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Rearing codling moth for the sterile insect technique

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Rearing codling moth for the sterile insect technique

by
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V.A. Dyck

Preface

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

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

In the last years, in view of the above described problems, there has been an increasing interest by Member States to develop codling moth SIT for integration with other control tactics. The development of this document is a response to this increased interest, and it compiles and summarizes available information on the rearing of the codling moth, be it in the laboratory or on a larger scale. The information in this document deals with aspects such as colonization, adult and larval diet, sexing, quality control, shipment, disease control, data recording and management. It is not intended to be read from cover to cover, but the information is presented so that individual sections can be consulted by the reader when necessary. Hence, the document does not provide guidelines ***per se***, nor is it a compendium of standard operating procedures, as these will need to be developed for each rearing facility based upon local needs and availability of materials and ingredients. The list of references in this document is exhaustive, and an attempt has been made to be complete. The document is unique as, for the first time, it brings together all existing information on the rearing of the codling moth.

Contents

1. Introduction

1.1. SCOPE

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

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

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

1.2. RATIONALE

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

1.3. BACKGROUND

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

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

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

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

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

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

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

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

2. History of Rearing the Codling Moth

2.1. REARING AND MASS-REARING

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

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

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

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

2.2. IMMATURE APPLES

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

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

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

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

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

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

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

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

2.3. DIETS THAT STAY MOIST AND SOFT

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

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

2.4. DIETS THAT DRY OUT AND HARDEN

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

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

3. Colonization from Field-Collected Material

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

3.1. COLLECTING LARVAE IN DIAPAUSE

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

3.2. COLLECTING LARVAE IN SPRING

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

3.3. COLLECTING ADULTS

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

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

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

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

3.4. PREVENTING INTRODUCTION OF VIRUS

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

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

3.5. USING AN ESTABLISHED COLONY

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

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

3.6. NUMBER OF INSECTS REQUIRED

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

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

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

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

3.7. INTRODUCTION OF WILD GENOTYPES

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

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

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

3.8. BACK-UP STRAIN/COLONY

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

4. Diet Ingredients

4.1. ARTIFICIAL DIETS FOR INSECTS

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

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

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

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

4.2. DIETS FOR LEPIDOPTERA

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

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

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

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

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

4.3. DIETS FOR CODLING MOTH

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

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

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

TABLE 2
The Singh General-Purpose Diet[1]

Composition			Preparation
Ingredients	*Amount*	*%*	**Composition of the stock mould inhibitor solution:** Methyl *p*-hydroxybenzoate, 37.5 g; sorbic acid, 50.0 g; 95% ethyl alcohol, 425 mL. **Preparation of stock 4*N* KOH solution:** 56 g KOH is added carefully to 250 mL distilled water. **Preparation of dry mix:** Mix the agar, casein, cellulose powder, Wesson's salt mixture and the finely ground wheat germ together. Dissolve cholesterol and linoleic acid in dichloromethane, then mix thoroughly with the dry ingredients. Leave in fume cupboard for 2–3 days, stirring periodically to allow solvent to evaporate. **Preparation of finished diet:** Mix the dry mix, distilled water and KOH together, cover and sterilize in autoclave at 121°C for 20 min. Combine 600 mL distilled water, vitamin mixture, sucrose, glucose, streptomycin, penicillin and prochloraz together. Mix thoroughly and add to autoclaved diet (cooled to 60°C) along with mould inhibitor. Adjust the pH of the diet between 4.5 and 5.0. The diet is dispensed into test tubes with a diet dispensing machine or is manually dispensed into waxed Lily[3] cups or plastic containers.
Dry mix to prepare 6.5 kg finished diet			
Agar	150 g	12.32	
Casein	210 g	17.24	
Cellulose powder	600 g	49.25	
Wesson's salt mix	60 g	4.93	
Wheat germ	180 g	14.78	
Cholesterol	3 g	0.25	
Linoleic acid	15 g	1.23	
Dichloromethane[2] (evaporates)	50 mL		
Finished diet			
Dry mix (see above)	1218 g	18.56	
Distilled water	4290 mL	65.33	
4*N* KOH	30 mL	0.46	
Vanderzant vitamin mix	120 g	1.83	
Sucrose	180 g	2.74	
Glucose	30 g	0.46	
Streptomycin sulphate BP[2]	900 mg		
Penicillin[2]	900 mg		
Prochloraz[2] (fungicide)	260 mg		
Distilled water	600 mL	9.15	
Mould inhibitor	90 mL	1.37	
Total diet (approximately)	6558 g	100	

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

[2] Not included in total weight.

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

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

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

TABLE 3
Ingredients in Artificial Diets that Stay Moist and Soft

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

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

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

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

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

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

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

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

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

[1] Ingredients are listed in no particular order.

[2] Torula yeast.

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

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

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

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

[7] Formaldehyde (40%) (Formalin®).

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

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

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

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

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

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

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

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

[16] Cost of ingredients only.

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

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

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

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

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

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

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

4.4. DIETS THAT STAY MOIST AND SOFT

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

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

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

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

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

TABLE 4
Ingredients in Artificial Diets that Dry Out and Harden

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

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

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

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

[1] Ingredients are listed in no particular order.

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

use hot water to reduce the diet preparation time.

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

[4] Torula yeast.

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

[6] Canola (soybean) meal.

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

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

[9] Fumaric acid used instead of citric acid.

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

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

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

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

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

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

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

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

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

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

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

[21] High pupal mortality occurred in this experiment.

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

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

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

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

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

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

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

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

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

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

4.5. DIETS THAT DRY OUT AND HARDEN

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

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

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

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

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

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

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

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

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

4.6. OBTAINING DIET INGREDIENTS

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

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

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

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

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

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

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

4.7. LOCALLY OBTAINED INGREDIENTS

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

4.8. STORING DIET INGREDIENTS

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

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

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

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

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

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

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

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

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

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

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

FIGURE 2
Sawdust storage building (separate building on left with large white door) – OKSIR facility, Osoyoos, Canada.

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4.9. DIET YIELD

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

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

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

4.10. COST OF DIET

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

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

4.11. COST OF PRODUCTION

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

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

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

4.12. MARKING USING A DYE IN THE DIET

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

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

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

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

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

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

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

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

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

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

5. Nutritional Aspects of Diets

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

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

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

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

5.1. PROTEINS

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

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

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

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

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

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

5.2. CARBOHYDRATES

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

5.3. LIPIDS

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

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

TABLE 5
Vitamins in Artificial Diets (Part 1)

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

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

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

[1] Vitamins are listed in no particular order.

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

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

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

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

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

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

[7] Product is 60.69% wt/v.

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

5.4. VITAMINS

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

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

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

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

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

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

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

TABLE 6
Vitamins in Artificial Diets (Part 2)

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

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

TABLE 6 (continued)
Vitamins in Artificial Diets (Part 2)

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

[1] Vitamins are listed in no particular order.
[2] Commercially available vitamin mixture from Hoffman-LaRoche, Inc.
[3] Ascorbic acid used in premix as a bulking agent.
[4] There is 50% aureomycin in an animal feed additive called Chlorachel-50.

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

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

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

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

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

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

5.5. MINERALS

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

TABLE 7
Minerals in Artificial Diets (Part 1)

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

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

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

[1] Minerals are listed in no particular order.

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

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

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

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

TABLE 8
Minerals in Artificial Diets (Part 2)

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

[1] Minerals are listed in no particular order.

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

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

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

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

5.6. OTHERS

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

Preservatives are added to prevent microbial contamination or oxidation:

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

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

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

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

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

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

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

Gelling agents are expensive but they improve diets by:

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

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