, 1981). Nevertheless, to provide insurance against high adult mortality from various environmental factors (e.g. low or high RH, high temperature), many rearing programmes continue to supply water to adults in mating and oviposition cages.

12.4. OVIPOSITION CAGES

To maintain a rearing colony, about 10% of produced adults are needed (Bloem et al. 1997, 2000); other authors report similar figures, 8% (Batiste and Olson 1973) and 15–20% (Mani et al. 1978).

Howell (1981) stated that "for economy in rearing massive numbers of codling moths, one must have an efficient means of obtaining the largest possible number of eggs per female in the shortest possible time with a minimum of labour and without sacrificing viability or vitality of the resulting insects. Also, with higher fecundity, fewer moths are needed to maintain a colony. Thus, over the years, a variety of oviposition cages has been developed, all of which provide a method of caging the moths and easy retrieval of eggs."

Oviposition in a laboratory cage proceeds without the need for special stimulation. However, in nature, female adults are stimulated to lay eggs when they detect apple odour (Wearing et al. 1973).

Types of cages and other oviposition substrates are described below:

FIGURE 21
Oviposition cage resting on slowly turning rollers. Constructed from aluminium and using brown paper waxed on both sides (OKSIR facility, Osoyoos, Canada)

M.J.B. VREYSEN

FIGURE 22
Rows of oviposition cages on slowly turning rollers (OKSIR facility, Osoyoos, Canada)

M.J.B. VREYSEN

- Cylindrical plastic cages lined with waxed paper (Dickson et al. 1952; Hamilton and Hathaway 1966)
- Waxed paper or cellophane from roll threaded through a plastic-screen oviposition cage (Jermy and Nagy 1971)
- Cellophane paper; eggs removed by gently crinkling the paper (Rock 1967)
- Cellophane or polythene covering a beaker or tube (Huber et al. 1972; Bathon et al. 1991)
- Waxed paper bags or plastic bags (White and Hutt 1970, 1971; Hathaway et al. 1971, 1972, 1973; Howell and Clift 1972; Bathon et al. 1991; Toba and Howell 1991)
- Wire cages and plastic pellets (Hathaway et al. 1972, 1973; Toba and Howell 1991)

Proverbs and Logan (1970) developed a wooden cage, and each day a sheet of paper with eggs could be removed and a new sheet inserted, without removing the moths. A very similar all-metal cage was developed for the OKSIR facility, except that at the centre of one of the round side disks a water bottle and wick were inserted to provide water (Dyck et al. 1993) (**Figures 21–23**). Similar oviposition cages have also been constructed (Batiste and Olson 1973; Carlyle et al. 1975; Mani et al. 1978; Guennelon et al. 1981; Mediouni and Dhouibi 2007). Guennelon et al. (1981) claimed that their cage was more practical than that developed by Proverbs and Logan (1970).

Except for the waxed paper, other surfaces on the inside of the cage are screen wire mesh or roughened to discourage oviposition. To ensure a uniform distribution of eggs on the waxed paper sheet, the drum-like cage is slowly rotated/tumbled by two parallel rods (covered with rubber to grip the cage) rotating under

FIGURE 23
Oviposition cages on slowly turning rollers. (South Africa in 2007)

MURIEL KNIPE, PLANT PROTECTION DIVISION, ARC INFRUITEC

the cage (Proverbs and Logan 1970, about four revolutions of the cage/h; Batiste and Olson 1973, about one revolution of the cage/h).

The number of moths per cage, that each day will produce the desired number of eggs/cm^2 of egg sheet, is not a constant; it depends on several factors, especially the size of the cage and area of egg sheet, fecundity of the females and temperature (section 19.8). Each laboratory must determine the optimum adult density for its oviposition cage.

The number of males in a mating/oviposition cage can be reduced by one-third to one-half without decreasing fecundity and the quantity of hatched eggs. Therefore, some male adults can be used for other purposes, e.g. release in the field (Pristavko and Boreyko 1971; Hathaway et al. 1973).

12.5. INCUBATING EGGS

The daily removal of egg sheets from oviposition cages, and incubating them at the appropriate temperature and in a systematic way, will provide the needed number of eggs in the blackhead stage (just prior to hatching) on the days when fresh diet should be infested. To prevent desiccation, sheets with eggs should be placed in plastic bags along with wet wicks to keep the humidity high during incubation (Huber 1972).

Egg development can be synchronized with the diet preparation schedule by altering the temperature. The eggs can be stored for up to seven days at 13ºC (Huber et al. 1972) and up to two weeks at 11ºC (Reed and Tromley 1985).

Egg mortality is about 15–30%, even under apparently suitable conditions for mating, oviposition and egg incubation (**Figure 20**).

12.6. TREATING EGGS

To control microbial contaminants, it is necessary to surface-sterilize the eggs (Theron 1947). This permits larvae to eclose in a sterile environment before seeding the diet (Howell 1970, Proverbs 1982, Taret et al. 2007). Egg treatment is common in insect rearing (Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994a, b; Sikorowski et al. 2001).

Egg sheets for seeding fresh diet need to be cut to fit the length and width of the diet tray (**Figure 24**).

For immersion and surface-sterilizing, egg sheets can be placed into a custom-made ‘book’ consisting of wire-mesh ‘pages’, one sheet between two opposing pages. The wire mesh between each egg sheet permits the liquid to penetrate the stack of sheets and thoroughly wet both sides of each sheet (Fisher 1984a) (**Figure 25**).

In general, the procedure is as follows:

- The book of egg sheets is immersed into the disinfecting solution for several minutes in a stainless-steel tank (**Figure 26**), then taken out and rinsed in water. After a second rinse in water, the book of sheets is placed on a rack to allow the water to drain.

FIGURE 24
Cutting egg sheets into a size suitable for placement over trays of diet (OKSIR facility, Osoyoos, Canada)

M.J.B. VREYSEN

FIGURE 25
Egg sheets being placed between wire mesh (OKSIR facility, Osoyoos, Canada)

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FIGURE 26
Stainless-steel tanks (3) for immersion of egg sheets in a disinfecting solution and then rinsing in water (OKSIR facility, Osoyoos, Canada)

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FIGURE 27
Stainless-steel cart, specially designed to hold disinfected egg sheets, for transporting egg sheets from the egg-sheet preparation room to the diet-dispensing room. (OKSIR facility, Osoyoos, Canada)

M.J.B. VREYSEN

- A specially constructed cart with doors, developed at the OKSIR facility, is used to hold the clean egg sheets in the books and transport them to a sterile holding room (**Figure 27**).

Procedures used to treat egg sheets:
- Place egg sheets in a solution of 0.1% NaOCl for 2 min, rinse with sterile water for 30 sec and dry in air at 27°C (Hamilton and Hathaway 1966; Hathaway et al. 1972; Toba and Howell 1991).
- Place egg sheets in a solution of 6.5% formalin for 30 min, rinse three times in distilled water and dry in air (Rock 1967).
- Place egg sheets in an aqueous solution of 3% NaOCl (commercial bleach that contains 5.3% available chlorine) and 0.1% wetting agent (e.g. Triton X-100) for 3 min, rinse in water for 1 min and dry in air at 27°C (Brinton et al. 1969; Batiste and Olson 1973).
- Place egg sheets for 5 min, with periodic agitation, in 0.164% NaOCl solution (13.83 mL of 12% commercial bleach in each litre of water) and a few drops of liquid soap. Rinse in tap water for 5 min with periodic agitation and dry in air (PARC 2007).
- Place egg sheets in 3% NaOCl solution for 3–4 min, wash in tap water for 10 min and dry in air between layers of paper towelling (Bathon 1981).

- Place egg sheets in 1.3% NaOCl and a wetting agent for 1 min, rinse under running water and dry in air.
- Place egg sheets in a 4% solution of formaldehyde for 5 min, rinse with sterile water (Howell 1970) (section 21.2). Surface sterilization in formaldehyde dislodged a few eggs from the sheet (Toba and Howell 1991).
- Place egg sheets in a solution of 4% formalin, rinse twice in distilled water and dry in air (Hathaway et al. 1971).
- Place egg sheets in formalin (1 part 37% formaldehyde to 10 parts water) for 5 min, rinse in 70% isopropyl alcohol to facilitate rapid drying and dry in air (Howell and Clift 1972).
- Place egg sheets in a 10% formalin solution for 2–3 min, rinse in distilled water and dry in air (Huber et al. 1972).
- Place egg sheets for 5 min in 4% aqueous formaldehyde, rinse in water and dry in air (Hatmosoewarno and Butt 1975).
- In a fume hood, place egg sheets in a tray with 5% formalin (or 2% formaldehyde) and agitate gently for 10–15 min with forceps, rinse sheets in running tap water for 10 min, rinse sheets again in sterile water for 5 min and dry sheets at 25°C between layers of soft tissue paper (Ashby et al. 1985).
- Place polythene strips with eggs into a nylon gauze bag and submerge in a 5% formaldehyde solution for 20 min. Rinse the bag in sterile water for 20 min and then dry the strips in a fume hood (Bathon et al. 1991).
- Fumigate egg sheets in formaldehyde vapour for 90 min at 23°C (Toba and Howell 1991) (section 21.2). Fumigation with formaldehyde is an excellent treatment to control CpGV.
- Place egg sheets in a container for 6–8 h with formaldehyde vapour arising from one drop of 5% formaldehyde in each 200 mL air (Bathon 1981; Reiser et al. 1993).
- Place egg sheets in a 1 L closed jar containing a filter paper, moistened with five drops of 5% formaldehyde, at 22–23°C for 6–8 h (Bathon et al. 1991).

Fumigating eggs of the cabbage moth *Mamestra brassicae* (L.) with formaldehyde vapour for 6 h prevents trans-ovum transmission of nuclear-polyhedrosis virus (Bathon and Gröner 1977).

13. Diapause

Diapause "represents a syndrome of developmental, physiological, biochemical and behavioural attributes that together serve to enhance survival during seasons of environmental adversity" (Denlinger 2003).

The codling moth is multivoltine with a facultative diapause (Jermy 1967; Wildbolz and Riggenbach 1969; Ashby and Singh 1990; Brown 1991), responding to environmental cues (Denlinger 2003). In the field, 5th instar larvae in the last generation enter diapause, induced principally by a shortening of daylength (Wildbolz and Riggenbach 1969). The critical photoperiod is that which induces diapause in 50% of the population (Brown 1991); in Hungary, this is between 16 and 17 h, and local strains show a clear tendency to polyvoltinism (Jermy 1967). In southern California, the critical photoperiod is 13.5 h (Peterson and Hamner 1968). A low light intensity, e.g. 10 lux, is enough for the photoperiodical reaction (Wildbolz and Riggenbach 1969).

Temperature also plays a role in diapause induction; the critical photoperiod decreases with higher temperatures and lower temperatures, especially during the scotophase, promote diapause induction (Brown 1991; Beck and Chippendale 1968; Denlinger 2003). Temperature and photoperiod work interactively to regulate diapause (Singh and Ashby 1986). Howell and Neven (2000) observed that 15% of larvae entered diapause at a long daylength (17L:7D) but at a low temperature (14.8°C). Diapause induction is only influenced by temperature when the photoperiod is marginal (Wildbolz and Riggenbach 1969). However, in Hungary, the photoperiod reaction of larvae was practically temperature independent (Jermy 1967).

Nutrition affects diapause (Jermy 1967; Beck and Chippendale 1968; Ashby and Singh 1990; Brown 1991) as does larval crowding (Brown et al. 1979; Ashby and Singh 1990). Marked geographical differences in diapause induction have been described (Beck and Chippendale 1968; Wildbolz and Riggenbach 1969; Ashby and Singh 1990) with Hungarian strains differing considerably from those in other geographical regions (Jermy 1967).

Regardless of voltinism, a portion of each generation enters diapause under all conditions of light and temperature (Brown 1991). Diapausing larvae overwinter in cocoons, in crevices in the bark of trees (Singh and Ashby 1986) and in wooden harvest bins (Proverbs and Newton 1975; Bloem et al. 1999a; Higbee et al. 2001).

Breaking diapause is a process that occurs over time when diapausing larvae are kept in the cold (section 13.2). In the field this happens during winter (Peterson and Hamner 1968). Development, i.e. pupal development and adult emerghence, resumes in spring as daylength increases and temperatures rise (Hansen and

Harwood 1968; Wildbolz and Riggenbach 1969; Wildbolz and Mani 1971; Sieber and Benz 1980; Ashby and Singh 1990).

Young larvae are more sensitive to photoperiod than older larvae (Jermy 1967; Hansen and Harwood 1968) but the particular environment present during the early part of the fifth instar has a strong influence on the induction of diapause (Sieber and Benz 1980; Brown 1985).

Obligate diapause is a barrier to efficient laboratory rearing but for the codling moth most individuals reared under conditions of long daylength, e.g. 18 hours, and warm temperatures, e.g. 25–30°C, do not enter diapause (Dickson et al. 1952; Beck and Chippendale 1968; Peterson and Hamner 1968; Wildbolz and Riggenbach 1969; Mani et al. 1978; Ashby et al. 1985; Ashby and Singh 1990). However, there is some evidence that insects that have gone through diapause have a higher competitiveness.

In the laboratory, the quality of diapaused adults compares well with standard adults, e.g. size and mating ability, but male longevity was shorter and female fecundity reduced for diapaused adults (Bloem et al. 2000; Neven et al. 2000).

Bloem et al. (1998a, 2002, 2004, 2007) found that reared moths that have undergone diapause have a high 'field quality', especially in spring when temperatures tend to be rather low, compared with non-diapaused insects. Research is needed to determine if including a period of diapause in the mass-rearing procedure can and should be implemented on a routine basis (Proverbs 1971; Singh and Ashby 1986; Dyck and Gardiner 1992; Anisimov 1993; Dyck et al. 1993; Bloem and Bloem 1995, 2000; Bloem et al. 1997, 1998a, 1999a, 1999c, 2000, 2004, 2005, 2007; Judd et al. 2004; K. Bloem et al. 2005; Judd and Gardiner 2005; Vreysen and Hendrichs 2005; Judd et al. 2006a, b; Vreysen et al. 2006; Wood and Arthur 2006; OKSIR 2007) (sections 9.1, 14, 16 and 19.4). An insect that has undergone diapause might be better able to withstand the adverse effect of gamma radiation on field quality (Bloem et al. 2004, 2007).

Another potential benefit of producing diapausing larvae is for stockpiling, permitting the release of large numbers of sterilized adults in a short time if there are unexpected increases in the natural population (Jermy and Nagy 1971; Singh and Ashby 1986; Dyck et al. 1993; Bloem and Bloem 1995; Bloem et al. 1998a, 2000; Parker 2005).

Rearing larvae in diapause can serve as a back-up to the main colony, in case of a major disaster (Ashby et al. 1985; Singh and Ashby 1986), and would also permit year-round production, efficient use of a rearing facility and facilitate the export of larvae (Bloem et al. 1997). Diapause-destined larvae are more easily infected with CpGV (Brassel 1978).

Diapause rearing costs are 64% higher than standard rearing, mainly due to materials, labour and cold storage (Bloem et al. 1997). The production of one million standard adults cost USD 1572, compared with USD 4400 for diapause adults, plus additional handling and utility costs for cool and cold storage. Also, 50% fewer trays of diet can be held on a cart (due to space needed for C-fluted

corrugated cardboard rolls) and additional temperature-controlled storage space is required (Bloem et al. 2000).

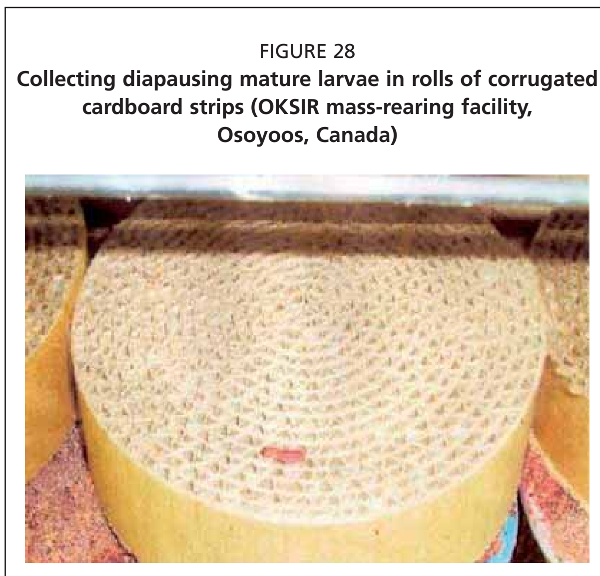
Costs can be reduced by enclosing trays in fibreglass mesh bags which prevent exiting larvae from wandering; they form cocoons in or on the diet (Bloem et al. 2000). However, increased cold storage space is needed.

13.1. INDUCING DIAPAUSE

To induce diapause in larvae, they must be reared with a short daylength, i.e. between 8L:16D and 12L:12D with at least 12 h at a relatively low temperature between 15 and 25°C and 55-65% RH (Hansen and Harwood 1968; Butt et al. 1970; Mani et al. 1978; Ashby et al. 1985; Singh and Ashby 1986; Ashby and Singh 1990; Bloem et al. 1997, 1998a, 1999a, 2000, 2004; Neven et al. 2000).

Storing diapaused larvae in diet is problematic since fungi may develop. In the sawdust diet, unexpectedly most of the larvae left the diet, suggesting that the environmental conditions that induce diapause also change the behaviour of mature larvae (Bloem et al. 1997). Diapausing mature larvae are captured in rolls of corrugated cardboard strips when they exit the diet (Wood and Arthur 2006), but 12% of larvae can still be lost (Bloem et al. 2000). Larval wandering can be limited by providing extra cardboard rolls on top of and below the diet trays, and cardboard barriers and double-sided tape on the floor around the cart (Wood and Arthur 2006) (**Figures 28 and 29**). Rolls with diapausing larvae are placed in black polyethylene bags and transferred to a storage room (Bloem et al. 1997).

FIGURE 28
Collecting diapausing mature larvae in rolls of corrugated cardboard strips (OKSIR mass-rearing facility, Osoyoos, Canada)



S. WOOD AND S. ARTHUR

FIGURE 29
Cart with trays of diet to collect diapausing mature larvae in rolls of corrugated cardboard strips on each tray.

S. WOOD AND S. ARTHUR

To limit larval wandering, note rolls on top of cart, cardboard and rolls on floor around cart, and two-sided tape on floor (OKSIR mass-rearing facility, Osoyoos, Canada).

13.2. BREAKING DIAPAUSE

To break diapause, larvae require two types of storage environments, one to condition the larvae for cold storage and second the actual cold storage (but researchers have reported somewhat different procedures).

Procedure for conditioning mature larvae:

100 days at 15°C, 50% RH, in the dark (Ashby and Singh 1990; Bloem et al. 1997); 2 weeks at 10°C in the dark (Neven et al. 2000).

Procedure for cold storage of mature larvae:

Chilling for 30–50 days at 0–2°C, 50% RH, in the dark (Ashby and Singh 1990; Bloem et al. 1997); holding at 4°C for three months (Wildbolz and Riggenback 1969); storing at 2°C for 2–4 months was ineffective, but storage at 4, 6 and 9°C broke diapause (Wildbolz and Mani 1971); holding at 0, 1 or 6°C in the dark (Butt et al. 1970; Mani et al. 1978; Neven et al. 2000).

Sub-zero cooling of diapausing larvae (–10 or –15°C for 2–6 days) reduced the amount of time to adult emergence, and also its span, i.e. more synchronous emergence. Sub-zero cooling increased the longevity of females and the number of spermatophores produced by males (Neven et al. 2000).

Diapausing larvae can be stored with good survival (≥ 92%) for 6–18 months at 15°C in the dark and ambient RH (Ashby et al. 1985; Singh and Ashby 1986; Bloem et al. 2000), and diapausing larvae can be kept for up to two years at 0–2°C (Neven et al. 2000). Synchronicity of emerging adults is greater if the cold storage period is longer (Ashby and Singh 1990; Bloem et al. 2000).

13.3. PUPAL DEVELOPMENT AND ADULT EMERGENCE

The procedure for continued development (pupal stage) and adult emergence is summarized below (but researchers have reported somewhat different procedures).

At 16L:8D, 25–28°C and 30% RH adults start emerging after about 16 days, with peak emergence on days 18–20, but emergence may continue for up to 50 days (Bloem et al. 1997; Taret et al. 2006); 18L:6D, 25°C and 60% RH with 93% diapause termination occurring after 25 days (Ashby and Singh 1990); 16L:8D, 23°C and 60% RH (Neven et al. 2000); 14L:10D, 26°C and 65–70% RH (Blomefield et al. 2006); 16L:8D or longer photophase at 26–27°C (Hansen and Harwood 1968; Butt et al. 1970); 18L:6D (Wildbolz and Riggenbach 1969).

14. Shipping Codling Moth

It is easy to ship codling moth eggs, larvae and pupae (Ashby et al. 1985; Addison and Henrico 2005). However, shipping chilled adults is more difficult given the risk of high mortality if the travel time is long. Shipping relatively few codling moths between laboratories is routine but for SIT operational programmes shipping large numbers of insects may be needed. Recent shipments of chilled adults from Canada to South Africa (Blomefield et al. 2006; Wood and Arthur 2006; Bloem et al. 2007) demonstrated that this is possible. The risks to the receiving country associated with the shipment of sterile insects are considered negligible (Enkerlin and Quinlan 2004; IAEA 2008).

14.1. EGGS

Waxed paper sheets with freshly laid eggs are rolled up (not too tightly), inserted with a source of moisture into a cardboard mailing tube and shipped via a courier service to the destination. Since the development time of these eggs may be no more than five days (depending on the ambient temperature), the distance over which a shipment can be made is relatively short, e.g. within Europe or the Americas. However, eggs have been shipped from Canada to South Africa (Addison and Henrico 2005).

Alternatively, eggs sheets are cut into pieces and packed into polystyrene Petri dishes lined with cotton wool. The dishes are taped together, surrounded with packing material and placed in a suitable-sized box. Eggs are held at 10–15ºC and 60–75% RH (Ashby et al. 1985).

14.2. LARVAE

The codling moth is most safely shipped long distances (overseas) as larvae (Ashby et al. 1985). Larvae are individually packed in polystyrene tubes with an adequate supply of freshly-prepared diet. Tubes are tightly bundled and packed between cushions of packing material such as cotton wool or styrofoam beads. Larvae should be sent as 1st or 2nd instars, and the mode of shipping should be expeditious to prevent diapause. Neonate larvae may be held for up to 36 h in egg-storage containers at 15ºC.

Shipping mature diapausing larvae is easier than shipping non-diapausing larvae (Singh and Ashby 1986; Taret et al. 2006). Diapausing larvae, in cocoons in corrugated cardboard strips, are a convenient stage and they can be shipped in a chilled container (Wood and Arthur 2006).

14.3. PUPAE

Young pupae are packed between cotton wool cushions in Petri dishes or rolled between layers of absorbent paper. RH can be increased by placing a small piece of lightly moistened filter paper in the bottom of each Petri dish. The time between dispatch and arrival should not exceed eight days (Ashby et al. 1985).

Pupae may be held in rearing containers at 15°C for 10–14 days; pupae held at temperatures lower than 15°C may desiccate if they are reared at 20–25°C as larvae (Ashby et al. 1985).

14.4. ADULTS

Shipping chilled adults from Canada to South Africa for field release was successful (Blomefield et al. 2005, 2006; Wood and Arthur 2006). However, the first shipments were kept below 0°C with a consequent reduction in quality of the adults. Increasing the temperature reduced this problem (Wood and Arthur 2006). Initial data showed that air-freighting (67–93 hours in duration) appeared to have little effect on the longevity and mating of the adults (Blomefield et al. 2006).

14.5. LOW-TEMPERATURE EFFECTS ON ADULTS

Ashby et al. (1985) recommended that adults can be stored at 15°C in polystyrene tubes for up to ten days (but fecundity is reduced if adults are held for more than 3–4 days). The quality of chilled insects deteriorates over time (Calkins and Parker 2005; Leopold 2000, 2007; Parker 2005).

Handling procedures (storage, packaging, transport and release) for chilled adults reduced trap catches of the moths by 25–50% (Bloem and Bloem 1995, 2000; K. Bloem et al. 2002; S. Bloem et al. 1998a, 1999c, 2002).

The recapture rate of codling moth males in pheromone-baited wing traps was not affected by cold storage at –2°C for 1 h. However, cold storage at – 2°C for 6 or 12 h for moths reared under different strategies, fluctuating temperatures, constant temperature conditions or diapause rearing, did reveal differences (Jallow and Judd 2007) (section 9.1).

15. Microbial Contaminants, Pests, Pathogens and Parasitoids

There is a constant risk of contamination with microbes or pest insects that attack the diet or with insect pathogens and parasitoids. The most important pathogen for codling moth is the CpGV virus, and it is vital that regular precautions be taken to avoid an outbreak, e.g. covering diet (Howell 1967; Brinton et al. 1969; Batiste and Olson 1973; Addison and Henrico 2005), regulating air pressure, filtering air (Gast 1968), isolating parts of a facility, isolating workers, using antimicrobial chemicals and applying stringent sanitation measures for both equipment and workers.

Sikorowski and Lawrence (1994a, b) defined microbial contamination as harbouring of, or having contact with, micro-organisms without symbiotic or pathogenic relationships. Pathogens are micro-organisms capable of producing disease under normal conditions of host resistance, and which rarely live in close association with the host without producing disease. The most important microbes are contaminant fungi, e.g. *Aspergillus niger* van Tieghem growing in the diet and CpGV that infects larvae (Howell 1972c; Proverbs 1982).

Some bacteria and fungi produce toxins that may harm insects (Sikorowski 1984a). Microbes induce biochemical changes that alter the nutritional value of a diet, reducing insect production and quality; if an epizootic occurs, the colony can be destroyed (Brinton et al. 1969; Howell 1971; Singh 1977; Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994a, b).

Diets are sterilized by cooking, autoclaving, flash-sterilizing, etc. (Shapiro 1984; Cohen 2004) and antimicrobials are added, e.g. methyl paraben, sorbic acid, antibiotics, formaldehyde and NaOCl (Shapiro 1984; Cohen 2004) (section 4.). To maintain insect quality, the smallest effective amounts of an antimicrobial compound should be used. However, heat and antimicrobials do not guarantee freedom from contaminants (Sikorowski and Lawrence 1994a, b). Lowering pH also helps to reduce contamination (Navon and Moore 1971).

UV light (about 260 nm) kills bacteria and thus helps to sterilize surface areas of laboratory benches (Sikorowski 1984a). Due to health hazards, UV lamps can only be used in special rooms or when workers are not present.

Workers are a major source of microbes in a rearing facility and an important source of diet contamination (Sikorowski 1984a, b; Sikorowski and Lawrence 1994a, b) (section 22.7).

According to Stewart (1984) "every failure to meet production quotas … has been directly or indirectly related to the dominating, harmful effects of microorganisms."

There are general reviews of microbial contaminants and pathogens that affect insect rearing (Gast 1968; Goodwin 1984; Shapiro 1984; Sikorowski 1984a, b; Singh 1984; Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994a, b; Sikorowski et al. 2001; Cohen 2004). Goodwin (1984), and Sikorowski and Goodwin (1985) provided keys to assist in recognizing insect diseases in a laboratory.

15.1. MICROBIAL CONTAMINANTS

15.1.1. Bacteria

Bacteria are usually not a problem in mass-rearing the codling moth. However, an antibiotic, e.g. Aureomycin® (chlortetracycline) is often included as a precaution. Prolonged use of antibiotics may lead to the selection of resistant strains of bacteria. The wise use of antibiotics is not a substitute for, but a complement to, good sanitation for disease control (Sikorowski 1984a, b)

15.1.2. Fungi

A . niger is a major threat to codling moth diets (Brinton et al. 1969; Proverbs et al. 1982; Shapiro 1984). It appears as a black powder and spreads rapidly through a tray of diet and to other trays. Larvae usually do not survive in diet that is infected with this fungus. This fungus has two effects (Howell (1971):

- Larvae abandon the affected areas as the hyphae penetrate the diet.
- Larvae become covered with spores and, if extensive, die.

Formaldehyde, methyl paraben and sorbic acid all give good suppression of microbes and are not harmful to adults (Howell 1971). However, it is also very important to control RH and maintain air movement (Howell and Clift 1972). A film of paraffin wax on the diet surface retards dehydration and fungal development (Howell 1967; Ashby et al. 1985) (section 6.1). Also, the addition of propylene glycol retards desiccation. Good sanitation practices (section 22.7) in the rearing areas are important to suppress this fungus (Howell 1971; Wildbolz and Mani 1971; Mani et al. 1978; Shapiro 1984; Ashby et al. 1985; Reed and Tromley 1985).

Spot treatments can be used to treat infection and prevent surface recontamination (Chawla et al. 1967; Wildbolz and Mani 1971). The infected area can be sprayed with 1% sorbic acid in acetone from an atomizer (Howell and Clift 1972). Other materials can also be used, e.g. NaOCl in ethyl alcohol, methyl paraben in ethyl alcohol, or a 1:20 mixture of propionic acid and ethyl alcohol (Mani et al. 1978). The infected area can also be cut out and the exposed edges of the remaining diet swabbed with a mould inhibitor solution (Ashby et al. 1985). Sawdust-based diets are more resistant to bacterial and fungal contamination than agar-based diets (Brassel 1978).

15.2. PEST ARTHROPODS

Scavenger mites (family Ascaidae) can become a problem when rearing on immature apples. To prevent mite contamination, the rearing rooms are emptied after use, cleaned with household ammonia and heated to about 49°C for two days (Toba and Howell 1991).

Severe infestations of an acarid mite *Tyrophagus putrescentiae* (Schrank) can occur which can only be dealt with by isolating adult emergence from larval rearing to prevent movement of mites from old to new diet (Batiste and Olson 1973).

Fruit flies infesting the diet can become a major problem (Mani et al. 1978; Moore 2003), especially if the rearing facility is located near an orchard where fruit flies are abundant at harvest. Production can drop rapidly, even to zero, in cultures infested with *Drosophila* spp. These flies are difficult to eliminate without terminating all rearing (Howell 1971; Howell and Clift 1972).

15.3. VIRAL PATHOGENS

Viruses are a significant threat to successful mass-rearing of lepidopteran insects, especially on artificial diet (Wildbolz and Mani 1971; Brassel 1978; Mani et al. 1978; Bathon 1981; Guennelon et al. 1981; Proverbs et al. 1982; Reed and Tromley 1985; Marti et al. 2007).

The occlusion bodies of the virus that infect the codling moth are granular and hence the name granulosis virus (CpGV). This virus usually has only one rod-shaped virion in an occlusion body (Zimmermann and Weiser 1991). Larvae become infected when they ingest virus particles and they die within a few days.

Bathon et al. (1991) summarized the threat: "Viruses can be transmitted from one generation to the next on the egg surface, externally on contaminated adults and through unhygienic laboratory conditions and techniques. They may infect any larval stage. Common symptoms of the virus diseases are that young larvae die after they have ingested virus from their contaminated eggshell, and that mature larvae show a reduction in feeding and a general sluggishness and may become discoloured. Moribund larvae become flaccid, rupture easily and sometimes turn black at death."

CpGV can be transmitted transovarially. In the OKSIR mass-rearing facility, heat treatment (60°C for three days) of spent diet (section 7.2) was inadequate to inactivate the virus, and even autoclaving the diet at 121°C for 20 min was insufficient. The virus particle is about 314 × 31 nm and the granules are 314 × 208 nm, which is close to the filtering limit of high-efficiency particulate air (HEPA) filters (300 nm). Virulent CpGV was found in the spent diet after disposal even though there was a low incidence of larvae with symptoms in diet trays. It was assumed that most of the virus within the colony was latent, and possibly the heat treatment given to spent diet activated the virus (Cossentine et al. 2005).

15.3.1. Eggs on Sheets

The surface sterilization of eggs is vital to protect against virus infection (Shapiro 1984; Stewart 1984; Reed and Tromley 1985; Bathon et al. 1991; Toba and Howell

1991; Rogers and Winks 1993) (section 12.6). Sikorowski and Goodwin (1985) and Sikorowski and Lawrence (1994a, b) reviewed procedures for sterilizing insect eggs.

15.3.2. Larvae

There is no curative treatment for infected larvae and they die. Rearing larvae individually (and destroying any that show infection) can reduce the level of infection (Rogers and Winks 1993).

15.4. OTHERS

These include infection of larvae with *Bacillus thuringiensis* Berliner (Guennelon et al. 1981) and a microsporidian (*Nosema* sp.) (Bathon 1981). *Trichogramma platneri* Nagarkatti (Bloem et al. 1998b) is a naturally occurring parasitoid of codling moth eggs, and strict precautions must be taken to prevent its entry via air intake ducts or doors.

16. Quality Control

In the past there was a tendency to mass-rear as many insects as possible, as cheaply as possible, with little regard for the quality of those insects (Singh 1977; Proverbs 1982). However, it is essential that mass-reared insects are of high quality, ensuring the efficacy and efficiency of the irradiated insects when released in the field (Beck and Chippendale 1968; Calkins and Ashley 1989; Bloem et al. 1998a, 1999c; Calkins and Parker 2005; Vreysen et al. 2007a). Quality control (QC) is a 'fitness for use' philosophy (Rogers and Winks 1993) and relates to 'biological vigour' (Moore 1985). Effective methods for monitoring and providing feedback on the quality and competitiveness of sterile insects are critical to success (Huettel 1976; Singh and Ashby 1985).

Laboratory colonization, due to natural inbreeding, genetic drift, inadvertent selection and adaptation to rearing conditions, can unknowingly affect insect behaviour in the field and produce insects of reduced quality (Shorey and Hale 1965; Gast 1968; Ferro and Harwood 1973; Bush 1975; Huettel 1976; Pashley and Proverbs 1981; Nunney 1982, 2002; Proverbs 1982; Proverbs et al. 1982; Bartlett 1984, 1985; Collins 1984; Joslyn 1984; Mangan 1992; Calkins and Parker 2005; Parker 2005; Rull et al. 2005; Liedo et al. 2007). Chambers (1977) stated that the "processes most contributory to genetic decay have been identified as the founder effect, inbreeding, genetic drift and selection. In the large populations used as factory breeding stock, selection can be considered to have the greatest impact, and the primary result is a quantitative rather than qualitative change in behaviour. Thus, it may be expected that behavioural thresholds and frequencies are more likely to be altered than are the kinds of behavioural traits themselves."

The measurement of behavioural changes in codling moth colonies should be conducted primarily in the field with emphasis on male dispersal, olfactory response and mating success. Selection and inbreeding tend to affect most deleteriously those traits with the highest adaptive value (Proverbs 1982). Genetic variability in a colony should be maintained (Joslyn 1984). It may also be possible to select for a desired biological trait (Wajnberg 1991; Leppla 1993).

As an integral part of the production system, QC provides a means of optimizing insect mass-rearing by identifying and gradually correcting deficient production processes, thereby preserving the quality of the strain (Leppla and Ashley 1989).

Using allozymes (Bush 1975; Huettel 1976; Chambers 1977; Moore et al. 1985), Pashley and Proverbs (1981) monitored genetic stability in a newly established colony for 25 generations. Significant allozyme frequency changes occurred at three of the five loci surveyed, but no significant decrease in average

heterozygosity with colonization was observed. Four reproductive traits also measured showed no directional changes.

Insect quality **must** take precedence over insect quantity, and facility and programme managers must accept this principle. Measurement of insect behaviour in the laboratory must be linked to its ability to function in the field (Leppla and Ashley 1989; Lux 1991; Leppla 1993; Mutika et al. 2001).

There is a need to establish internationally accepted standards for the quality of mass-reared codling moths (Singh 1977; Rogers and Winks 1993; Cohen 2004) with the wild insect being the standard for comparison (Chambers 1975; Huettel 1976).

The development of adequate standards depends on the ability to measure biologically meaningful traits and relate them to insect performance. These traits must be identified before production specifications or quality-control standards can be established (Webb et al. 1981).

For a tortricid and a pyralid lepidopteran, Ochieng'-Odero (1991) developed mathematical relationships among larval, pupal and adult weights, and between pupal or adult weights and the fecundity of female adults. This information was used to describe pupal and adult indices of quality for laboratory-reared insects.

Insufficient research and methods development have been done on the parameters of competitiveness for male codling moths such as adult longevity, male-female attraction, flight ability, mating compatibility and sperm transfer (Trécé 2007a). However, a parameter that is easy to measure is not necessarily important in the context of the insect's intended role in the field (Huettel 1976). Expensive information that cannot be handled should not be collected, and unneeded tests should not be made just because they are available.

Diet can influence the quality of an insect, e.g. ability to produce sex pheromones and enzymes, and vision (vitamin A in the diet) (Singh 1984).

Many parameters of codling moth development and behaviour that are aspects of process and product control have been noted, e.g. egg hatch, larval survival, duration of larval and pupal development, pupal weight, adult emergence, sex ratio, adult longevity, response of males to pheromone trap, male and female mating ability and fecundity. Tests used to monitor the quality of laboratory-reared insects must be reproducible, economical and simple (Huettel 1976).

A very important aspect of QC is routine monitoring of quality parameters (Huettel 1976; Hathaway et al. 1973; Rogers and Winks 1993). "However, monitoring itself does not correct problems. Each rearing facility should develop SOPs for rearing operations, QC operations and finally responses to adverse QC findings" (Parker 2005).

Rogers and Winks (1993) illustrated case studies of successfully identified problems such as diet deficiency, material contamination, and a lack of response to pheromones and a larval attractant (α-farnesene). These case studies illustrated how an Insect Rearing Management system identified, solved and helped to prevent quality problems.

16.1. CONCEPT OF QUALITY CONTROL

The concept of QC in insect mass-rearing became critical when the field behaviour of released insects was the most important element of a field SIT programme (Webb et al. 1981).

A working group exists that advocates the rearing of quality insects — Arthropod Mass Rearing and Quality Control Working Group (Boller 2002; AMRQC 2007). There is a publication relating to the quality of mass-reared fruit flies (FAO/IAEA/USDA 2003). Boller et al. (1981) developed the 'RAPID quality control system for early warning', a standardized programme for the establishment of quantitative quality profiles for mass-reared Mediterranean fruit flies. The RAPID data were displayed graphically in Shewhart control charts (Calkins and Parker 2005). Chambers et al. (1983) described a series of biological assays of behavioural competence of Mediterranean fruit flies that complements the RAPID quality control system. It was demonstrated that variations detected by the RAPID laboratory tests can be confirmed in field-cage tests.

QC is divided into three categories (Leppla and Ashley 1989; Leppla and Fisher 1989; Bigler 1992; Leppla 1993; Bernon and Leppla 1994; Calkins and Parker 2005, Parker 2005) (section 25):

- Production control: inputs to rearing — diet ingredients, materials, schedules, personnel, equipment, environmental conditions, etc.
- Process control: how things are done — diet preparation, seeding the diet, insect holding and collection, etc.
- Product control: adult insects — evaluation of effectiveness in completing the purpose for which they were reared (in both the rearing facility and the field).

Chambers and Ashley (1984) discussed process analysis (study of the variability of the process) and process-control charts (chronological graphical comparisons of measured product characteristics). Leppla and Ashley (1989) described both Shewhart and three-dimensional graphs. It is essential to relate changes in quality to specific insect stages and associated rearing processes (Leppla and Ashley 1989). Bruzzone et al. (1993) described process control activities for the mass production of Mediterranean fruit flies in Guatemala and used charts to plot data from quality assessments.

For data analysis and charting, expected means and variances are derived from either historical data or an initial large sample taken when the monitored processes and resulting products meet specified tolerances (Leppla and Ashley 1989: Dowell et al. 2005). Charted mean and range values are bound by lines representing the upper and lower control limits, positioned (as in industrial process control) at no more than ± 3 SDs. If production needs to be stable, values from QC tests consistently above or below these limits warn that some part of the production process needs attention. This condition may also exist when test points are not distributed randomly about the expected mean or range.

'Total quality control' (Leppla and Fisher 1989; Leppla 1993; Rogers and Winks 1993) has evolved to encompass the structure and associated mechanisms

for developing and improving product quality and productivity, and involves eight elements: management, research (Huettel 1976; Dame 1989), methods development, material, production, utilization, personnel and QC. Total quality control makes it possible to optimize an entire pest management programme. It provides direction in the persistent search for ways to perfect all of the associated science and technology. Total quality management is a proven and powerful management tool that can provide significant quality control over living organisms (Burt 2002).

The first part of QC as a concept is quality assessment of parameters, e.g. insect weight, flight ability, mating ability. Should any parameter not be satisfactory according to a predetermined standard, then the 'control' in QC follows. The 'control' in QC is a management tool (Calkins and Parker 2005) consisting of:

- Setting quality standards
- Appraising conformance to (or deviations from) these standards (quality assessment)
- Acting when standards are breached
- Planning for improvements in standards

'Control' is achieved when there is constructive feedback, from the QC workers through the programme manager, on activities that may be responsible for the lack of quality (Chambers and Ashley 1984; Calkins and Parker 2005). In well-managed insectaries, problem-solving protocols are outgrowths of the QC programme. The combination of a good QC/problem-solving plan, a good sensitive problem-detection system and a well-oiled feedback system is the heart of high-quality systems operations (Cohen 2004).

The purpose of a QC programme is to maintain quality during the rearing process and not to correct low quality after insects have been reared. However, some remedial actions to reduced quality of reared insects may be possible (Leppla and Ashley 1989; Calkins and Parker 2005).

Production can be improved through testing and acting on feedback (Leppla and Ashley 1989). A consistent schedule for measuring variables relating to QC is needed so that specifications and tolerances are established (Moore et al. 1985). Significant changes signal that something is wrong and needs correcting. However, in some cases, there may not be a direct effect of a change in a parameter on the performance of the adult insect.

QC should be used by production personnel to measure and control their own activities (Chambers and Ashley 1984). This regulatory capability requires routinely gathered accurate information which must be used not only in decisions on whether to accept or reject the product but also in managing the processes that produce the product.

QC procedures (Calkins and Parker 2005) "both incur costs and provide benefits. The costs are in the appraisal costs of evaluating [assessing] inputs, processes and product quality, while benefits accrue from savings associated with avoiding: (1) defects during the rearing process, (2) internal failure costs caused by defective equipment, materials, or substandard rearing ingredients, and (3)

external failure costs caused by allowing defective products to reach a customer, e.g. insects that are incompatible with the target population...”

In case of a conflict of interest, the facility workers who make product quality evaluations report to the programme manager, not the rearing manager. However, those working on product QC must work closely with rearing personnel involved in production and process QC evaluations and provide continuous feedback to maintain an effective rearing process (FAO/IAEA/USDA 2003).

QC publications especially for mass-reared tephritid fruit flies are available (Prokopy et al. 1975; Huettel 1976; Boller and Chambers 1977; Boller et al. 1977, 1981; Chambers 1977; Leppla and Guy 1980; Webb et al. 1981; Chambers et al. 1983; Chambers and Ashley 1984; Webb 1984; Moore et al. 1985; Schwarz et al. 1985; Calkins and Ashley 1989; Dame 1989; Leppla and Ashley 1989; Leppla and Fisher 1989; Lux 1991; Ochieng’-Odero 1991; Wajnberg 1991; Bigler 1992, 1994; Bruzzone et al. 1993; Leppla 1993; Maki and Gonzalez 1993; Bernon and Leppla 1994; Bigler 1994; Boller 2002; Burt 2002; Nakamori 2002; Nunney 2002; FAO/IAEA/USDA 2003; Calkins and Parker 2005; FAO/IAEA 2006; AMRQC 2007; Barnes et al. 2007; Cáceres et al. 2007).

Except for Hathaway et al. (1973) and Rogers and Winks (1993), little substantial QC work has been done on the codling moth. Nevertheless, many workers have reported on various quality parameters (Brinton et al. 1969; Hathaway et al. 1971; Navon and Moore 1971; Pristavko and Boreyko 1971; Wildbolz and Mani 1971; Howell 1972c, 1981; White et al. 1972; Butt 1975; Proverbs et al. 1975; Robinson and Proverbs 1975; Singh 1977; Mani et al. 1978; Pristavko et al. 1978; Guennelon et al. 1981; Ashby et al. 1985; Reed and Tromley 1985; Bathon et al. 1991; Reiser et al. 1993; Bloem et al. 1997, 1998a, 2000, 2002, 2004; K. Bloem et al. 2002; Vreysen and Hendrichs 2005).

16.2. ROUTINE VS PERIODIC QC TESTS

Routine QC tests are those tests that are very important for product QC and are relatively easy to do in a rearing facility, e.g. pupal weight, percentage adult emergence and percentage egg hatch. Periodic QC tests are those tests that, because of the nature of the test, can be made only periodically when conditions are suitable, e.g. tests in the field and tests involving wild insects. Field measurements are expensive, time consuming and not readily adaptable to routine quality testing (Stewart 1984).

17. Production Quality Control

17.1. DIET INGREDIENTS

Since the source, content and consistency of dietary ingredients change over time, the suitability of these ingredients can change, but if the concept of an 'ingredient cycle' (section 4.8) is followed the problem is minimized. Also, for any new format or supplier of an ingredient, preliminary testing (bioassay) on a small scale should be done to ensure acceptability.

Moore et al. (1985) recommend the following:

- Ingredients are purchased from the same supplier, in quantities that last for a minimum of six months.
- On arrival of a new batch, a sample of the ingredient is stored at –6°C. In the event of a diet contamination problem, appropriate samples can be examined for bacteria and fungi to determine the source of the contamination (Brewer and Lindig 1984; Shapiro 1984).

It is important to obtain information from suppliers about the quality of their products (Bernon and Leppla 1994). Most suppliers will furnish, on request, detailed technical data on products, product quality, and recommended shelf life and storage conditions (Brewer and Lindig 1984).

As diet components age, they deteriorate due to changes in water content and activity, oxidation, loss or gain of volatile components, microbial growth and enzyme-mediated chemical changes. Therefore, where feasible, the chemistry of ingredients should be checked (Cohen 2004). Physical and chemical tests on diet ingredients can be made to ensure quality:

- Meals (especially wheat germ) and flours from plant materials are subject to oxidation, peroxidation, enzymatic destruction and microbial deterioration, etc. Tests of these materials include water content and water activity tests, lipid peroxidation, microbe counts and sensory qualities (visual inspection for crusting, gumming, caking and moisture; off-odours). Also, they should be monitored for pesticide contamination (Brewer and Lindig 1984; Rogers and Winks 1993).
- There are specific tests for vitamins, minerals, sugar, gelling agents, amino acids, sterols (especially cholesterol), buffers and antimicrobial agents. The most likely to degrade are vitamins (especially ascorbic acid) and the gelling agents. The FRAP (ferric-reducing antioxidant power) test can be easily modified to assess the total antioxidant level and the ascorbic acid content.
- Long-chain fatty acids, especially unsaturated, are very susceptible to lipid peroxidation; this can be determined using the thiobarbituric acid (TBA)

test. In this test the number of µg/g diet of malondialdehyde (a primary breakdown product of peroxidation) is measured.

- Gelling material (agar or carrageenan) can be tested for gel temperature and gel strength. For the former, a sensitive thermometer is placed in a container with a standard mixture of recently heat-activated and molten material, and the temperature at which the gel forms is noted. The gel strength test uses a special device that measures the pressure (in g/cm^2) required to penetrate the surface of a formed gel.

For Mediterranean fruit fly rearing in Guatemala, the quality of the agricultural by-products used in the diet (sugar cane bagasse and wheat bran) was assessed regularly for density, porosity, particle size characterization, acidity, pH and moisture content (Bruzzone et al. 1993).

Pharmaceutical-grade materials are used to rear the pink bollworm (Stewart 1984). When feasible, and before being used, each lot or batch is sampled and assayed by QC personnel or by a commercial laboratory. The quality of wheat germ is a problem because it is relatively unrefined, and prolonged storage causes it to become unstable. It is the most variable ingredient (in terms of protein and oil content) in the diet. Agar is another highly variable ingredient, and gel strength, gel temperature, viscosity and water-holding capacity should be tested, as well as moisture content and particle size of the dry material.

Animal feed-grade items are cheaper than pharmaceutical-grade items but will likely not have the same guarantee of quality.

The amount of testing that is appropriate is a judgment that must be made by rearing facility managers. If the rearing programme is critical, all incoming shipments of ingredients should be tested (Brewer and Lindig 1984). Bioassays are easier to conduct than chemical analyses, and they measure how the ingredient affects the insect rather than simply defining the ingredient.

17.2. ENVIRONMENTAL CONDITIONS

Sometimes environmental conditions are selected more with regard to economics of production and human convenience than for insect quality. This may affect development rates, circadian rhythms, mating habits, flight capabilities, etc. and hence quality (Moore et al. 1985).

17.2.1. Temperature

Recording (e.g. every 15 min) the temperature in all rearing rooms, and keeping these records on file indefinitely, is vital to being able to determine the cause of a temperature-related problem and correct it. Computer-operated temperature controllers and digital recorders are nowadays common features of rearing facilities. Temperature records can be obtained using a data logger which shows the time associated with all recordings of environmental parameters.

17.2.2. Relative Humidity

Control of RH is vital to regulate the rate of drying of the diet, to prevent the growth of mould in the diet, and to create a physical environment suitable for egg hatch, adult emergence and oviposition. Immediately after diet preparation, evaporation of water from the diet requires dehumidification in the rearing room. Also, incoming air may require dehumidification. At other times, humidification may be needed using steam rather than water because it is sterile. As for temperature, the RH in each rearing room must be monitored and recorded.

17.2.3. Light and Photoperiod

Light in a rearing room establishes the photoperiod, and light intensity does not need to be high (sections 9.1 and 13) to regulate diapause development. Lamps should be placed vertically so that light illuminates all trays on a cart (**Figure 9**). It is important to set the photoperiod, especially the time of sunset, to coincide with that existing in the field where the insects will be released. In the codling moth, sensitivity to the photophase appears to occur in the late larval stages (Chambers 1977). The light regime for each rearing room must be monitored and recorded.

17.2.4. Air Movement and Pressure

Air movement is critical to creating conditions for the appropriate rate of drying of the diet and to prevent the growth of mould. The air speed over the trays of diet, and the number of air changes per hour, are important physical parameters of air-handling (section 9.1).

Preventing the entry of contaminants and pathogens into the facility is very important. The air entering a room must be filtered with a HEPA filter (sections 15.3, 17.2.5, 22.6 and 22.7). Positive air pressure is also needed in rooms where fresh diet is dispensed and larvae are reared, i.e. the 'clean' part of the facility. Whenever a door or pass-through between the clean part and the 'dirty' part or the outside are opened, positive pressure moves the air from the clean area to the dirty area and not vice versa.

17.2.5. Cleanliness of Air and Surfaces

Monitoring microbial levels evaluates the effectiveness and quality of contamination control activities (Sikorowski 1984b). Properly cared-for equipment, instruments, walls, floor, etc. are minor sources of microbial contamination (Sikorowski 1984a). The microbial content of the air in an area usually reflects the total microbial contamination of the surrounding area. Air sampling is the best indicator of microbial contamination in a rearing facility (Sikorowski and Lawrence 1994a). Air samples should be taken once a month in several locations (Moore et al. 1985).

The greatest number of microbes in the air normally occurs during peak human activity, and the highest number of microbes can be detected on equipment, walls and floors in the morning (Sikorowski and Lawrence 1994a).

Procedures have been established to check air quality for aerobic microbial contaminants (Roberson and Wright 1984; Sikorowski 1984a, b; Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994a, b; Moore 2003; Cohen 2004):

- Prepare trypticase soy agar (TSA) in Petri dishes and place in rearing rooms and the diet dispensing area. Uncover the dish for 10 min and then recover. Incubate the dishes for 48 h at 36°C and then record the number of colony-forming units (CFUs) per dish. A colony count of more than ten CFUs per dish indicates an air-quality problem (Cohen 2004). Identification of the microbe may help to find the source of the problem (Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994b).

  Sikorowski and Lawrence (1994b) extended the test: The Petri dishes are uncovered for 2–12 h, then covered; half of the dishes are incubated for 24–48 h at 36°C for bacteria, and half are incubated for seven days at room temperature for fungi (fungal mycelia can usually be detected in 2–3 days).
- Sikorowski (1984a, b) and Sikorowski and Lawrence (1994a) described a procedure to monitor the microbial content of air. Air, 100 L, is drawn through a membrane filter (0.45 μm), and the filter is placed in a dish with bacteriological or mycological broth and incubated at 35–36°C for 24 h for bacteria and at room temperature for 48 h for fungi. The filters are removed, dried, stained with methylene blue solution (0.5% methylene blue in 100% ethyl alcohol) for 30 sec and redried. The filter is examined with a microscope at a 100x magnification.

TSA nutrient agar detects bacteria and Sabouraud maltose agar detects fungi (Sikorowski 1984b).

For the pink bollworm, it was found that moth scales could carry pathogens (Stewart 1984), and a simple air-sampling technique was used to monitor rates of scale fallout in various areas of the rearing facility. Filter papers (with grid lines) in Petri dishes were exposed to the air for 24 h and then scales on these papers were counted. Also, on a regular basis, dishes with agar were exposed to the air to monitor bacteria.

It is important to keep the working surfaces and equipment in the diet preparation area clean and free of microbial contaminants and pathogens. A surface assay should be made once a week (Cohen 2004). Two types of surface assessment can be made, the second being the simplest but only 50% as efficient as the first (Cohen 2004):

- Using sterile materials, wet swabs with sterile water and swab surfaces. Then streak the swabs on TSA with lecithin and polysorbate 80 medium, cover the dish and incubate at 26°C for 48 h. [Lecithin and polysorbate 80 inactivate residual disinfectant collected with the specimen.]
- Replicate organism direct agar contact (RODAC) method (also used with TSA and lecithin and polysorbate 80 medium). The dish full with medium is opened and placed flat onto the surface to be checked, then covered and incubated at 26°C for 48 h.

Three replicates of each surface are made.

Earlier work (Sikorowski 1984a, b; Sikorowski and Goodwin 1985; Sikorowski and Lawrence 1994a, b) described two similar methods of making surface assays — swab-rinse method (as above except that the swab is rinsed in sterile dilutant and this rinse fluid is plated on an appropriate culture medium) and agar contact method (RODAC method described above except that the incubation temperature is 36–37°C for bacteria and room temperature for fungi). The agar contact method produced more reproducible results than the swab-rinse method.

When observations are completed, microbial cultures must be killed in an autoclave or pressure cooker and disposed of using biohazard containers.

Roberson and Wright (1984) prepared touch plates with TSA growth medium and pressed them against surface areas of materials and equipment to show levels of microbial contamination.

17.3. EQUIPMENT OPERATION

Engineering staff maintain environmental control equipment and the machines that prepare and dispense diet, hold and transport infested diet, collect adults and eggs, etc. Equipment that is used for diet preparation and dispensing must be sterile (disinfected regularly, autoclaved, etc.).

Daily checks of records of the environment of each rearing room are required, and any greater-than-expected deviation in the environmental conditions must be investigated; if needed, the relevant equipment must be repaired to prevent a breakdown.

Regular maintenance work must be done on a timely basis to ensure quality production:

- service systems for lighting, heating/cooling, humidification/dehumidification, air handling and refrigeration;
- service air filters and replace as appropriate;
- service equipment for preparing and dispensing diet;
- service equipment for adult collection, egg collection, tray/cart washing and waste disposal.

17.4. PERSONNEL

The training, skill and attitude of workers in a mass-rearing facility are critical to achieving high-quality production. They must be trained, highly skilled and motivated individuals who understand and appreciate that quality is more important than quantity. Special training in quality assessment procedures, e.g. microbial contamination (Sikorowski 1984b), is necessary.

Workers in QC must be dedicated to that activity and not be influenced or distracted by the daily routine demands of production work. The QC team should be supervised by the programme manager and not the rearing manager, thereby avoiding a potential conflict of interest between quality and quantity (section 16.1.).

Clean areas of a rearing facility should have only the required number of workers who are dressed in appropriate clothes, and other workers in the facility should not be allowed to enter these areas.

18. Process Quality Control

The process of mass-rearing insects involves diet preparation, diet seeding, larval and pupal rearing, and collection of larvae, pupae, adults and eggs. This section discusses QC of these rearing activities.

Process control is regulated by check lists and colony records. For example, if low larval establishment is recorded, information should be available about who inoculated the larvae, which batch of diet was used, the rearing conditions and which generation the larvae came from. These data are then used to investigate quality problems (Rogers and Winks 1993).

Tables 9, 10, 11, 12, 13 and 14 provide published data on quality assessments. Table 13 covers rearing on immature apples. Standards for some quality parameters are suggested.

18.1. DIET PREPARATION

Besides QC tests of diet ingredients, Cohen (2004) listed five useful tests on the prepared diet: water or dry matter content, antioxidants (by the FRAP test), microbe counts, a visual (macroscopic and microscopic) inspection to determine homogeneity of particles and pH. Physical characteristics of the prepared diet, such as viscosity, colour and texture, can be checked (Brewer and Lindig 1984).

18.1.1. Water Content

Water added to the diet is usually a fixed volume, but the finished diet from batch to batch will not have a constant water content due to varying levels of moisture in the diet ingredients, e.g. sawdust and soybean meal. Therefore, the finished diet should periodically be monitored for water content. Diet subsamples can be pre-weighed and then post-weighed repeatedly after storage in a drying oven until they reach a steady weight (Cohen 2004).

18.1.2. Viscosity

Water content of the diet is not constant from one batch to another and viscosity will vary. Nevertheless, workers making diet should seek to produce consistent batches with the same viscosity. It is most important to get the correct consistency, and this can be achieved only through experience (Brinton et al. 1969).

18.1.3. pH

Even though the pH of the diet, e.g. 4.6 (Moore 2003), should remain rather constant from batch to batch, it is advisable once a day to check pH. A standard pH meter is used with either a probe that is capable of measuring gels or a standard probe for liquid diets (Cohen 2004).

TABLE 9
Quality Assessment Data on Artificial Diets that Stay Moist and Soft (Part 1)

Parameter	Publication									
			Rock 1967		Information from Butt (1975) and Hatmosoewarno and Butt (1975)					
	Redfern 1964	Hamilton and Hathaway 1966 (Nr 2)	Casein diet	Wheat germ diet	Shorey and Hale 1965	Navon and Moore 1971	Bulytginskaya (Russia)	Falcon (USA)	Anderson (Australia)	Howell (USA)
Diet										
Yield (pupae/kg diet)					206	250	230–250	150	238	100 (larvae)
Eggs										
Egg hatch (%)	65.5[1]				53.1	61, 74.5				
Larvae										
Survival (%)	45[1]				89	50				
Larval duration (days) [°C]		34 [27.8]			25[2] [25]	16 [27]	15 [25]	10–14 [25]	24.7 [24]	18.9 [28]
Pupae										
Survival (%)	74[1]					100				
Pupal weight (mg) male	29[1]				24–25					34.7
Pupal weight (mg) female	38.3[1]				28–32					44.5
Adults										
Egg to adult duration (days) [°C]		29–54 [27.8]					30–32 [25]			

Larva to adult duration (days) [°C]	male	33[1] [26.7]		28.2 [27.2]				
	female	33.2[1] [26.7]		28.1 [27.2]				
Survival from neonate larva to adult (%)		60 33[1]	60	79	55–65	83		
Adult weight (mg)	male	16[1]						
	female	25[1]						21–23
Sex ratio (male:female)						1.7:1	1.2:1	
Mated females (%)						40		
Longevity (days) [°C]	male	6.6[1] [26.7]					4–15[3] [27]	
	female	9.5[1] [26.7]						
Fecundity (eggs/female)		38.8[1]				42.1	48.5 118.7	

[1] Data obtained by Hathaway et al. (1971) when the diet of the listed publication was tested.

[2] Data from Hatmosoewarno and Butt (1975) — larval development of males was 28.2 days, and that of females was 32 days (at 26°C).

[3] Sex not stated in publication.

TABLE 10
Quality Assessment Data on Artificial Diets that Stay Moist and Soft (Part 2)

Parameter		Navon 1968	Sender 1969	Sender 1970	Huber et al. 1972	Shumakov et al. 1974 (see Singh 1977)	Bathon 1981	Guennelon et al. 1981
Diet								
Yield	adults/kg diet					230	57.2[1]	333 250 156 510
	adults/L diet							375[2]
Eggs								
Egg hatch (%)		68.8[3]	73	70				75
Egg duration (days) [°C]			7 [20]					
Larvae								
Survival (%)		37[3]			70–80			
Pupae								
Survival (%)		74[3]						
Pupal weight (mg)	male	24.3[3]					37	
	female	28.8[3]					47	
Pupal duration (days) [°C]			11[4] [25]					
Adults								
Egg to adult duration (days)						29		
Larva to adult duration (days) [°C]	male	35[3] [26.7]	31[4] [25]	32[4] [25]				
	female	35.1[3] [26.7]						
Survival from neonate larva to adult (%)		20–30 28[3]	88	83				77 44 17 40
Adult weight (mg)	male	13.7[3]				14.3		24[2]
	female	20.1[3]				21.7		37[2]
Mated females (%)			65	86				68
Longevity (days) [°C]	male	6.5[3] [26.7]	15 [20]	15 [20]				
	female	8.3[3] [26.7]	20 [20]	20 [20]				
Longevity in the field (days)	male							
	female							9.3
Fecundity (eggs/female)		73.9[3]	160	130		144	230	90–150 39.4

[1] Data obtained by Reiser et al. (1993) when the diet of the listed publication was tested.
[2] Data obtained by D. Stenekamp (pers. comm.) of South Africa when the diet of the listed publication was used.
[3] Data obtained by Hathaway et al. (1971) when the diet of the listed publication was tested.
[4] Sex not stated in publication.

TABLE 11

Quality Assessment Data on Artificial Diets that Stay Moist and Soft (Part 3)

Parameter		Publication						
		Hatmosoewarno and Butt 1975 (CW) (Vanderzant 1966)	Hatmosoewarno and Butt 1975 (MB) (Burton 1969)	Reed and Tromley 1985	Howell 1970	Howell 1971	Howell and Clift 1972	International Minerals and Chemical Corp. (Information from Hathaway et al. 1971 and Butt 1975)
Diet								
Yield	pupae/kg diet							100–150
	adults/L diet				250[1]	80[2]	81 39	
Eggs								
Egg hatch (%)		57.9	55.6	85	80.3 82[3]	71.6 12[4] 50.6[4]	72.2 45.6	76.5
Egg duration (days) [°C]				5 [28]	5–7 [27]			
Larvae								
Survival (%)		85	82		91.7 61[3]	25.9[4] 47[4]		61
Larval duration (days) [°C]	male	22.7 [26]	26 [26]	15[5] [28–29]	18.9[5] [27]			
	female	22.3 [26]	28.5 [26]					
Pupae								
Survival (%)					96 78[3]	100[4] 92.7[4]		77
Pupal weight (mg)	male	29.6	31.5	23.3	34.7 28.5[3]	34.7[4] 36.1[4]		30.7
	female	39.1	37.7	35.8	44.5 36.6[3]	44.5[4] 43.6[4]		41.2
Pupal duration (days) [°C]	male			9 [28–29]	9.1–18.4[5] [27]			
	female			7 [28–29]				
Adults								
Egg to adult duration (days) [°C]	male			24 [28–29]		31–32[5] [27]		33.1[6] [26.7]
	female			22 [28–29]				33.6[6] [26.7]
Larva to adult duration (days) [°C]	male				30.6[3]♂ [26.7]		30.8[5] [28]	32.6 [26.7]
	female				31.2[3]♀ [26.7] 28.9[5]♀ [27]			32.9 [26.7]
Survival from neonate larva to adult (%)		76	65	97	88.3 47[3]	91.7 20.9		47[3] 48[7]

TABLE 11 **(continued)**
Quality Assessment Data on Artificial Diets that Stay Moist and Soft (Part 3)

Parameter		Publication						
		Hatmosoewarno and Butt 1975 (CW) (Vanderzant 1966)	Hatmosoewarno and Butt 1975 (MB) (Burton 1969)	Reed and Tromley 1985	Howell 1970	Howell 1971	Howell and Clift 1972	International Minerals and Chemical Corp. (Information from Hathaway et al. 1971 and Butt 1975)
Adult weight (mg)	male				15.1 15.3[3]			17.7
	female				25.5 24[3]			27.8
Sex ratio (male:female)		0.8:1	0.8:1	1:1				
Mated females (%)		46.7	66.7	98				
Longevity (days) [°C]	male				7[3] [26.7]			6.5 [26.7]
	female			6–7 [28]	6.1[3] [26.7]			8 [26.7]
Fecundity (eggs/female)		36.8	73	90–100	131.1 76.4[3]	88.1 53.9[4] 71.4[4]	109	69.7

[1] Howell (1967) reported that about 250 larvae needed 1 L diet (in trays).

[2] If reared on immature apples, 1 L apples yielded 42 adults.

[3] Data obtained by Hathaway et al. (1971) when the diet of the listed publication was tested.

[4] Information from Howell 1972c.

[5] Sex not stated in publication.

[6] Average time (oviposition to adult emergence) on six different diets. Information from Howell 1991.

[7] For comparison, insects reared on immature apples could be considered as ideal; Hathaway et al. (1971) observed that the weight of female pupae was 43.4 mg, and of female adults 29.1 mg; Rock (1967) observed 90% survival on immature apples.

18.1.4. Microbial Contamination

Most rearing facilities use a combination of heat (about 80°C) and antimicrobials in the insect diet to render it partially free of contaminants (sections 15 and 15.1). It is crucial to assess the diet for excessive amounts of microbial contamination. The protocols for assessing microbial counts in diets are more complex than those used for counts of microbes in the air and on laboratory surfaces (section 17.2.5). Cohen (2004) recommends two approaches:

- Through regular and careful observations (visual and olfactory), workers in the diet and larval rearing areas should learn to detect, at an early stage of infection, the presence of bacteria or fungi in the diet. Also, regular inspection of spent diet should be made, looking for larvae that have died from CpGV (Moore 2003).
- Use microbiological media:

1. Using sterile instruments, apply freshly made diet to a plate containing trypticase soy agar (TSA) and cover.
2. Incubate the plate for 48 h at 36°C.
3. Inspect the plate for the formation of microbial colonies next to the diet.
4. If microbe colonies are too numerous to count, serial dilutions may be required.

Bruzzone et al. (1993) described a regular sampling programme in which diet was sampled five times: after diet mixing and dispensing, after 72 h, after 120 h, prior to thermal treatment in a cooking (100/110°C) extrusion device (approximately 192 h) and after this treatment. The following parameters were then assessed:

- Microbiological assay on larval diet: total count of mesophilic micro-organisms (CFU/g).
- Analytical assays on larval diet: density, porosity, acidity, pH and moisture content.

18.1.5. Yield per Kilogram of Diet

Tables 3, 4, 9, 10, 11, 12, 13 and 14 give figures for the yield of various diets. Even though a yield of 200 pupae or adults/kg or /L of diet would be a good objective for a mass-rearing programme (section 4.9), yields are only one indicator of productivity. Other important indicators are the cost per 1000 adults (Tables 3 and 4) (section 4.11) and the quality of produced adults (section 19).

18.2. EGGS

18.2.1. Eggs/cm^2 of Egg Sheet

Data on mean egg density on egg sheets, and on mean percentage egg hatch, are needed to calculate the mean number of neonate larvae that infest a tray of diet. These should be calculated at least once/week, and they can be related later to larval survival and adult production in the relevant batches of diet. To optimize adult production, the optimum mean number of eggs on an egg sheet should be determined for each rearing system, and then practices relating to mating and fecundity and to handling oviposition cages can be adjusted accordingly.

18.2.2. Percentage Egg Hatch

The most important factors affecting egg hatch are temperature, RH and the age of the female. The number of inviable eggs increases as the female ages (Howell 1981) (**Figure 20**). Some reduced egg hatch is presumably due to non-fertilization of eggs. In South Africa, the egg hatch is usually between 70 and 80% (D. Stenekamp, pers. comm.) and in Syria between 78 and 85% (Mansour 2002).

Bloem et al. (1997) described a procedure for assessing fecundity and fertility. Individual newly emerged females were paired with virgin males in clear 200 mL plastic cups with lids and a wick for water. The pairs were allowed to mate and oviposit at 25°C, 16L:8D and 50% RH until the female died. The cups were

TABLE 12
Quality Assessment Data on Artificial Diets that Dry Out and Harden

Parameter		Publication										
						Information from Butt (1975)					Mani et al. 1978	
		Brinton et al. 1969[11]	Brinton et al. (1969) diet tested by Hathaway et al. (1971)	Brinton et al. (1969) diet used by Dyck in 1993 (Bloem et al. 2007; OKSIR 2007)[3]	Howell 1972c	Howell 1972c	Anderson (Australia)	Brinton et al. 1969	Mani/Charmillot (Switzerland)	Wearing (New Zealand)	Nr 1 (Brinton et al. 1969)	Ashby et al. 1985
Diet												
Yield	pupae/kg diet						81		100-200			
	adults/kg diet							121		77	170	178
	adults/L diet	200[1] 201[2] 380[2]		263[3] 441[3]	18[4] (larv.)							
Eggs												
Egg hatch (%)		87 74 75[10]	72		33.3						89	95
Egg duration (days) [°C]		7[9] [25]										6.5 [25]
Larvae												
Survival (%)			39		57.4							
Larval duration (days) [°C]		22–26[9] [25]					29.5 [26]					24 [25]
Pupae												

Survival (%)			75		93.8						
Pupal weight (mg)	male	26–40 40.3[10]	24.8		39.9			32.2 27.6		29.1	29.9
	female	30–50 49.9[10]	29.1		52.2	52.2		40.4 32.2	37.2	35.6	36.5
Pupal duration (days) [°C]		8[9] [25] 8.8[10] [28]									8 [25]
Adults											
Egg to adult duration (days) [°C]		28 [27]					32 [28]			31 [27]	
Larva to adult duration (days) [°C]	male		36.7 [26.7]		24–36[5] [31]			30[5] [27]	31[5] [28]		32[5] [25]
	female		37.6 [26.7]								
Survival from egg to adult (%)		52 56[10]								30	
Survival from neonate larva to adult (%)		16[2]	30[6]	39[7] 52[7]	41						
Adult weight (mg)	male	17.3 20.7 16.6[2]	12.8				18.5				
	female	26.7 31.9 23.7[2]	19.1				28		24.7		
Sex ratio (male:female)		1.08:1									1:1
Spermatophores received (nr/female)		2 2.4								0.9	
Response of male to calling female (%)		24[8] 20[8]									

TABLE 12 (**continued**)
Quality Assessment Data on Artificial Diets that Dry Out and Harden

Parameter		Publication										
						Information from Butt (1975)					Mani et al. 1978	
		Brinton et al. 1969[11]	Brinton et al. (1969) diet tested by Hathaway et al. (1971)	Brinton et al. (1969) diet used by Dyck in 1993 (Bloem et al. 2007; OKSIR 2007)[3]	Howell 1972c	Howell 1972c	Anderson (Australia)	Brinton et al. 1969	Mani/Charmillot (Switzerland)	Wearing (New Zealand)	Nr 1 (Brinton et al. 1969)	Ashby et al. 1985
Longevity (days) [°C]	male	15 [26]	5.4 [26.7]									
	female	10 [26]	6.5 [26.7]									
Fecundity (eggs/female)		114 145 146[9] 86[9]	44.4		80						105	97

[1] If reared on immature apples, 1 L apples yielded 50 adults.

[2] Data obtained by Batiste and Olson (1973) when the diet of the listed publication was tested. 380 adults/L diet is calculated from 0.38 adults/mL diet.

[3] It is assumed that the diet used in the OKSIR Program is the same as, or similar to, that used by Dyck in 1993 (Proverbs et al. 1982; Bloem et al. 1997; Bloem and Bloem 2000; K. Bloem et al. 2005). Values of yield (Bloem et al. 2007) are for 1994 and 2004.

[4] Howell (1972c) estimated larval production at 18/L diet, and also at 42/L apples.

[5] Sex not stated in publication.

[6] High pupal mortality occurred in this experiment.

[7] In 1993, 39%, and in 1997, 52% (Bloem et al. 1997).

[8] Irradiated males released.

[9] Data obtained by Singh and Ashby (1985) when the diet of the listed publication was tested.

[10] Data obtained by Taret et al. (2007) when a modified version of the diet of Brinton et al. (1969) was tested.

[11] Using a modified version of the diet of Brinton et al. (1969), Taret et al. (2007) obtained a mean adult (sex not stated) weight of 29.2 mg.

incubated at the same conditions for a further seven days when the total egg number and number of hatched eggs were counted. Rogers and Winks (1993) used a similar procedure.

18.2.3. Duration of Egg Development

Depending on the incubation temperature, eggs develop in about 5–7 days.

18.3. LARVAE

This section describes procedures that can be used to determine the number of mature larvae (or the number of pupae formed) per unit of diet (sections 4.9 and 18.1.5).

18.3.1. Larval Survival

The percentage of larval survival (percentage pupation) is obtained by counting the number of pupae formed from a known number of neonate larvae placed on the diet. These data are not easy to obtain in mass-rearing so average numbers, or special trays with a known number of larvae placed onto the diet, may have to be used. If the diet is dry and hard following pupation, it is broken open and the pupae are extracted and counted. However, if the diet is moist and soft and the larvae exited the diet and spun cocoons in provided corrugated cardboard strips, then pupae in the strips can be extracted and counted.

If neonate larvae do not infest fresh diet and instead wander from the diet and die, the attractiveness of the diet should be investigated (section 5.6). However, it is also possible that insects 'lose' the ability to be attracted to a feeding stimulant, e.g. neonate larvae from a laboratory colony appeared to have a weak ability to respond to α-farnesene, an apple volatile to which wild insects showed a much stronger response (Rogers and Winks 1993; Bradley and Suckling 1995).

A larval survival of 80% is acceptable.

18.3.2. Duration of Larval Development

Even though temperature and the diet strongly influence the rate of larval development, development can be quite prolonged when mass-rearing in trays of diet because there are always stragglers that increase the average duration.

If the diet is moist and soft and larvae exit and spin cocoons in cardboard strips, the daily provision of new strips (and examination of removed strips) gives an opportunity to measure the duration of larval development by determining each day how many cocoons are formed.

However, if larvae spin cocoons and pupate in the diet, it is not possible to see when individual larvae form a cocoon and then pupate. Instead, the combined survival of larvae and pupae has to be determined.

The usual duration of larval development is about 15–25 days.

TABLE 13
Quality Assessment Data on Rearing on Immature Apples

Parameter	Publication										
	Dickson et al. 1952	Hamilton and Hathaway 1966	Rock 1967	Brinton et al. 1969	Howell 1970	White et al. 1970	Pristavko and Boreyko 1971	Fossati et al. 1971	Hathaway et al. 1971	Howell 1971	White and Hutt 1971
Diet											
Yield (adults/L apple)				50						42	
Eggs											
Egg hatch (%)	60			77 71.3	76.5	66.2	75.8	87.2	82		66.4 63.4
Egg duration (days) [°C]					5–7 [27]						
Larvae											
Survival (%)									55		
Larval duration (days) [°C]					17 [27]						
Pupae											
Survival (%)									87		
Pupal weight (mg) male				24–40	34.1 35.9				34.5		
Pupal weight (mg) female				28–53	42.2 42				43.4		
Pupal duration (days) [°C]					10.5–18.8 [27]						

Adults										
Egg to adult duration (days) [°C]		28 [29]		27 [27]						
Larva to adult duration (days) [°C]	male		27.9 [27.2]						31.1 [26.7]	
	female		29.1 [27.2]						32.1 [26.7]	
Survival from neonate larva to adult (%)			90					57.7	48	
Adult weight (mg)	male			20.8	18.8				19.8	
	female			32.2	28.6				29.1	
Sex ratio (male:female)				1.02:1			1.1:1	1.1:1		
Spermatophores received (nr/female)				2.4 3.1 3.6		2.62	1.8	2.4		
Response of male to calling female (%)				18[1] 17[1]						
Longevity (days) [°C]	male			18 19 [26]		7.88 [26]	13.5 [23]	14.7 [27]	7 [26.7]	9 8.8 [26]
	female			13 [26]		9.02 [26]	14.3 [23]	11.4 [27]	7.1 [26.7]	9.2 [26]
Fecundity (eggs/female)		20	20 80	199 132	117.8	102.8	184.5		75.7	121.8 121.6

TABLE 13 (CONTINUED)
Quality Assessment Data on Rearing on Immature Apples

Parameter		Publication						
		Howell 1972c	White and Hutt 1972	White et al. 1972	Hathaway et al. 1973	Charmillot (Switzerland) from Butt (1975)	Hutt and White 1975	White 1975
Eggs								
Egg hatch (%)		47.6	54.3 66.4	66 81	53 67			84
Larvae								
Survival (%)		62						
Larval duration (days) [°C]						28 [25.5]		
Pupae								
Survival (%)					95			
Pupal weight (mg)	male	35.8			39.7	34.5		
	female	43.9			48.9	42.4		
Adults								
Rate of population increase					8			
Adult weight (mg)	male				22.8			
	female				35			
Mated females (%)					91.7		90	
Spermatophores received (nr/female)			1.6 1.5	1.54 2	2.3		1.4	
Longevity (days) [°C]	male		10.1 7 [26]	7 7.4 [26]				
	female		10 7.4 [26]	7.4 7.2 [26]				
Fecundity (eggs/female)		82.4	112.6 119.3	119 135	94			

[1] Irradiated males released.

18.4. PUPAE

18.4.1. Number of Pupae/cm^3 of Diet

Sections 4.9, 18.1.5 and 18.3.1 discuss material relevant to this topic.

18.4.2. Pupal Weight

The procedure described in section 18.3.1 can be used to obtain pupae for weighing. Pupae are separated by sex (section 10.3) and weighed (individually or as a group), and the mean weight per pupa of each sex is recorded. Examples of graphing pupal weights (Shewhart chart) are shown in Bruzzone et al. (1993) and Rogers and Winks (1993).

The mean weight of male pupae should be about 31–35 mg or heavier and that of female pupae about 39–43 mg or heavier.

18.4.3. Empty Pupal Cases/cm^2 of Diet Surface

This parameter is a measure of adult production when using trays of diet that dry out and harden (sections 2.4, 4.5 and 6.4). About four trays are randomly selected from each day's spent diet, and the empty pupal cases that project above the diet in a portion (e.g. one quarter) of each of these trays are counted. This number can then be used to calculate the mean adult production per tray of diet. These data can then be related to the number of moths collected from the emergence room (sections 11.1 and 18.5.2).

18.4.4. Pupal Survival

Pupal survival (section 10.2) should be 80% or higher.

18.4.5. Duration of Pupal Development

Depending on the rearing temperature, the duration of pupal development is about 8–10 days.

18.5. ADULTS

18.5.1. Number of Adults Collected

The number of adults collected on each day should be recorded (section 11.1). When this number is related to the number of trays of diet from which the adults came, the number of adults produced per tray can be calculated (also section 18.4.3).

Singh (1985) suggested that artificial diets should produce an average yield of adults of at least 75% from initial viable eggs. The figures shown in Tables 9–14 indicate that this has rarely been achieved. At a temperature of 27°C, the duration of development from neonate larva to adult is about 32 days.

TABLE 14
Quality Assessment Data

Parameter		Publication												
		Proverbs and Newton 1962a	**Hathaway et al. 1972**	**Batiste and Olson 1973**	**Proverbs 1982**	**Bathon et al. 1991**	**Howell 1991**	**Reiser et al. 1993**	**Rogers and Winks 1993**	**Bloem et al. 1997**	**Bloem et al. 1998a**	**Bloem et al. 1999b**	**Keil et al. 2001**	**Gu et al. 2006**
Diet														
Yield	adults/kg diet				100–200			281 57						
	adults/L diet			201 380										
Eggs														
Egg hatch (%)		55 77 50	74.1 78.2	74.4			38–88 93–96	82.4	66.5 80 70.3 69.5	72.2 81 77.2 77.5 88.9 84.2	88 82 87	71.8		
Egg duration (days) [°C]							16–17 [15] 8–9 [20] 5–6 [25] 4–5 [30]							
Larvae														
Survival (%)												80		
Larval duration (days) [°C]						25 [24]								14.74 15.4 [25]

Pupae											
Survival (%)		86–93									
Pupal weight (mg)	male				33.6	7–34		25.4 26.4 27.1 31.7	30.9 36.7 31.1[1]		
	female				39.9	21–52		34.3 36 38.9 40.5 40	39.2 40.6 40.5[1]		
Pupal duration (days) [°C]											13.38 12.88 [25]
Adults											
Egg to adult duration (days) [°C]							41 [25]				
Larva to adult duration (days) [°C]			30–31 [28]								
Survival from egg to adult (%)							57.8				
Survival from neonate larva to adult (%)			15.7				70.2		39 52.3		
Rate of population increase				3–4 10 (field)					10		see publ.
Adult weight (mg)	male		16.6						19.7 19.1 18.9[1]	19.3 19.8 17.7	21.85 22.31
	female		23.7						29.8 30 30.9[1]	30 31.7 27.5	29.13 32.7

TABLE 14 (CONTINUED)
Quality Assessment Data

Parameter		Proverbs and Newton 1962a	Hathaway et al. 1972	Batiste and Olson 1973	Proverbs 1982	Bathon et al. 1991	Howell 1991	Reiser et al. 1993	Rogers and Winks 1993	Bloem et al. 1997	Bloem et al. 1998a	Bloem et al. 1999b	Keil et al. 2001	Gu et al. 2006
		Publication												
Sex ratio (male:female)				1.07:1								1:1		
Mated females (%)		19 84 90									17–90 (field)			
Spermatophores produced (nr/male)										4.7 4.51				
Mobility/flight ability/dispersal					see publ.								see publ.	see publ.
Longevity (days) [°C]	male	10 14 12 [21]						8 [25]		14.4 11.5 10.3[1] [25]	14.8 13.9 12.8 [25]			13.57 16.7 [25]
	female	15 14 13 [21]						10.7 [25]		11.2 10.7 9.3[1] [25]	11.8 12.1 9.7 [25]			16.63 19.9 [25]
Fecundity (eggs/female)		55 34 66	92 95	114.6		230	132–162 43–130 53	130	184.7 107.5 164.3 161.8	216.3 181.7	152 193 187	200		206.47 249.37

[1] Data from wild insects (insects obtained in the field as cocooned larvae).

18.5.2. Percentage Recovery of Adults (Capable of Flight) from Emergence Room

If the adult collection system is such that adults are attracted to a UV light and are collected in a cold room (sections 11.1 and 19.5), it is important to know if, over time, adults continue to fly to the light and are trapped. Checking the emergence room visually (at the time of disposing of the spent diet) provides some information. A routine comparison should be made between the estimated number of adults produced (based on empty pupal cases, section 18.4.3) and the actual number as recorded per day in the adult collection system. If the percentage of adults recovered declines, it could mean that the proportion of the emerged adults that are capable of flight is declining.

18.5.3. Sex Ratio

The sex ratio is calculated from a sample of adults that are collected after emergence and then sexed (section 11.4). This ratio should be determined about once a week. The sex ratio is about 1:1. However, Pristavko and Boreyko (1971) found a tendency over time for the ratio to slightly favour males.

18.5.4. Adult Weight

The mean weight of male adults should be 18–20 mg or heavier and that of female adults 28–30 mg or heavier.

18.5.5. Handling Procedures

Handling procedures (storage, packaging, transport and release) for chilled adults can reduce the performance of the moths by 25–50%, as measured by trap captures (Bloem and Bloem 1995, 2000; S. Bloem et al. 1998a, 1999c, 2002; K. Bloem et al. 2002; Jallow and Judd 2007). Tween and Rendón (2007) discussed the use of cryogenics in sterile insect delivery systems.

19. Product Quality Control

An important challenge is to identify and rank the importance of the behavioural traits that contribute to the required function of released insects (Chambers 1975; Leppla et al. 1982). Tables 9, 10, 11, 12, 13 and 14 provide data on quality assessments drawn from various publications. Table 13 covers rearing on immature apples only. Standards for some quality parameters are suggested.

19.1. MATING ABILITY AND SPERMATOPHORE TRANSFER

Not all copulations lead to the transfer of a spermatophore, and proof that a female has mated successfully is the presence of a spermatophore in the bursa copulatrix (Proverbs and Newton 1962a; White et al. 1970, 1972, 1975; Pristavko and Boreyko 1971; Howell 1988; Neven et al. 2000; Judd et al. 2006a; Trécé 2007a) (section 12.2). In the field in the USA, the number of spermatophores per female averaged 1.08 at one location and 1.76 at another location, and cool temperatures reduced the number of spermatophores, reflecting reduced mating (Howell 1988). Howell suggested that spermatophore size might be a useful criterion for judging the quality of sterilized males.

The number of spermatophores per female increased if the number of males in a mating cage increased (Hathaway et al. 1973). Data in Tables 12 and 13 show that the number of spermatophores in mated females tends to be greater than one and sometimes two or three.

Laboratory-reared males mated less frequently than native males and transferred fewer eupyrene sperm, but colonization increased the mating frequency of females (Proverbs 1982).

The field vigour of laboratory-reared males, as measured by mating activity with wild females, was significantly reduced (by 29.6%) when compared with wild males (White and Mantey 1977). Mating tables (section 19.4) can be used to assess the mating ability of reared males with wild females.

Increased temperature favours mating; a higher percentage of females mated at 26.7ºC (80.3%) than at 23.9ºC (58.9%) (Howell 1981). Data from Tables 9, 10, 11, 13 and 14 show values for percentage mated females ranging from 19 to 98% (most values ≥ 65%). Results from a field cage showed that the mating percentage of laboratory-reared adults was about 43% (White et al. 1977).

Daily flight and mating activities began about 3 h before sunset and extended to about 2 h after sunset (Batiste et al. 1973). High and low temperatures limited these activities and so the periodicity varied considerably with the time of the season and location (Bloem et al. 2004). Most released males were recaptured in sex traps during sunset (Wildbolz and Mani 1971), and the first matings in a day

occurred during the hour preceding sunset (Bloem et al. 1999c). Wong et al. (1971) found that peak mating activity occurred in the hour immediately after sunset. An important aspect of quality is that the activity is the same for reared and wild insects (Huettel 1976).

Bloem et al. (1997) described a procedure for assessing male mating ability. Newly emerged males were paired with virgin females in clear 200 mL plastic cups with lids and a wick with water and allowed to mate for 48 h. The males were paired with a new virgin female every 48 h until death. The females were dissected and the total number of spermatophores produced by each male was determined (Table 14). Males usually mate several times (section 12.2) and produce several spermatophores in their lifetime.

19.2. MATING COMPATIBILITY

When insects originate and are reared in one area and then are shipped to another area, they are not necessarily sexually compatible with wild insects in the new area (Cayol et al. 1999, 2002; Mutika et al. 2001; FAO/IAEA/USDA 2003; Calkins and Parker 2005). However, research to date using field cages indicated that there is no mating incompatibility among codling moths of different geographical origins (Robinson and Proverbs 1973; Blomefield et al. 2005, 2006; Taret et al. 2006; Vreysen et al. 2006; Bloem et al. 2007; Taret et al. 2010).

19.3. FEMALE CALLING BEHAVIOUR AND PRODUCTION OF SEX PHEROMONE

Prolonged colonization can decrease the activity periods of moths, but colonization may increase the attractiveness of the female adult (Proverbs 1982). There are laboratory tests to monitor pheromone production by female insects (Moore et al. 1985).

19.4. RESPONSE OF MALES TO CALLING FEMALES

It is possible to measure male response to a sex pheromone using an olfactometer, flight tunnel (Moore et al. 1985) or electroantennogram. However, most tests are done in the field (Tables 12 and 13).

In a field-cage competition experiment comparing laboratory-reared (for 3 years on artificial diet) codling moths and wild moths, laboratory males still appeared to be responsive to the sex pheromone secreted by wild females, notwithstanding the long period of laboratory rearing (Robinson and Proverbs 1975). Electrophysiological and sex trap data indicated that reared males had an enhanced olfactory sensitivity (Proverbs 1982).

The response of reared males to sex-pheromone traps may be similar to that of wild males (Wildbolz and Mani 1971; Dyck et al. 1993), but reared males do not always respond as well as wild males (White and Hutt 1975).

Rogers and Winks (1993) showed that reared male adults placed in field cages did not respond to a tethered female on a mating table. This poor response, and

possibly a disinclination to fly, required that a new colony was started from wild insects.

A good method to measure the response of males to the female sex pheromone is to tether virgin females to a mating table (or clip part of one wing) and visually monitor any males that come to the female and mate (Chambers 1977; McBrien and Judd 1996; Bloem et al. 1999c, 2004; Judd et al. 2006a, b; Jallow and Judd 2007). Judd et al. (2006a) provided photos of a mating table suspended in a tree, showing a male mating a tethered female. The mating pair can be captured and brought to a laboratory, and the female given an opportunity to lay her eggs which can be checked for fertility (Vreysen 2005). If the female is wild, this technique can measure the level of sexual and flight competitiveness of released sterile males against wild males. An advantage of a mating table over a virgin female-baited trap is that the table is more selective — only the most competitive male, i.e. the one that reaches her the quickest and interacts using the appropriate behaviours, will be able to successfully mate (Bloem et al. 2004).

Bloem et al. (1998a, 2005) showed that males reared as larvae in diapause were recaptured in passive interception traps, pheromone-baited traps and virgin female-baited traps in significantly higher numbers than non-diapaused males reared under a constant temperature (section 13). Also, in general, recapture in pheromone-baited and virgin female-baited traps of both non-diapaused and diapaused males was lowest in spring and highest in autumn.

Judd et al. (2006a) found that, in spring (sections 9.1, 13 and 16), significantly more wild diapause males mated with tethered wild females than did non-diapause mass-reared males. Wild males mated approximately 45 min earlier than mass-reared males, with most wild males (70.5%) mating before sunset and mass-reared males mating at or shortly after sunset. The superior mating competitiveness of wild males in spring was mirrored by greater recapture rates in pheromone-baited traps. However, in summer (with warmer temperatures prevailing), the mating competitiveness of mass-reared males improved relative to wild males.

19.5. MOBILITY, FLIGHT ABILITY AND DISPERSAL

Dispersal has two major traits, flight propensity and flight ability (Huettel 1976). Both males and females travel farther in the warm summer than in the cool spring (Bloem et al. 1998a).

Batiste et al. (1973) recorded daily flight and mating activities, and found that they began about 3 h before sunset and extended to about 2 h after sunset. High and low temperatures limited these activities, and so the periodicity varied considerably with the time of the season and location. In the field, wild males showed a greater tendency to disperse than laboratory-reared males (White and Hutt 1975; White and Mantey 1977). The flight ability of adults shipped from Canada to South Africa was good (Blomefield et al. 2006) (sections 11.1 and 18.5.2).

Flight ability in a laboratory can be measured by using adults emerging from pupae placed in open cups that have unscented talcum powder coating the inside walls of the cup to prevent adults from crawling out of the cup (FAO/

IAEA/USDA 2003). This test also provides data on pupal survival (sections 11.1 and 18.4.4).

Carpenter and Blomefield (2007) conducted a flight ability test using a cylinder 8-cm high and 16 cm in diameter and test duration of 48 h. However, this test was not as robust as trials conducted in the field. Observations suggested that this test did not measure ability to fly from the cylinders, but rather the propensity to initiate multiple flights or the degree of irritability of the moth.

For the codling moth, long-distance dispersal is not unusual, but involves only a small percentage of the population; populations tend to remain localized (Howell and Clift 1974). Schumacher et al. (1997) reported that flight behaviour and dispersal are subject to genetic variation, with about 10% of the genotypes being mobile and the remaining 90% sedentary. Biological traits, e.g. body weight, size, longevity, fertility and the intrinsic rate of increase could be correlated with mobility. The efficacy of an SIT programme might be increased if mobile- versus sedentary-type males in a colony could be selected (Vreysen and Hendrichs 2005, Vreysen et al. 2006). However, there appear to be consequences in terms of reduced fitness (smaller size, lower fecundity, shorter longevity, and lower net reproductive rate and intrinsic rate of increase) if the mobility trait is selected (Dorn and Gu 2004, 2006; Gu et al. 2006).

Bloem et al. (2006) reported that diapaused females were significantly more mobile than standard females, whereas no differences were detected in male mobility because of rearing strategy.

Mated females were significantly more mobile than virgin females, whereas no difference in mobility because of mating status was detected for males. Mated females were significantly more mobile than mated males, but virgin females were significantly less mobile than virgin males (Bloem et al. 2006).

The dispersal propensity and capacity of males is monitored by releasing marked moths at a release point and then trapping moths using sex pheromone, virgin female or passive interception traps in the following evenings at various distances from the release point (Bloem et al. 1998a, 1999c, 2001, 2004; Tabashnik et al. 1999; Keil et al. 2001). A mating table (section 19.4) can also be used to trap males.

In the laboratory, mobility can be measured using an actograph (Chambers 1975; Moore et al. 1985; Keil et al. 2001; Bloem et al. 2006; Gu et al. 2006) and flight ability using a flight mill (Chambers 1975; Huettel 1976; Moore et al. 1985; Schumacher et al. 1997) or a flight tunnel (Suckling et al. 2007). However, Stewart (1984) claimed that measurement of flight ability with flight mills is not a practical system because of size and fragility of the moths and the non-reproducibility of the results.

19.6. MALE COMPETITIVENESS

Competitiveness is a general concept, and several parameters measure different components of competitiveness. In relation to applying the SIT, especially

important components are flight ability and dispersal, response to the sex pheromone, mating ability and longevity (Suckling et al. 2007).

19.7. ADULT LONGEVITY

Longevity is affected by the presence or absence of food or water (section 12.3), RH and temperature (Howell 1981). Giving food or water to moths increases their longevity. Howell (1991) reported that longevity is reduced as the rearing temperature rises. At 26.7ºC the longevity of females was five days if starved, 13 days if provided water and 17 days if given sugar water. Females oviposited for 4–7 days (section 12.3) (see **Figure 20** for comparison) and lived about four days after the last oviposition. Gu et al. (2006) provided a graph showing the decline over time, starting at nine days, in adult longevity.

Tables 9–14 show longevity data for the codling moth. The holding temperatures are shown but the feeding status is not, and therefore the results are quite variable. Nevertheless, as the holding temperature increased, there was a decrease in the average longevity (across all publications):

- Males: 25ºC–12.9 days, 26ºC–10.9 days, 27ºC–7.7 days
- Females: 25ºC–12.4 days, 26ºC–9.1 days, 27ºC–8.1 days

To measure longevity in the laboratory, newly emerged adults were placed individually in small plastic cups (29.5 mL) with a water-moistened cotton wick and kept at 25ºC, 16L:8D, 50% RH. The cups were checked daily and daily mortality recorded (Bloem et al. 1997, 1998a).

Guennelon et al. (1981) provided an estimate of female longevity in the field — 9.3 days (Table 10). However, the longevity of released adults in the field has rarely been measured *per se*, but the decline in trap catch after the release of marked adults provides a partial measure of longevity in the field (but is confounded by a decline in the number of insects available to trap).

Longevity outside can be measured by placing newly emerged adults into cages and placing them outdoors and then monitoring daily survival. However, this does not necessarily reflect environmental conditions in the field, where predation, rainfall and other factors not measured in an outdoor cage could affect longevity.

19.8. FECUNDITY

Fecundity of wild females (Howell 1991) is low when compared with laboratory (mean 132–162 eggs/female) or mass-reared females (range 43–130 eggs/female). If mating is delayed after emergence, females lay fewer eggs. There is a positive correlation between adult or pupal weight and fecundity; however, this was not found by Hathaway et al. (1973). These authors found no correlation between weight and the number of eggs/female, and also none between the number of spermatophores and the number of eggs/female.

Pristavko and Boreyko (1971) obtained a fecundity of 184.5 eggs/female if the male:female sex ratio was 1:1 (Table 13). However, if the sex ratio favoured males 2:1, female mating frequency increased but female longevity decreased and fecundity decreased to 127.8 eggs/female (sections 12.2 and 12.4). Bloem et

al. (1998b) reported a fecundity of 216 eggs/female. Tables 9–14 show data on fecundity ranging from 20 to 249 eggs/female. However, most values range from 34 to 135 eggs/female, with quite a few cases of higher fecundity in the range of 144 to 230 eggs/female.

Bathon et al. (1991), Rogers and Winks (1993) and Bloem et al. (1997, 1998a, b) described procedures to assess fecundity and fertility (section 18.2.2).

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 ± 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.

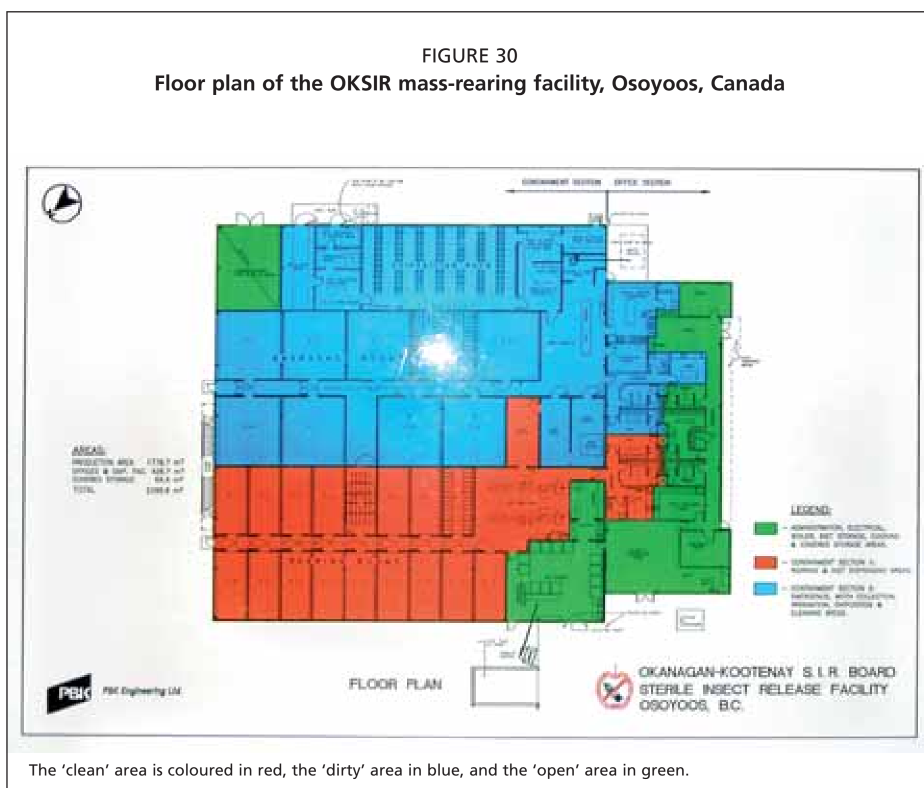
In the early stages of the design of the OKSIR facility (**Figure 7**), a major decision was taken to provide separate environmental controls for each rearing and emergence room. This was an 'expensive' decision, but it permitted clean air to be brought into each room separately and air could not move 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.

FIGURE 30
Floor plan of the OKSIR mass-rearing facility, Osoyoos, Canada



The 'clean' area is coloured in red, the 'dirty' area in blue, and the 'open' area in green.

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).

FIGURE 31
Cart washer (also used to wash oviposition cages) – OKSIR mass-rearing facility, Osoyoos, Canada

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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).

FIGURE 32
Rearing room with vertical fluorescent tubes in the air plenum behind clear-plastic walls – OKSIR mass-rearing facility, Osoyoos, Canada.

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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 (180ºC 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 115.5ºC 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 cm^3/m^3).
- Every three months each rearing room is emptied and cleaned. After applying formaldehyde vapour, the room is heated to 45ºC for three days.
- Diet trays are washed, disinfected in a cleaning solution and dried at 50ºC. 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 mass-rearing 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 mass-rearing 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 mean- or 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 self-replicating biotic entity (FAO 2007)

multivoltine – having many generations per season or year (Gordh and Headrick 2001)

natural diet – natural food of an animal

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
Trays (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.

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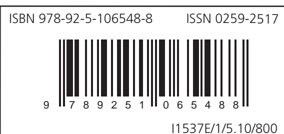

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