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 (14.8ºC). 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).

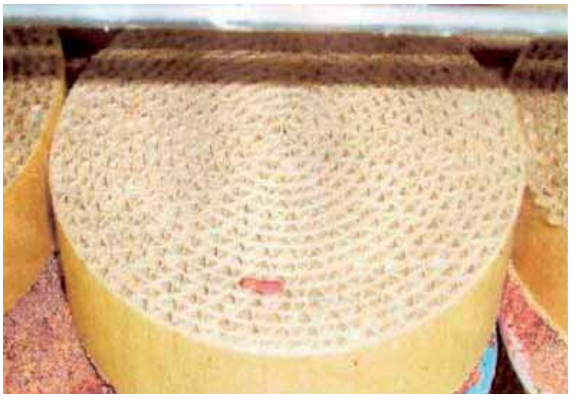
Costs can be reduced by enclosing trays in fibreglass mesh bags which prevent exiting larvae from wandering; they form cocoons in or on the diet (Bloem et al. 2000). However, increased cold storage space is needed.

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 25ºC 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 15ºC, 50% RH, in the dark (Ashby and Singh 1990; Bloem et al. 1997); 2 weeks at 10ºC in the dark (Neven et al. 2000).

Procedure for cold storage of mature larvae:

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

Sub-zero cooling of diapausing larvae (–10 or –15ºC 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 15ºC 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 0–2ºC (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, 25–28ºC 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, 25ºC and 60% RH with 93% diapause termination occurring after 25 days (Ashby and Singh 1990); 16L:8D, 23ºC and 60% RH (Neven et al. 2000); 14L:10D, 26ºC and 65–70% RH (Blomefield et al. 2006); 16L:8D or longer photophase at 26–27ºC (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 15ºC for 10–14 days; pupae held at temperatures lower than 15ºC may desiccate if they are reared at 20–25ºC 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 0ºC 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 15ºC 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 –2ºC for 1 h. However, cold storage at – 2ºC 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 micro-organisms."

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 49ºC 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 (60ºC for three days) of spent diet (section 7.2) was inadequate to inactivate the virus, and even autoclaving the diet at 121ºC 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 36–37ºC 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		Publication						
		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]			32.6 [26.7]
	female				31.2[3]♀ [26.7] 28.9[5]♀ [27]		30.8[5] [28]	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											
pupae/kg diet						81		100-200			
adults/kg diet							121		77	170	178
Yield											
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		
	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		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
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).