Optimization of a mass-rearing system to produce codling moth, *Cydia pomonella*, for a Sterile Insect Release programme in South Africa

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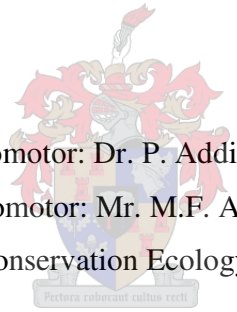

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Abstract

Codling moth, *Cydia pomonella*, is a worldwide pest and of major economic importance to the South African pome fruit industry. Sterile insect release is applied as a component of area-wide integrated pest management and includes the mass-rearing, sterilization and the release of the sterile insects. For sterile insect release, the improvements of rearing methods in terms of the quality of the diet ingredients and the economical aspect of the rearing method are examined. The effect of genetically modified maize meal, containing the *Bacillus thuringiensis* gene, in an artificial medium for codling moth rearing, is determined. The use of even a small amount of *Bacillus thuringiensis* resulted in larval mortality and prolonged development. These results are detrimental to a mass-rearing facility and must be considered by any rearing facility that uses genetically modified maize meal if the insect is sensitive to the gene. An alternative to maize meal in the artificial medium was tested and whole wheat flour was considered to be a suitable replacement. Agar agar is an expensive gelling agent used in the artificial medium. An alternative for agar agar (Kelcogel, Elastigel and carrageenen) is tested and the biological effect on codling moth is determined. Factors such as mortality, pupal and moth weight, longevity, fecundity and development time were used as quality parameters. Results showed that Elastigel was a suitable replacement for agar agar, with bigger pupae and moths, higher fecundity and increased longevity. The economical advantage of the replacement is a 40.91% reduction of the diet cost. The other gelling agents tested also gave acceptable results and can be considered if shortages of agar agar or Elastigel occur. A new method of mass-rearing codling moth larvae in a closed rearing system using large trays placed in a ventilated box is designed. This method is more cost and space effective as a smaller area is needed to rear a large number of moths. The risk of diet contamination is less because of the closed environment and more economical and effective air handling. This is the first report of its kind to describe the mass-rearing of codling moth in a closed environment and the risks involved in using genetically modified maize meal in an artificial diet for the codling moth. These results should be incorporated into existing mass-rearing facilities or taking into consideration when designing new mass-rearing facilities.

Opsomming

Die kodlingmot, *Cydia pomonella*, is van ekonomiese belang vir die Suid-Afrikaanse kernvrugte bedryf. Die steriele insek tegniek word gebruik as ‘n komponent in area-wye geïntegreerde plaagbeheer en sluit in die massa-aanteel, sterilisering en vrylaat van steriele insekte. Vir die steriele insek tegniek is die verbetering van die massa-aanteel van die kodlingmot in terme van kwaliteit van die dieet en die ekonomiese aspek van die aanteel metode ondersoek. Die effek van genetiese gemanipuleerde mieliemeel wat die *Bacillus thuringiensis* geen bevat, in ‘n kunsmatige voedselmedium vir die aanteel van kodlingmot, is bepaal. Daar is gevind dat die gebruik van selfs ‘n klein persentasie *Bacillus thuringiensis* in die mieliemeel, mortaliteit en ‘n verlengde lewenssiklus in kodlingmot veroorsaak. Die gevolge is nadelig vir ‘n massa-aanteel fasiliteit en behoort in ag geneem te word vir enige insek wat op ‘n kunsmatige medium, wat mieliemeel bevat, geteel word, mits die insek sensitief is vir *Bacillus thuringiensis.* ‘n Alternatiewe bestanddeel vir mieliemeel, volkoringmeel, word aanbeveel. Agar agar is ‘n duur verdikkingsagent wat in kunsmatige mediums gebruik word. ‘n Alternatief vir agar agar (Kelcogel, Elastigel en carrageenen) is getoets en die biologiese effek op die kodlingmot is bepaal. Faktore soos mortaliteit, papie en mot gewig, langlewendheid, vrugbaarheid en lengte van lewenssiklus was gebruik as kwaliteit parameters. Resultate het getoon dat Elastigel ‘n geskikte plaasvevanger is van agar agar, met groter papies en motte, groter vrugbaarheid en langlewendheid. Die ekonomiese gevolg van die plaasvervanger, is ‘n vermindering van 40.91% van die dieetkoste. Die ander verdikkingagente wat is getoets is, het aanvaarbare resultate gelewer wat noodsaaklik is indien daar ‘n tekort van Elastigel of agar agar ontwikkel. ‘n Nuwe metode van massa-aanteel van kodlingmot larwes is bepaal. Die metode behels ‘n geslote sisteem, waar groter aanteel bakke in ‘n geslote, geventileerde boks geplaas word. Die metode is koste en spasie effektief en ‘n kleiner area word benodig om ‘n groter aantal motte te lewer. Die risiko van kontaminasie van die dieet word verminder as gevolg van die geslote sisteem wat gebruik word en meer ekonomiese en effektiewe lugversorging word gebruik. Hierdie is die eerste verslag van sy soort wat die massa-aanteel van kodlingmot in ‘n geslote sisteem beskryf en wat die risiko aandui van geneties gemanipuleerde mieliemeel in ‘n kunsmatige medium vir die kodlingmot. Hierdie resultate behoort in ag geneem te word

vir reeds bestaande massa-aanteel fasiliteite of met die ontwerp van nuwe massa-aanteel fasiliteite.

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CHAPTER 1

Development of artificial diets for mass-rearing codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae)– a review

1.1 Introduction

1.1.1 Codling moth: a pest in South Africa

Codling moth, *Cydia pomonella* (Linnaeus) (CM), is a major pest on apples and pears in the Western Cape, South Africa (Nel 1983, Pringle et al. 2003, Addison 2005, Timm et al. 2006) and was first recorded in the country in 1885 (Lounsbury 1898). Codling moth infestation in South Africa is one of the highest in the world and is capable of infesting 80% of an apple crop (Myburgh 1980, Pringle et al. 2003, Timm et al. 2006). It occasionally attacks stone fruit (Blomefield 1989, Blomefield & Giliomee 2009), walnuts, almonds, pecan nuts and pomegranates (Nel 1983, Sæthre & Hofsvang 2002). There are three to four generations a year, starting from September until April resulting in moths being active for almost 8 months of the year (Nel 1983, Pringle et al. 2003, Timm et al. 2006). Codling moth has a facultative diapause and overwinters as fifth instar larvae in cracks and bark on the tree (Nel 1983, Ashby & Singh 1990, Bloem et al. 1997, Bloem et al. 2000). Temperature, relative humidity, food quality and photoperiod affect CM development (Hathaway et al. 1971).

1.1.2 Control methods

Many control strategies have been used against the CM with little effect and it remains a problem. Conventional control programmes in the past have relied on the use of single control tactics namely broad-spectrum insecticides (Riedl et al. 1998, Pringle et al. 2003). Calendar spray programmes were used prior to the 1970's and during the 1970's, pheromone baited CM traps were used to monitor the activity of the moths (Pringle et al. 2003). In using the traps, the

timing of the sprays were improved, resulting in reduced spray programmes (Pringle et al. 2003). Chemical control was a reliable control strategy for several years until resistance against organophosphates occurred (Blomefield 1994, Pringle et al. 2003, Bloem et al. 2010). Environmental and human health concerns along with resistance against organophosphates were a good reason to search for alternative, integrated control strategies. Insect growth regulators, attract and kill methods and biological control agents such as CM granulosis virus are used as integrated control methods (Riedl et al. 1998, Pringle et al. 2003). Pheromone mating disruption is widely used at present and low population levels are a prerequisite in using mating disruption (Riedl et al. 1998, Pringle et al. 2003, Addison 2005, Timm et al. 2006). Monitoring of CM populations in the orchards using fruit damage assessments and trap counts are intensified to make the programmes successful and to reduce fruit damage. The direct cost of CM control is significant and populations remain high enough in the orchards to cause extensive damage if control measures are not applied effectively (Addison 2005).

The South African pome fruit growers are looking for sustainable control alternatives. The isolation and the separation of the different growing areas in South Africa and a lack of wild hosts makes the conditions for the use of Sterile Insect Release (SIR) very favorable (Riedl et al. 1998). Sterile Insect Release is a method of pest control using area-wide inundative releases of sterile insects to reduce fertility of a field population of the same species (Knipling 1955, Klassen 2005). Sterile Insect Release includes the mass-rearing of the insects on an artificial diet and maintaining a laboratory colony of insects before being sterilized and released. Released sterile moths mate with the fertile wild moths, resulting in a sterile progeny. The aim of this technique is to suppress the wild population below the economic threshold or to eradicate the pest and to enhance the efficacy of a non-pesticide approach.

The Sterile Insect Technique was pioneered in the 1950's by American entomologists Dr. Bushland and Dr. Knipling and they developed the technique to eliminate screwworms ("Sterile insect technique 2010"). Sterile insects have been used in many area-wide integrated pest management programmes against *Helicoverpa zea* (Boddie) (Carpenter 2000), tsetse flies (*Glossina spp*), fruit flies, screwworm fly, *Cochliomyia hominivorax* (Coquerel) (Botto & Glaz 2009), pink bollworm (*Pectinophora gossipiella* (Saunders)) and other insect pests. Sterile

Insect Release on CM was started in the Similkameen Valley in British Columbia, Canada in 1976 (Proverbs et al. 1982). Mass-rearing and mass-release operations of the SIR programme in British Columbia started in 1992 and the first sterile moths were released in 1994 (Calkins et al. 2000). The growers were required to supplement the SIR programme with the use of organophosphate in 1995. The programme was deemed successful in 1997 as CM populations were effectively suppressed and fruit damage significantly reduced (Bloem et al. 1997, Calkins et al. 2000). At present, the Canadian insect rearing facility produces more than 14 million moths per week (Bloem et al. 1997, Bloem et al. 2004).

In South Africa, area-wide integrated pest management programmes with a SIR component have been implemented against Mediterranean fruit fly, *Ceratites capitata* (Weidemann), and false codling moth, *Thaumatotibia leucotreta* (Meyrick). A CM SIR pilot project was started in 2002 in Elgin, Western Cape, South Africa (Addison 2005). Two thousand sterile male and female moths per hectare per week were released from September to March in mating disruption orchards (Addison 2005) and the moth population and damage in these orchards have decreased significantly over the past years (Personal communication, M. Addison). The cost associated with SIR is high compared to conventional chemical control and requires intensive management (Addison 2005). Due to financial constraints, rearing capacity of the insectary, inconsistent rearing, rearing temperature, humidity control problems and insect quality concerns, the initial pilot was limited to 120 ha. "Start-up problems are not uncommon for a facility of this type" (Bloem & Bloem 2000) and despite the initial problems, the CM production in a 100 m^2 building and three permanent staff members increased to 300 000 moths per week.

1.1.3 Rationale of thesis

Various research projects on CM rearing have been done over the years and while all stages of CM are needed for research, having a laboratory colony is important. Artificial diets for rearing insects are a very important aspect in insect research and the advantage of having a laboratory colony reared on an artificial diet is the ability to produce insects throughout the year. Most of the information available on CM rearing methods is on small- or large scale rearing but not mass-rearing. Mass-rearing is: "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" (Chambers 1977). The major use of mass-reared insects has been in SIR (Singh 1983). No review on mass-rearing CM had been undertaken until Dyck 2010 did an extensive review intended for SIR. Canada is the only country currently mass-rearing CM, whereas South Africa and Argentina have large-scale CM rearing facilities. Therefore, information on artificial diets and rearing methods for a mass-rearing CM facility for SIR is lacking.

South Africa is in the process of commercializing a mass-rearing CM SIR programme, but some difficulties have arisen. Inconsistent insect rearing numbers, the cost of the artificial diet used for CM rearing and the safety of the diet ingredients were a concern to the programme. Diet is the most important component of rearing insects and together with labour, constitutes the main costs (Parker 2005). Minimizing the cost of the diet ingredients can help to make this control method viable but a balance has to be achieved between cost and the performance of the insects (Parker 2005). The focus of this thesis will be on optimizing artificial diets and rearing methods used for mass-rearing CM for SIR.

1.2 Application of artificial diets for codling moth

The successful formulation of an artificial medium depends on the chemical composition, nutrition of the diet and knowledge of the feeding behaviour of the insect (Singh 1977).

1.2.1 Feeding biology and ecology of codling moth

CM is a direct pest, but only the immature stages cause damage. This makes it possible to release adults of both sexes for SIR. Adult moths lay eggs singly on the fruit or foliage near the fruit (Nel 1983, Hughes et al. 2003). The larvae have to locate the food source and penetrate into the fruit via calyx or stalk ends while the fruits are small or through the sides later in the season (Blomefield et al. 1986). Larvae penetrate the fruit, feed on the core and thus making the fruit

unmarketable. The larvae have chewing and biting mouthparts and begin feeding within two hours of hatching (Hughes et al. 2003). The adult and the neonate larvae use kairomones to orient anemotactically to apple fruit (Zalucki et al. 2002) and the main attractant is (E,E)-α-farnasene, present in the wax and peel of apple fruit (Hern & Dorn, 1999, Hughes et al. 2003, Witzgall et al. 2005). This acts as an attractant to the neonate larvae and as an oviposition stimulant to the female moth, but there might be other compounds present in apple extracts necessary to stimulate feeding (Hughes et al. 2003).

In the CM SIR facility, the eggs are laid on wax paper and the paper is placed above the diet. The diet is scarified to make larval penetration easier. Therefore, the larvae do not have to locate the food source and they have no choice in food selection and food acceptance. It is known that diet texture is a key factor in the attractiveness to the insect, but there is a lack of research on the contributions of texture to the phagostimulation of insect diets (Cohen 2004). Ascorbic acid (vitamin C) might play a role in phagostimulation (Cohen 2004) as well as sugars (Singh 1984, Bernays & Chapman 1994) Suski et al. (1985) determined that adding α-farnasene to an artificial diet did not improve food acceptance but caused a positive locomotory response of newly hatched CM larvae. However, Bradley & Suckling (1995) noticed that CM larvae derived from a laboratory colony showed less response to α-farnasene and lower walking speeds than wild larvae. This might be due to selection pressure on laboratory reared larvae in the absence of this volatile. Larvae feeding on artificial diets might induce a change in adult behavior causing a selection for females that oviposit in the absence of an odour stimulus (Witzgall et al. 2005) which is advantageous in laboratory rearing.

1.2.2 Nutritional information for apples

Information on the composition of the host material can be used to determine the essential elements of an artificial medium and in the case of CM the predominant host material is apples and pears. About 10% of an apple consists of carbohydrates, 4% of vitamins and minerals and 80% of water. The skin and core contains dietary fiber. Apples contain all the essential amino acids such asisoleucine, leucine, lycine, methionine, cystine, phenylalanine, tyrosine, threonine, thryptophan, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, proline, serine and

saturated, mono-unsaturated and polyunsaturated fats (Paul & Southgate 1978). Apples are rich in ascorbic acid and contain about 6 – 12 mg/100 g fruit (Coultate 1989, Besler 1999). Vitamins found in apples are carotene, thiamine, riboflavin, nicotinic acid, vitamin E, B_6, folic acid, pantothenic acid and biotin (Paul & Southgate 1978).

1.3 Artificial diets used for rearing codling moth

Artificial diets have been defined as any diet that is not the natural food of the insect (Vanderzant 1974). An artificial diet provides continuous availability of food for the insects compared to the use of host plant material (Howell 1967). Insect diets must be stable, nutritious, and fulfill the sensory requirements of the insects but remain economically feasible (Cohen 2004).

Insects are very adaptable and successful organisms and they can modify their metabolism to develop on sub-optimal diets (Gordon 1972). Insects need energy to perform their basic life processes, which they obtain in the form of chemical bonds within carbohydrates, proteins and fat from the food consumed (Downer 1981). Components usually added to the artificial diets include carbohydrates, vitamins and minerals, proteins and lipids (Brewer & Lindig 1984; Cohen 2004). Gelling agents, fillers, pH stabilizers and preservatives can also be added (Cohen 2004).

Many artificial diets have been used for CM. Some diets did not work satisfactory because of a vitamin deficiency (Coutin 1952). Other CM diets were modified from other insect's diet e.g. the boll weevil's, *Anthonomus grandis* (Boheman) diet (Redfern 1963; Redfern 1964), the red-banded leafroller, *Argyrotaenia velutinana* (Walker) (Rock et al. 1964; Rock 1967), a diet for oriental fruit moth, *Grapholita molesta* (Busck) and a diet for noctuid species (Cossentine, Jensen & Eastwell 2005). Some researchers used a general diet to rear CM for research purposes (Ashby & Singh 1990, Hansen et al. 2004, Eberle & Jehle 2006). Boncheva et al. (2006) used a diet to rear a small colony for research on *Bacillus thuringiensis* and this diet is similar to the diet described by Guennelon et al. (1981).

Brinton et al. (1969), Howell (1972), Guennelon et al. (1981), Bloem et al. (2000), Botto (2006) and Hansen and Anderson (2006) described diets used for large- or mass-rearing CM (Table 1.1a) and will be discussed in this chapter. These diets are unique because CM has been reared for a few generations on these diets and large numbers of insects were produced. The Canadian SIR facility uses a modified diet described by Brinton et al. (1969) and Botto (2006) used a modified diet described by Guennelon et al. (1981). Howell (1970) described a diet used at Yakima Arid Areas Deciduous Fruit Insects Investigations, which he modified in 1972 for because it was too expensive. Hansen and Anderson (2006) described a diet used for rearing CM at Yakima Agricultural Research Laboratory in Washington very similar to Howell's diet (1972). There are similar ingredients used by many of the researchers and the key factors are vitamin mixtures, antimicrobial agents and some of the protein sources such as wheat germ. There are also variations in the amount and type of carbohydrates used such as the different types of flour and water. The rationale for decisions to use one diet over the over would be the cost of the ingredients, safety and quality of the ingredients, using local suppliers, availability of the ingredients and the quality of the insects reared on the diet.

Table 1.1a: Ingredients used in artificial diets for large- or mass-rearing *Cydia pomonella.*

Diet ingredients (g/kg)	Brinton et al. 1969	Howell 1972	Geunnelon et al. 1981	Botto 2006	Canada SIR 2003	Hansen & Anderson 2006
Moisture/solvents						
Water	717ml (71.7%)	788ml (78.8%)	755.5ml (75.5%)	477ml (47.72%)	650ml (65%)	832ml (83.2%)
Apple pulp		12 (1.2%)				
Ethanol						11.9ml
Propylene glycol		7.2ml				7ml
Oil				1ml (0.19%)		
Binding agents						
Agar			20 (2%)			4.2 (0.42%)
Paper/wood pulp	12.4 (1.24%)			16 (1.62%)	11.72 (1.17%)	
Proteins						
Yeast			37.8 (3.78%)	52 (5.25%)		
Casein	26.9 (2.69%)					
Milk powder				6 (0.63%)		
Wheat germ	9 (0.9%)	36 (3.6%)	35.5 (3.55%)	52 (5.25%)	8.13 (0.81%)	42.8 (4.28%)
Wheat gluten		36 (3.6%)		62 (6.20%)	4.69 (0.47%)	
Wheat bran	18 (1.8%)					
Carbohydrates						
Sugar		18 (1.8%)		31 (3.1%)	24.37 (2.44%)	
Sucrose	26.9 (2.69%)					20 (2%)
Cellulose powder		1.8 (0.18%)				
Canola flour					121.88 (12.19%)	
Corn flour			141 (14.1%)	94.1 (9.41%)		
Soybean flour		109 (10.9%)		62 (6.2%)		85 (8.5%)
Whole wheat flour	98.6 (9.8%)	18 (1.8%)			62.5 (6.25%)	19 (1.9%)
Vitamins and minerals						
Ascorbic acid	11 (1.1%)	1.94 (0.194%)	5 (0.5%)	6.3 (0.63%)	3.54 (0.35%)	3 (0.3%)
Vitamin mixture	6.1 (0.61%)	6.06 (0.61%)		0.33 (0.03%)	5.21 (0.52%)	7.8 (0.78%)
Choline chloride	1 (0.1%)				1.79 (0.18%)	
Wesson's salt mixture	6.2 (0.62%)	1.2 (0.12%)			4.92 (0.49%)	1.28 (0.13%)
Antimicrobial agents						
Aureomycin ®	4.94 (0.49%)					0.76 (0.08%)
Benzoic acid			2.3 (0.23%)	2.8 (0.28%)		
Formaldehyde		1.8 (0.18%)	1.3ml	0.76ml (0.08%)	1ml	
Methyl-p-hydroxybenzoate		0.7 (0.07%)	1.8 (0.18%)	2 (0.25%)	1.44 (0.14%)	0.7 (0.07%)
Norflaxina				0.1 (0.01%)		
Sorbic acid	2.7 (0.27%)	6 (0.6%)				0.64 (0.06%)
Benlate						0.1 (0.01%)
Fillers						
Sawdust	68.9 (6.89%)			131 (13.12%)	97.9 (9.79%)	
Ph						
Fumaric acid					7.27 (0.73%)	
Citric acid	9 (0.9%)					

1.4 The role of diet ingredients used in artificial diets

1.4.1 Yeast and wheat germ as protein source

Most insects use proteins as a source of nitrogen (Cohen 2004). Proteins are composed of amino acids, which are linked by a single peptide bond (Coultate 1989). Amino acids are important to support optimal growth and the quantity must be efficient for the insect. Some insects are able to distinguish artificial diets that are high in protein from those that are not by associative learning (Bernays & Chapman 1994). Amino acid requirements can vary according to the age of the insect and the quantitative relationship between other nutrients (McGinnins & Kasting 1972). Amino acids can be absorbed by the insect cells and resynthesized into proteins that make up the insect's body. Insects require eight to ten amino acids (methionine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, lysine, arginine and histidine) (Gilmour 1965; Cohen 2004). Other amino acids that can be synthesized by the insect's metabolic pathway include serine, asparagines, aspartic acid, glutamine, glutamic acid, alanine, cysteine, glycine, tyrosine and proline (Cohen 2004). Extremes of pH and temperatures in the diet preparation process can lead to denaturation of protein.

Yeast is an important source of proteins. Artificial diets that include yeast as an ingredient usually do not need a vitamin and salt mixture. Different types of yeast have been used in CM diets such as baker's yeast; torula yeast and the more common, Brewer's yeast. Diets that include yeast are those described by Guennelon et al. (1981) and Botto (2006).
Wheat germ is a very important ingredient and is used in all the diets discussed. It has a high protein content of about 23%, a high mineral and iron content and a high lipid content that is rich in polyunsaturated fatty acids (Cohen 2004). It contains the essential and nonessential amino acids, lots of fiber and vitamins with the exception of vitamin A and ascorbic acid (Cohen 2004).

Casein is a milk protein, very rich in amino acids (Dyck unpublished 2010) and can help with growth performance (Rodrigue 1972). Marwick et al. (1995) found that there was a linear relationship between the mean CM larval weight and the casein content of the diet. Casein levels

of < 0.9% limited larval weight, increased developmental time and caused higher mortality than diets with more casein (Marwick et al. 1995). Brinton et al. (1969) describe CM diets that include casein while Botto (2006) used milk powder.

There are two types of protein in flour – one type (7 - 15%) consists of the cytoplasmic proteins, which are soluble in water. The remaining 85% are the storage proteins of the seed. This protein is responsible for dough forming and is called gluten (Coultate 1989). The Canadian SIR facility uses gluten as a binding agent in the diet and whole wheat flour contains gluten.

1.4.2 Flour as a source of carbohydrates (monosaccharides, oligosaccharides, polysaccharides)

The principal components of carbohydrates are sugars, such as sucrose and glucose, together with polysaccharides such as starch and cellulose (Coultate 1989). Carbohydrates are important as building materials and energy for insects (Friend 1958, Downer 1981, Cohen 2004). Some insects cannot digest some carbohydrates (e.g. cellulose), but it can be used as a bulking ingredient (Cohen 2004). Some insects need a diet with at least 50% carbohydrates (Cohen 2004).

Polysaccharides are high-molecular-weight polymers of monosaccharides (Coultate 1989). Polysaccharides occur in plants and have two major roles. The first as a carbohydrate reserve in tissues such as seeds and is almost always filled with starch and secondly providing structure to the plant and cells (Coultate 1989). Starch is a major plant polysaccharide. Undamaged starch granules are insoluble in cold water, but as the temperature is raised, water begins to be imbibed. Initial gelatinisation temperatures lie in the range of 55 – 70˚C (Coultate 1989). As the granules begin to swell, the viscosity of the suspension rises. Wheat, maize and soya beans contain starch. Availability of these products can play a role in deciding which flour to use e.g. the availability and quality of canola meal throughout the year in South Africa can be a problem. Genetically modified Bt-maize cultivars are another factor that has to be taken into consideration as these can have a negative effect on insect production of (Personal observation).

Sucrose is a disaccharide and is extracted from sugar cane or sugar beet, but is also abundant in plant materials and fruit (Coultate 1989). Sucrose is not a reducing sugar and under mild acid conditions is hydrolyzed to its component monosaccharides. This is termed inversion and the resulting mixture is invert sugar (Coultate 1989). Howell (1972) used invert sugar instead of crystallized sugar in this diet. Sucrose can be a feeding stimulant and is nutritive (Singh 1984). An apple contains about 2.47 g sucrose per 100 g fruit (Besler 1999).

1.4.3 Vitamins and minerals

Vitamins are essential components of the biochemical and physiological systems of life. Vitamin B and ascorbic acid and some compounds such as choline, are water-soluble. Vitamin B is important in energy utilization (thiamine, riboflavin, niacin) and folic acid and biotin are important for growth (Cohen 2004). A deficiency of biotin can result in slow larval growth and a decrease in adult fertility.

Ascorbic acid is used in all the diets (Table 1.1a and 1.1b) and it can stimulate feeding and serves as an antioxidant. Rock (1967) found that ascorbic acid had an effect on growth and development of CM. On diets without ascorbic acid, no moths emerged (Rock 1967). The ascorbic acid requirement for that study was between 0.4 and 0.8 g per 100 g diet (Rock 1967). Redfern (1964) also did a study on the ascorbic acid requirement and showed that adult emergence increased with an increase in ascorbic acid (Rock 1967).

As a general rule, thiamin (vitamin B_1) is present in food that is rich in carbohydrates e.g. the embryo (germ) component of grains (Coultate 1989). The association of thiamin with carbohydrates is related to its role in metabolism. Thiamin is a co-factor in biochemical pathways of energy transduction from the chemical bonds of carbohydrates and lipids to high-energy phosphates, ATP (Cohen, 2003). Apples contain about 35 µg of vitamin B_1 and 30 µg of vitamin B_2 per 100 g fruit (Besler 1999). Dried brewer's yeast and yeast extracts contains riboflavin (Vitamin B_2) and other vitamins (Coultate 1989). Riboflavin is essential in the energy metabolism pathways involved in ATP production and niacin is involved in the energy transduction pathways (Cohen 2004). Niacin is the collective name for nicotinic acid (Coultate

1989). Fruits and vegetables can be useful sources of niacin. Whole wheat flour can contain high niacin levels but milling can have negative effects on the nutrition (Coultate 1989). Pyridoxine is involved in amino acid metabolism, but it is not essential to all insect species. Pyridoxine is involved in processing tryptophan into various pigments and a deficiency can result in abnormal pigmentation and frass colour (Cohen 2004). Wheat germ is rich in pyridoxine (Coultate 1989). Guennelon et al. (1981) did not use a vitamin mixture (Table 1.1a) but ascorbic acid and the brewer's yeast included in that diet contains many of the vitamins needed. No information was available on the vitamin mixtures used by Hansen and Anderson (2006) and Botto (2006).

Table 1.1b: Components of the vitamin mixture used in artificial diets.

	Binton et al. 1969	Howell 1972	Canada SIR
g/kg	6.10g	6.06g	5.2g
Vitamin mixture			
Niacinamide	5.00		1.875
Calcium pantothenate	5.00	12	7.875
Thiamin hydrochloride	1.25	3	0.465
Ribivlafin	2.50	6	0.938
Pyridoxin hydrochloride	1.25	3	0.465
Folic acid	1.25	3	0.465
Biotin	0.10	0.24	0.0375
Vit B12	1.00	24ml	3.75mg
Choline chloride		750ml	
Nicotinic acid		12	
Inositol		240	
Ascorbic acid	1804	1.94	680.93
Alpha tocopherol		96	
Tween 80		200	
Mannitol			0.375
Sorbic acid	449	6.06	169.5

Minerals cannot be biosynthesized thus they must be present in the diet and can be added to insect diets as salt mixtures (Cohen 2004). Guennelon et al. (1981) and Botto (2006) did not use any salt mixtures. A common salt mixture used by many entomologists is Wesson's salt mixture (Table 1.1c). Potassium is involved in chemical reactions and appropriate ratios of potassium to sodium or magnesium to sodium stimulates insect feeding (Cohen 2004). Magnesium is widely distributed in foods and in whole wheat flour there can by over 100 mg magnesium per 100 g

flour (Coultate 1989). Apples contain about 6 mg magnesium per 100 g fruit (Besler 1999). Magnesium functions in the glycolysis pathway involved in the conversion of carbohydrates to yield energy (Cohen 2004). Chloride is involved in the maintenance of the membrane potential and is required by all organisms (Cohen 2004). Chloride, potassium and sodium are involved in water regulation processes. Calcium is involved with the regulation of muscle responses to stimuli (Cohen 2004). Apples do not contain large amounts of calcium (less than 10 mg calcium per 100 g fruit) (Coultate 1989). Whole wheat flour has about 35 mg calcium per 100 g flour and white flours have added calcium in the form of carbonate (Coultate 1989).

Table 1.1c: Wesson's salt mixture (Cohen 2004).

	Amount (%)
Salt mix (Wesson's)	
Calcium carbonate	21.00
Copper sulphate	0.04
Ferric phosphate	1.47
Magnesium sulfate	0.02
Manganese sulfate	9.00
Potassium aliuminium sulfate	0.01
Potassium chloride	12.00
Potassium phosphate monobasic	31.00
Potassium iodide	0.01
Sodium chloride	10.50
Sodium fluoride	0.06
Tricalcium phosphate	14.90

1.4.4 Gelling agents

Gelling agents are important ingredients in insect diets because they keep water in a solid state, prevents reactions between ingredients, preserve the mixed state of the ingredients and some gelling agents can be utilized (proteins, starches and pectin) while some are nondigestible (agar and carrageenens) (Cohen 2004). Carbohydrates are the most common gelling agents in food and include gums such as guar gum and carboxymethylcellulose, carrageenan, agar, starch, alginates and pectins (Cohen 2004). Guar gum is obtained from the seeds of *Cyamopsis tetragonolobus* and has a water-soluble fraction (85%) called guaran, a nontoxic colloidal polysaccharide (Imeson 1997; Jain et al. 2005)

The alginates are a seaweed polysaccharide in brown algae and agar and carrageenans sulfated polysaccharides in red algae (Imeson 1997, Hilliou et al. 2006). Navon and Moore (1971) and Singh (1977) used sodium alginate as a binding agent. There are three types of carrageenen, kappa, iota and lambda, but the one most suitable for artificial diets is the kappa type (Imeson 1997). Agar agar is a very popular gelling agent used in many artificial diets. It is a dried, hydrophilic, polysaccharide extracted from red seaweed, *Gracilaria converfoides* (Roeper certificate of analysis). It has a lower sulphate content compared to carrageenens and has a reversible gelling capacity. Agar agar forms gels at very low concentrations and does not require the presence of other ions or products for gelation (Imeson 1997). A 0.05% agar agar solution will give a slightly viscous gel and a 3% solution will give a firm gel (Gillepsie 1993). Agar agar is easy to use and easy to mix with other ingredients. A disadvantage of agar agar is that it is very expensive. Agar agar was used as a binding agent in most of the other CM diets mentioned.

Brinton et al. (1969) described an agar-free diet used for mass-production of CM. He modified a diet used by Ignoffo (1963) who used a semi-synthetic diet for the cabbage looper, *Trichoplusia ni* (Hübner). The agar in the cabbage looper diet was replaced by whole wheat flour, wood pulp and wood sawdust. Water retention was improved by sawdust with a particle size of about 2 x 2 x 20 mm (Brinton et al. 1969). The sawdust used was from Douglas fir, *Pseudotsuga taxifolia* (Poir.). Sawdust also helps to provide the larvae with shelter in the diet, thus reducing cannibalism (Bloem et al. 1997). Howell found the medium too expensive and not acceptable enough for mass-rearing (Howell 1970, 1971). He composed an alternative diet that is less expensive. Agar and casein were replaced with wheat starch and soya flour resulting in better larval acceptance of the diet (Howell 1972).

The Canadian SIR facility uses a modified sawdust diet described by Brinton et al. (1969). This diet contains paper pulp and sawdust instead of agar as gelling agent (Bloem et al. 1997). Botto (2006) used a modified diet described by Guennelon et al. (1981) but replaced agar with paper pulp and sawdust.

1.4.5 Antimicrobial agents

Microorganisms in an artificial medium can be detrimental to insects. Mold, bacteria and viruses are common to mass-produced insects. Antimicrobial agents and preservatives used are methyl-parahydroxybenzoate, formaldehyde, sorbic acid, Streptomycin, Aureomycin and benzoic acid. Alternative methods for controlling the microbial contamination are to adjust the pH and sterilizing the diet and equipment used (Singh 1977). The pH of the diet used by Brinton et al. (1969) was about 3.5. The pH of the diet used in the Canadian CM mass-rearing facility is 4.6 (Personal communication, S. Taggart 2003) and the pH of the Guennelon et al. (1981) diet is 4.07 (personal observation).

1.4.6 Water content

Water is a very important nutrient in artificial diets. Water activity is needed in the chemical reactions and physical appearance of the diet (Cohen 2004). Cohen (2004) stated that the amount of water in the insect's natural food source is a good basis for water needed in the artificial diet. It is difficult to maintain a constant water percentage in the diet due to evaporation and dehydration. Howell (1972) used dried powdered apple pulp to retain moisture. Free water can also be a problem because the newly hatched larvae can drown. Apples contain about 80% water and therefore most of the diets in Table 1.1a contain more than 70% water.

1.5 Methods and preparation of diets

1.5.1 Containerization for rearing insects

Many kinds of containers have been used for rearing insects. Containers have been selected for suitability and availability. Containers used for rearing insects include milk bottles, metal trays, petri dishes glass jars and other consumables (Burton & Perkins 1984). Containerization became more standardized as the technology of insect rearing moved from natural diets to artificial diets (Burton & Perkins 1984).

An effective container must protect the food, present the food to the insect in an acceptable manner, separate cannibalistic insects, and provide the proper surfaces and atmosphere to the insects (Burton & Perkins 1984). Container size can have an influence on rearing because of the diet type, dehydration of the diet and the edge effects on the diets (Personal observation). Brinton had poor results in small cups but satisfactory results in a tray (Hathaway et al. 1971). Air and moisture exchange may be the most important physiological and ecological function of the rearing container, giving the insect a favourable microenvironment (Burton & Perkins 1984).). Light intensity experienced by the insects will vary depending on the type of container (Owens 1984). As the technology of insect rearing advances to mass-rearing, the type of container and size becomes more important as it must save labor cost and time. Brinton et al. (1969), Howell (1972), Guennelon et al. (1981), Bloem et al. (1997) and Hansen and Anderson (2006) used big trays for rearing CM (Table 1.2).

Table 1.2: Tray dimensions used for different diets for *Cydia pomonella*.

	Dimension	Material
Brinton et al. (1969)	30 x 46 x 2.5 cm	polystyrene
Howell (1972)	45 x 26 x 7 cm	
Guennelon et al. (1981)	27 x 25 x 10 cm	plastic boxes
Bloem et al (1997)	45x x29 x 2.5 cm	fiberglass
Hansen & Anderson (2006)	31 x 50.11 x 8.3 cm	aluminium

1.5.2 Diet preparation

There are many different ways and flexibility to prepare diets for rearing insects, but there are a few general practices that are used. Dyck (unpublished 2010) gave a list of guidelines for diet preparation methods, but only the general methods for the five diets (Table 1.2) described will be mentioned.

- Heating is required for destroying microbial contaminents, detoxifying soy proteins (Cohen 2004) and for activating starch formation and gelling agents. Heating can require up to 100°C for boiling water (Howell 1972, Guennelon et al. 1981), between 74°C and 94°C (SIR 2003, Brinton et al. 1969) and 52°C (Hansen & Anderson 2006).
- Gelling agents such as agar (Guennelon et al. 1981, Hansen & Anderson 2006) and fillers such as wood pulp, cellulose and paper pulp (Brinton et al. 1969,SIR 2003) must be added to water and heat to activate the gelling process or to hydrate the fillers such as dried apple pulp and soybean meal (Howell 1972, Hansen & Anderson 2006).
- Vitamins, especially ascorbic acid, should be added at a lower temperature of 60°C (Dyck unpublished 2010).
- Sucrose is mixed in the diet at relative higher temperatures (90°C – Brinton et al. 1969, 75°C - SIR 2003) and at lower temperature (52°C – Hansen & Anderson 2006).
- Wheat germ and flour are mixed in the diet at temperature 52°C - 65°C (Guennelon et al. 1981, Hansen & Anderson 2006) and at higher temperatures 84°C - 90°C (Brinton et al. 1969, Howell 1972, SIR 2003).
- Some ingredients must be dissolved before adding it to the diet. Howell (1972) and Hansen and Anderson (2006) dissolved methyl-hydroxyparaben and sorbic acid in ethyl alcohol and ascorbic acid in water. Guennelon et al (1981) and Brinton et al. (1969) mixed methyl-hydroxyparaben and ascorbic acid directly in the diet.
- Some diets use a wax film to cover the diet in the trays to prevent dehydration (Brinton 1969, Howell 1972, SIR 2003, Hansen & Anderson 2006).

1.6 Quality control in mass-rearing

1.6.1 Definition of quality

Chambers (1977) noted, "Quality is the degree of excellence in some skill relative to a reference". In terms of CM quality control, this means the performance requirement of the laboratory insect compared to the standard, which could be the wild CM in the field or CM reared on other successful diets. In the past, the aim of mass-rearing insects was to produce as many insects as possible (Proverbs 1982). However, in a SIR program it is essential that the insects reared be of high quality to ensure efficient competition in the field among the wild population. Thus, mass-rearing CM involves two major components: (a) production of the insects and (b) performance of the insects to accomplish the purpose of the program (Chambers 1977). Therefore, a Coordinated Research Project (CRP) entitled "Improvement of Codling Moth SIT to Facilitate Expansion of Field Application" was initiated in 2001 and completed in 2007. The CRP objective was to improve CM SIR for application and research focused on sterile moth quality and basic genetics of CM (Vreysen 2010).

A variety of values is determined to monitor the quality of the rearing process and to ensure continuity in production facilities (Chambers 1977). Quality control involves (1) process quality control, measuring how things are done, diet preparation, environmental conditions, irradiation dose etc., (2) production quality control, where the inputs to rearing are addressed, including equipment diet ingredients and (3) insect quality parameters (Calkins & Parker 2005, Parker 2005, Dyck 2010). The SIR facility in British Columbia, Canada, prepared a manual on mass-rearing CM, including quality control (SIR 2003).

1.6.2 Process quality control

Environmental control involves regulation of the external conditions that affect the growth, development and behavior of an organism (Owens 1984).

1.6.2.1 Temperature

Temperature affects insect development rate and in a mass-rearing facility, the rate of insect production is important. The longer the larval growth period, the more uniform the temperature has to be for development synchrony (Owens 1984). In addition, studies indicated that fluctuating rearing temperature could improve the quality of the insects rather than constant high temperatures (Proverbs 1982). Bloem et al. (1998a) found that fluctuating temperatures could cause maintenance problems (Dyck unpublished 2010). Accurate temperature control is critical in larval areas and unless large amounts of heat are dissipated, the quality of the insects will be lowered (Dowell et al. 2005). In melon-fly mass-rearing, the larval rooms are cooler than those that holds the adults for egg production and the temperature needs to be lowered as the larvae become larger as an increasing amount of metabolic heat is produced (Dowell et al. 2005).

1.6.2.2 Humidity

Humidity control is important for diets that dry out i.e. diets without a gelling agent and for the larvae to pupate inside the diet. Too high humidity can cause mold contamination. The amount of diet, number of insects per tray and the material of the container also affects humidity (Owens 1984). The Canadian mass-rearing facility as well as the large rearing facility in Argentina decreases the humidity from 75% to 50% in 21 days (Bloem et al. 2000, Taret et al. 2007).

1.6.2.3 Photoperiod

A long photoperiod is important to prevent diapause in the developing larvae. Generally, insects respond to very low light intensity (Owens 1984). Light and photoperiod need to be regulated to

keep the larvae from going into diapause. Light intensity experienced by the insects will vary according to the way the containers are distributed in the rooms (Owens 1984).

1.6.2.4 Air movement

Ventilation control in insectaries producing CM is problematic. Air handling and quality is important to control temperature and humidity, reduce airborne particulates and microbial contamination (Howell 1971, James et al. 1973, Dowell et al. 2005, Parker 2005). Codling moth shed scales that can be harmful to workers. High Efficiency Particulate Air (HEPA) filters are necessary to reduce the incidence of airborne bacterial, fungal and viral pathogens (Owens 1984). Therefore, air must be recycled through filters often enough to keep it clean (Griffin 1984). For diets without gelling agents, which tend to dry out quickly, air movement is critical (Griffin 1984, Dyck 2010). Too much airflow in a boll weevil, *Anthonomus grandis grandis* Boheman, development room dries the medium too fast and causes lower yield of insects (Griffin 1984). Horizontal airflow between trays on carts is necessary to control the rate of drying as in the Canadian CM rearing facility. Horizontal airflow is provided by air entering the room from small holes in the plastic sidewalls and each tray receives air from a hole adjacent to it (Dyck unpublished 2010).

A 2-3 minute air-exchange rate requires more airflow than is needed to maintain the temperature and humidity (Owens 1984). Brinton et al. (1969) used three air changes per minute, Howell (1971) used two air changes per minute and the Canadian facility 1.3 air changes per minute (Dyck unpublished 2010). Increasing the number of air changes per unit time requires more energy (Owens 1984). The use of large rooms requires the recirculation of air and the continuous filtering and adjustment (temperature and humidity) of the air.

1.6.3 Production control

The ingredients of an artificial diet must be of the quality needed to rear the insect for a particular purpose (Brewer & Lindig 1984). Flours should be insect free and should be monitored for chemical contamination from pesticides. The correct storage of the diet ingredients is very important and wheat germ should be stored in a moisture-proof container

(Brewer & Lindig 1984, Dyck 2010). Vitamins and agar should be stored according to the manufacturer's recommendation and not in a warm and humid environment, but in a closed, dark container. The quality of the diet ingredients can change over time and affect insect colonies drastically (Brewer & Lindig 1984). Storage of cereal grains for four months increase sugars and decrease starch content, but storage for a shorter period has no effect on the carbohydrate values (Jood et al. 1993).

1.6.4 Insect quality parameters

The routine insect quality parameters that are determined for most production facilities and to assess the efficiency of the diet, are egg mortality, survival and yield of adults, sex ratio, adult and pupal weight, longevity, and fecundity (Table 1.3). Characteristics of artificial diets, such as nutritive elements, contaminants, moisture, texture and pH can influence these insect quality parameters (Lance & McInnis 2005). Data are variable between diets and rearing methods (Table 1.3).

- Body weight is a quantitative adaptive trait and sufficient nourishment is needed for body size to evolve (Miller 1990). Pupal size is a good indicator of larval diet quality (Calkins & Parker 2005).
- Egg mortality is affected by temperature, humidity and the age of the females (Howel 1981). Changes in egg mortality may indicate problems in the colony or rearing facility.
- Percentage adult emergences determine the number of insects to be released. Eclosion may be affected by larval nutrition, affection pupal energy reserves, temperature and humidity (Calkins & Parker 2005).
- Development time of the larvae is affected by temperature and diet quality.
- Longevity is influenced by adult diet (water), temperature and humidity (Howell 1981). The ability of sterile insects to survive as long as the wild population is critical to the success of SIR. If the longevity of the sterile insects decline, the frequency of releases should be increased (Lance & McInnis 2005).

- Fecundity is the number of progeny produced per female and is affected by temperature, humidity, age and weight of the females (Gu et al 2006). There is also a correlation between the number of eggs laid and female longevity (Hagley 1972).

All the insect quality parameters usually examined are a result of the quality of the diet. A reduced survival and skewed development rate is found in the gypsy moth, *Lymantria dispar* (Linnaeus), offspring when there is a deficiency of available iron (Odell et al. 1997, Keena et al. 1998). Supplementing boll weevil, *Anthonomus grandis grandis* (Boheman) diet with beta-carotene increases dispersal and trap response (Reinecke 1991) and protein feeding improves pheromone production in Mediterranean fruit flies (Calkins & Parker 2005).

Table 1.3: A comparison of quality parameters for *Cydia pomonella* reared on different diets.

	Binton et al.	Howell	Geunnelon et al.	Bloem et al. 1997	Hansen & Anderson
QUALITY PARAMETER	1969	1972	1981	Canada SIR	2006
Adult emergence	52.00%	51.20%	77.00%	42.30%	
Pupale weight (mg) males	26-40	39.90		30.93	
Pupale weight (mg) females	30 - 50	52.20		39.22	
Adult weight (mg) males	17.30		32mg (ave)	19.70	38mg (ave)
Adult weight (mg) females	26.70			29.75	
Longevity (days) males	15.00			14.36	
Longevity (days) females	10.00			11.22	
Fecundity (eggs per female)		80		216	

1.7 Aims of study

The development of artificial diets has pioneered advancements in insect rearing. The emphasis on SIR in an integrated pest management program ensures that dietetics will continue to be important in the development of standardized rearing systems. The crucial areas in dietetics that need support are the quality and safety of the ingredients used and the effect of preparation procedures on diet quality and stability (Odell 1984).

A successful CM diet depends on chemical and physical factors and the texture of the diet must be acceptable for the larvae to penetrate and pupate in. The water content of the diet must be relatively high with no free water. Nutrients must be sufficient for the insects to complete their life cycle in the diet and microbial contamination must be limited. Preparation of the diet must be easy and each diet should fulfill the purpose for which it was developed.

There is a lack of documentation about the technology and methods use in SIR mass-rearing CM. The ultimate aim of this research is to develop an optimal rearing strategy for CM SIR, with the following objectives:

1. Determine the effect of genetically modified maize meal containing the *Bacillus thuringiensis* gene in an artificial diet on rearing CM, thus the safety of the maize meal
2. Assess the interaction between various gelling agents and carbohydrates in rearing CM on an artificial diet, thus the chemical and physical texture of the diet
3. Compare the quality of the moths and costs of mass-rearing CM on four different gelling agents
4. Designing an optimal closed-production system for rearing CM.

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CHAPTER 2

The effect of genetically modified maize meal containing *Bacillus thuringiensis* genes on rearing codling moth larvae, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), on an artificial medium

2.1 Abstract

Genetically modified maize contains the *Bacillus thuringiensis* (Bt) gene which is an important component in integrated pest management against maize pests. Large numbers of codling moth, a pome fruit pest, were reared for sterile insect release using an artificial diet containing maize meal as the main ingredient. However, most of the maize producers in South Africa use Bt maize. Bt is known to be toxic to codling moth. Five Bt maize meal concentrations and a control were used to rear the codling moth on an artificial diet for 44 days. The use of Bt maize meal resulted in prolonged larval development and high mortality. It is therefore important for an insect rearing facility to use non-genetically modified ingredients.

2.2 Introduction

Codling moth (CM) *Cydia pomonella* (L.) is a major pest on apples and pears throughout the world (Barnes 1991, Boncheva et al. 2006, Soleno et al. 2008). Codling moth is being laboratory-reared on a large scale in South Africa (Stellenbosch, Western Cape) for use in a Sterile Insect Release (SIR) program starting 2002 (Addison 2005). Economical production and efficient rearing of large numbers of insects are important in a SIR program. An artificial diet, described by Guennelon et al. (1981), is used for rearing CM and it includes maize meal as a major ingredient, making up 59% of the diet. Almost 30 years have passed since the Guennelon

diet was published and the availability, safety and quality of the original diet ingredients have changed.

The production of genetically modified (GM) cultivars in South Africa has increased from 34.9% in 2006 (Van Rensburg 2007) to 62% in 2008 ("Status of biotechnology by country" 2009). Genetically modified maize accounted for 64% of the total area in 2008 ("Status of biotechnology by country" 2009). The use of GM *Bacillus thuringiensis* (Bt) maize targets stemboring Lepidoptera and is an important component in integrated pest management (Gore et al. 2002). The Bt gene is engineered into the maize genome and marketed by a number of companies in South Africa. Recent studies have shown that non-target insects and beneficial insects can be negatively influenced by these GM cultivars due to exposure to insecticidal toxins (Vojtech et al. 2005, Walker et al. 2007). Availability of Bt-free maize meal is limited, as most of the maize producers in South Africa use GM Bt-maize cultivars and South African milling companies do not distinguish between Bt maize and non-Bt maize. *Bacillus thuringiensis* is known to be toxic to CM and Bt-crystal formations have been used as a control measure against CM in the field (Andermatt et al. 1988, Falcon & Huber 1991, Boncheva et al. 2006). The use of local maize meal in an artificial diet for rearing CM thus represents a high risk.

High larval mortality and fluctuations in rearing numbers were observed from 2005 to 2007 at the SIR facility (personal observation). Because of the high larval mortality, the University of Cape Town's Virology Departement tested CM in 2007 for granulosis virus. No virus infection was found. However, varying amounts of Bt were found in maize meal batches, which might explain some of the fluctuations in the number of insects produced at the rearing facility. Konecka et al. (2007) also observed high mortality in laboratory culture lines of a CM colony caused by Bt.

Bacillus thuringiensis is a gram positive bacterium which produces crystalline inclusions during sporulation (De Maagd et al. 1999, Boncheva et al. 2006, Konecka et al. 2007). These inclusions consist of delta-endotoxins or Cry proteins with insecticidal properties (De Maagd et al. 1999, Boncheva et al. 2006, Konecka et al. 2007). Bt crystal proteins destroy cells in the insect's midgut and gain entry only if the insect ingests Bt-contaminated food (Harris et al. 2006). The

insect ingests Bt crystals which dissolve in the midgut of the insect, releasing Cry proteins as protoxins. This results in direct mortality, as well as indirect effects such as cessation of feeding (Fast & Regniere 1984, Harris et al. 2006) and increased dispersal if larvae have recovered from the toxic effects (Halcomb et al. 2000, Gore et al. 2002, Harris et al. 2006).

In this study, the amount of Bt in the maize meal was quantified and the effect of it on the development and mortality of CM larvae feeding on an artificial medium containing various concentrations of Bt maize, was assessed.

2.3 Materials and Methods

2.3.1 Insects and maize meal

The CM colony originated from larvae that were collected in commercial apple orchards in Elgin, Western Cape in 2003. Codling moth eggs, on sheets of wax paper, were obtained from the SIR facility in Stellenbosch, Western Cape, South Africa where CM has been cultured on a diet described by Guennelon et al. (1981). Eggs were held at 25 ± 1ºC and a photoperiod of 16:8 (L:D) until the blackhead stage was reached. Eggs were sterilized in a 2% sodium hypochlorite solution to reduce the risk of fungal contamination. These sterilized egg sheets were air-dried using a ventilated plastic box and kept in a closed plastic container until egg hatch.

Bt maize (Monsanto, Gauteng, South Africa; event Mon810; cultivar DKC 8012B and DKC 7815B used together), expressing Cry1Ab protein was used and Bt free, organic maize meal (Bio-organic certified BDOCA 009GPhPrHa, Wensleydale Farms, Gauteng, South Africa) was used as the control.

2.3.2 Diet preparation and general methods

The diet was mixed in a stainless steel pot heated on a gas ring. Water (40 l) was boiled, agar agar (800 g) was added and the mixture brought to the boil again before cooling to 65°C. Some of the ingredients (ascorbic acid 266 g; benzoic acid 122.6 g; methylparaben 96 g; 4% formaldehyde 70 ml; wheatgerm 1.89 kg; brewer's yeast 2.02 kg) were subsequently added. To 1.5 liters of the diet, organic maize and the Bt maize were mixed as follows:

(i) 0% Bt, 100% organic maize
(ii) 20% Bt maize, 80% organic maize
(iii) 40% Bt maize, 60% organic maize
(iv) 60% Bt maize, 40% organic maize
(v) 80% Bt maize, 20% organic maize
(vi) 100% Bt maize, 0% organic maize

For each batch, 282 g of maize was used. The diet was dispensed into sterile 25 ml plastic cups. Once the diet cooled and hardened, it was scarified before putting one first instar larvae per container, using a small paintbrush, on the diet. These cups were covered with wax paper to maintain relative humidity and to minimize contamination and larvae walking around. Experiments were repeated twice on different dates using the five Bt maize concentrations, a control and 50 larvae per concentration; each in individual cups containing the maize meal diet. In Experiment 1 larvae were kept in a rearing room at 27 ± 2°C, 58 ± 10% relative humidity (RH) and in Experiment 2, 28.8 ± 2°C and 69 ± 10% RH. The photoperiod was 18:6 (L:D) and the experiments were terminated after 44 days. The temperature differences were seasonal. Under normal rearing procedures, the larvae are kept in the rearing room for 28 days before adult eclosion starts.

2.3.3 Quantification of Cry1Ab protein in maize meal

The concentrations of Cry1Ab protein in the maize was determined using a commercially available ELISA test kit (Agdia Inc., USA) and assayed according to the manufacturers' protocol. The positive control, which had a concentration of 40 µg/ml of Bt toxin, was suitably

diluted to generate a standard curve. Maize samples were prepared by adding 1 ml sample buffer to 1 g of maize and allowing the Bt toxin to dissolve. Aliquots of 100 µl were used in the Bt ELISA test. The concentration of Bt toxin in the maize was calculated using the absorbance values obtained by reading the unknown values of the standard curve. Bt toxin calculations were recalculated in µg.

Hoerls' function (Daniel & Wood 1980),
$y = a(x)^b.e^{[c(x)]}$,
was used to describe the relationship between the percent Bt maize meal (x) and the amount of Bt in µg/g (y). The regression constants are a, b and c and e is the base of the natural logarithms.

2.3.4 Insect bioassay and toxicity

Adult eclosion starts around day 30 and can last for two weeks (day 44) if the larvae are kept at 27°C, a long-day photoperiod 16:8 (L:D) and 75% RH (Bloem et al. 1997). In the current SIR rearing facility, trays are moved from the larval rearing room to the adult eclosion room on day 28 when adult eclosion is about to start and are kept until day 44 when most of the adults have emerged. Peak eclosion is around day 35 (Personal observation, Bloem et al. 2000, Bloem et al. 1997).

In these experiments, weekly inspections were done on day 28 (beginning of adult eclosion), day 35 (peak eclosion) and day 44 (end of adult eclosion). Only data for day 44 were used in the analysis. The responses that were measured were:

(i) larval mortality plus the number of larvae that did not reach adulthood by day 44, or delayed development. This is the most important parameter and can influence insect rearing numbers.
(ii) the number of larvae that did not reach adulthood by day 44. Bt can have a negative influence on CM larval development (Dandekar et al. 1998).
(iii) larval mortality plus the number leaving the diet. Larvae have an increased tendency to disperse when infected with the toxin (Harris et al. 2006).

The concentrations causing 50% mortality (LC_{50}), 95% mortality (LC_{95}) and the 90% or 95% fiducial limits were determined by probit analysis using PoloPC (LeOra software 1987). Abbots' correction (Finney 1971) was used if there was a response in the control. Probit lines were tested for equality and parallelism. If they were equal, the data were combined (Finney 1971).

2.4 Results

2.4.1 Quantification of Cry1Ab protein

The relationship, as described by Hoerl's function, between percent Bt maize meal and the amount of Bt in µg/g fitted the data extremely well. ($F_{3,6}$ = 397.37; P < 0.001; R^2 = 0.995). The fitted function was $y = 0.4012\ (x)^{0.7338}.e^{[-0.0276(x)]}$. Using this function, the amount of Bt in µg/g of maize meal for the five concentrations of Bt maize meal in the diet was estimated as follows:

- 20% Bt maize meal = 0.8789 µg/g
- 40% Bt maize meal = 1.7578 µg/g
- 60% Bt maize meal = 2.6367 µg/g
- 80% Bt maize meal = 3.5156 µg/g
- 100% Bt maize meal = 4.3944 µg/g

2.4.2 Effect of Bt maize meal on mortality and larval development

The lines for the two experiments were not the same (χ^2_2 = 15.74; p < 0.001), but they were parallel (χ^2_1 = 0.12; P = 0.734). The data fitted the probit model well (χ^2_7 = 6.80; P = 0.45). The regression formulae were: y = 2.31x + 4.16 and y = 2.31x + 3.61 for Experiments 1 and 2 respectively (Fig. 2.1a). The LC_{50} and LC_{95} values in Experiment 1 were higher than in Experiment 2 (Table 2.1). This difference was significant as the effect (mortality plus delayed larval development) in Experiment 1 relative to Experiment 2 was 1.7324. The 95% fiducial limits were 1.3124 to 2.4699. This range does not include 1. The positive relationship between

increasing Bt concentration and the response of the larvae indicated that CM larvae were susceptible to the Bt maize meal.

Table 2.1: The LC_{50} and LC_{95} values with their 95% fiducial limits for delayed development plus mortality when reared on a diet containing Bt maize meal.

	LC_{50} (95% fiducial limits)[a]	LC_{95} (95% fiducial limits) [a]
Exp 1	2.31164 (1.88941-2.782)	11.87314 (8.05326-23.516)
Exp 2	4.00477 (3.25224-5.238)	20.56947 (12.72112-48.256)

[a] Toxicity is indicated as LC_{50} or LC_{95} (in µg/g maize meal) on day 44.

2.4.3 Effect of Bt maize meal on larval development

The probit lines for the effects of Bt maize meal on larval development were not the same (χ^2_2 = 46.75; P = 0.001), but they were parallel (χ^2_1 = 2.04; P = 0.154). Probit regression lines were determined for both experiments and results showed that the data fitted this model well (χ^2_7 = 9.54; P = 0.216). The probit regression formulae were y = 1.89x + 3.79 and y = 1.89x + 2.85 for Experiments 1 and 2 respectively (Fig. 2.1b). Delayed development in Experiment 1 relative to Experiment 2 was 3.1496 (Table 2.2) and the 90% fiducial limits were 1.8467 to 6.9300, suggesting that the difference in delayed development was significant as the fiducial limits did not include 1. The upper 95% fiducial limit could not be determined.

All of the larvae developed to adults in the control by day 44, while 58% and 24% (Experiment 1 and 2 respectively) of the larvae failed to develop to adults in the 100% Bt maize by day 44.

Table 2.2: The LC_{50} and LC_{95} values with their 95% fiducial limits for delayed development when reared on a diet containing Bt maize meal.

	LC_{50} (95% fiducial limits)[a]	LC_{95} (95% fiducial limits) [a]
Exp 1	4.38292 (3.215-8.313)	32.64398 (13.756-365.775)
Exp 2	13.80461 (7.605-63.280)	102.81669 (31.127-2910.253)

[a] Toxicity is indicated as LC_{50} or LC_{95} (in µg/g maize meal) on day 44.

2.4.4 Effect of Bt maize meal on the mortality of CM

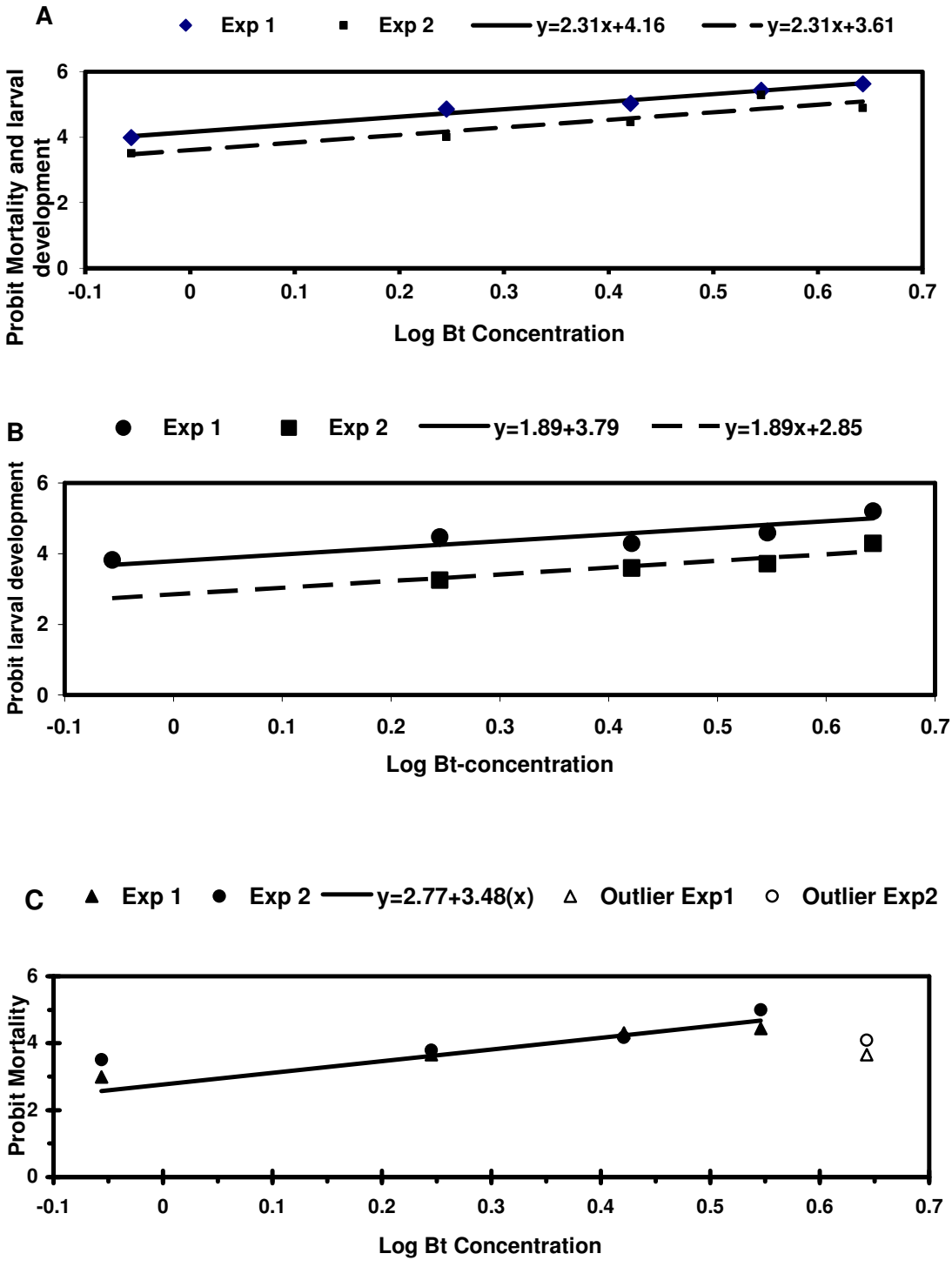
In both experiments mortality at the highest concentration (100% Bt maize meal or 4.3944 µg/g Bt) was lower than that of the second highest concentration (40% maize meal or 1.7578 µg/g Bt) (Fig. 2.1c). The regression lines of probit mortality on log concentration did not fit the data well (χ^2_3 = 7.73; P = 0.05 for Experiment 1 and χ^2_3 = 11.88; P = 0.01 for Experiment 2). When these points were omitted from the analysis the data fitted the probit well (χ^2_2 = 0.39; P = 0.82 for Experiment 1 and χ^2_2 = 1.37; P = 0.50 for Experiment 2). Therefore, these points were not included in the analysis

The slopes and intercepts were the same (χ^2_2 = 2.33; P = 0.312). The common regression formula was y = 3.48x + 2.77 (Fig. 2.1c). The data fitted the probit model well (χ^2_6 = 3.47; P = 0.352). The LC_{50} and LC_{95} values and their fiducial limits for both the experiments are given in Table 2.3.

Table 2.3: The LC_{50} and LC_{95} values with their 95% fiducial limits for mortality when reared on a diet containing Bt maize meal.

	LC_{50} (95% fiducial limits)[a]	LC_{95} (95% fiducial limits) [a]
Exp 1&2	4.389 (3.609-8.936)	13.051 (7.263-151.076)

[a] Toxicity is indicated as LC_{50} or LC_{95} (in µg/g maize meal) on day 44.



Fig

Figure 2.1: Probit response on log Bt concentration of *Cydia pomonella* larvae reared on a diet containing Bt maize where the responses were: (A) larval mortality plus delayed larval development; (B) delayed larval development; (C) larval mortality.

2.5 Discussion

From the above results, it is clear that there is a positive dose-response effect of Bt maize meal on CM larval mortality and a prolonged larval development. This is the first report quantifying these effects of Bt maize meal in an artificial diet on CM and it shows that larvae are negatively affected in terms of development and mortality by even small amounts of Bt in maize meal. This negative effect of Bt maize meal on CM is not surprising, as the toxicity of different δ-endotoxins and Cry1Ab to CM has been demonstrated by various researchers (Andermatt et al. 1988, Pasquier et al. 1997, Rang et al. 2000, Boncheva et al. 2006). Rang et al. (2000) determined the toxicity of Cry1Ab against CM with a LC_{50} of 2.92 x 10^{-1} µg/µl and Boncheva et al. (2006) at 78 ng/g of diet. That is 0.078 µg/g which is less than in the present study, despite the mortality being determined after only four days. They used solubilized protoxins of Cry1 δ-endotoxins in mixture with an artificial diet.

There was a difference in mortality and larval development, as well as in the larval development only, between the two experiments. Both were higher in Experiment 1 than in Experiment 2. The most probable reason for the difference in larval development, and therefore the difference in the potency of the toxin, is the temperature difference. Blomefield (2003) determined that the degree day units (DDU) for larval development from first to fifth instar at 25°C (at a lower threshold temperature of 10°C) were 321.6°D for males and 329°D for females. This results in a development time of 20–21 days. At the average temperature of 27°C (Experiment 1) the DDU were 357ºD at day 21 and at 28°C (Experiment 2), the DDU were 378ºD. Therefore, the larvae were expected to develop faster in Experiment 2 than in Experiment 1. By day 44, many of the larvae feeding on the Bt maize still had not reached the fifth instar in both experiments, although the DDU reached 748ºD. This is more than double the expected time of 321.6ºD for CM larvae (Blomefield 2003) to reach the fifth instar. Although there was a difference in temperature between the two experiments, the CM in the control (0% Bt maize) still maintained a higher percentage emergence and faster development compared to the Bt maize-fed larvae in both the experiments. We can assume that the delayed emergence of adults indicated that larval development was prolonged because of ingestion of a sublethal dose of Bt. Dandekar et al.

(1998) reported a decreased rate of larval development in CM feeding on transgenic somatic walnuts with high levels of CryIA(c) gene expression. Similar findings were reported with regard to the delayed developmental time of *Spodoptera littoralis* (Boisduval) larvae reared on leaves and stems from Bt maize plants (Vojtech et al. 2005), the soybean looper larvae, *Pseudoplusia includens* (Walker) reared on Bt-cotton (Ashfaq et al. 2001) and other lepidopteran larvae (Adamczyk et al. 2001).

Mortality can be the result of direct toxicity or due to a lack of feeding activity (Avilla et al. 2000). The mortality at the highest Bt concentration was lower than the second highest concentration. A possible reason is that the larvae stopped feeding because delayed larval development at that concentration was the highest, but larvae were still alive. Dispersion can be associated with acute toxicity (Harris et al. 2006). CM larvae dispersing from the diet were also observed and it was assumed that they would die. Harris et al. (2006) found that the light brown apple moth *Epiphyas postvittana* (Walker) larvae stopped feeding after ingesting food contaminated with Bt and there was an increased tendency to disperse after the larva has recovered from the toxic effects. If the dose of the toxin is not lethal, the midgut is repaired in time to resume larval development (Harris et al. 2006).

These results demonstrate that Bt maize has a deleterious effect on CM even at low concentrations. It has a direct toxic effect as well as an indirect effect resulting in delayed development. Both effects are of major concern in mass-rearing insects. There are other lepidopteran diets internationally and locally in which maize meal is an ingredient. The risk of using South African milled maize meal in an artificial diet is high unless the Bt status of every maize batch is known as well as the effect on the insects. It is difficult and time consuming for an insect mass-production facility to carry out Bt quantification tests. The best alternative is to find a suitable replacement for maize meal (Chapter 3). This includes organic maize meal, whole wheat flour (Brinton et al. 1969) or soybean flour (Howell 1972, Hansen & Anderson 2006). These ingredients are more expensive and extensive testing, evaluation and GM status of these products are needed before being accepted as a suitable replacement. The results of this study have important implications for the successful rearing of insects.

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CHAPTER 3

Comparison between two carbohydrates and various gelling agents in rearing codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae), on an artificial diet

3.1 Abstract

Information on the interaction between gelling agents and carbohydrates in artificial diets is lacking, despite their importance for rearing insects. Therefore, a combination of nine gelling agents (agar agar, carrageenen, Kelcogel, Aquagel, Elastigel, pectin, guar gum, Cekol, Kiccolate) and maize meal or whole wheat flour as sources of carbohydrates were tested for rearing codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in a diet described by Guennelon et al. (1981). There were interactions between gelling agents and carbohydrates on CM larval mortality but not on pupal or adult weight, development time or diet weight loss. The carbohydrates had a significant effect on mortality and pupal weight. Larval mortality was higher in the maize meal than in the whole wheat flour diet and pupal weight was higher in the whole wheat flour diet than in the maize meal diet. Therefore, whole wheat flour was considered to be a better source of carbohydrates than maize meal. The gelling agents had a significant effect on mortality, pupal and adult weight, percentage diet weight loss and larval development. Agar agar, carrageenen, Kelcogel, Aquagel and Elastigel gave the best overall results.

3.2 Introduction

The economical production and efficient mass-rearing of *Cydia pomonella* (CM) is a pre-requisite in a Sterile Insect Release (SIR) program in area-wide integrated pest management. One of the requirements in a SIR program is mass-rearing insects on an artificial diet that is nutritionally and physically acceptable to the insects (Howell 1970).

Various artificial diets have been used to rear CM (Brinton et al. 1969, Redfern 1964, Rock 1967, Guennelon et al. 1981, Howell 1972, Hansen & Anderson 2006) and can be categorized into those with or without gelling agents (Dyck unpublished 2010). A non-gelling agent diet for CM is described by Brinton et al. (1969) and is used by the SIR facility in Osoyoos, British Columbia, Canada (Bloem et al. 1997). This diet substitutes paper pulp for agar agar and uses sawdust to regulate moisture and to provide shelters for developing larvae (Bloem et al. 1997). Botto (2006) also used paper pulp and sawdust as substitutes for agar agar. Relative humidity and airflow are very important aspects when non-gelling agents are used as the diet tends to dry out rapidly if the air supply is not efficient enough. These diets need sufficient humidity and a reduction in humidity may be necessary, e.g. from 75 to 55% within three weeks (Bloem et al. 2000, Dyck 2010). With the air handling equipment available in the South African SIR facility and the experience gathered with insect rearing, it was decided to use an artificial diet containing a gelling agent.

Gelling agents improve insect diets by preventing reactions between ingredients, preserve the mixed state of diet components and keep the high water content mixture into a solid state (Cohen 2004). Gelling agents which have been used for insect rearing include starch, gelcarin, carrageenen, Water-Lock G-400 (Honda et al. 1996, Chaudhury & Alvarez 1999) carboxymethyl cellulose and pectin (Dyck unpublished 2010) and tapioca (Abbasi et al. 2007), but few have been used in CM diets. Use of sodium alginate and carrageenen to rear CM were reported by Navon and Moore (1971) and Butt (1975) and Hatmosoewarno and Butt (1975), respectively. Many CM diets contain agar agar as a binding agent (Redfern 1964, Rock 1967, Howell 1970, Boncheva 2006, Hansen & Anderson 2006). Agar agar reduces moisture loss and limits free water on the diet surface, mixes and binds easily with the other ingredients, is stable at high temperatures and has very high gel strength.

The CM SIR program in South Africa started a pilot program in 2002 (Addison 2005). The diet currently used by the South African rearing facility (Stellenbosch, Western Cape), is described by Guennelon et al. (1981). This diet is easy to prepare with few ingredients which are all locally available. The one disadvantage of this diet is the amount of agar agar as the gelling

agent, making up about 70% of the diet cost. Due to the high cost of agar agar and the large number of insects needed, a replacement of or reduction in the use of agar agar is important to save costs for the SIR program to be economically sustainable.

Flour is an important ingredient in artificial diets, being a source of carbohydrates and proteins. Whole wheat flour, maize meal and soybean flour are popular ingredients of CM diets and many researchers have used whole wheat flour instead of maize meal in their diet (Brinton et al. 1969, Howell 1972, Hansen & Anderson 2006). Guennelon et al. (1981) used maize meal or semolina, but 64% of the maize producers in South Africa use genetically modified maize cultivars ("Status of biotechnology by country" 2009). These cultivars often contain the *Bacillus thuringiensis* (Bt) gene, toxic to CM, which can influence the quality of insect rearing (Chapter 2). So, taking the suitability and economic viability of the carbohydrates into consideration, a decision has to be made as to their use, either singly or in combination.

Quality measurements provide the information that ensures efficient production of insect numbers (Chambers 1977). Tests used to monitor the quality of the laboratory-reared insects must be repeatable, economical and simple (Huettel 1976). There are two types of quality control tests: routine and periodic tests. Routine tests are easy to do in the laboratory and include percentage mortality, percentage egg hatch, pupal and adult weight while periodic tests are done when the conditions are suitable e.g. tests involving wild insects (Dyck unpublished 2010). Routine quality parameters that will be used in this chapter include percentage mortality, pupal and adult weight, development time and percentage diet weight loss. Dyck 2010 did a review on the guidelines for rearing CM and some of the information that he gathered from other researchers and rearing facilities will be used in determining the optimal quality parameters in Chapter 3 and 4.

The objectives in this study were to (1) determine the effect (percentage mortality, pupal and adult weight) of the various gelling agents on the insects, (2) compare two sources of carbohydrate, yellow maize meal and whole wheat flour, on the mortality and weight of the insects reared on artificial diets containing these ingredients, (3) determine whether an interaction between the gelling agents and the maize meal/whole wheat meal exists as well as to

compare the mortality and weight of the insects and (4) find the four best gelling agent/carbohydrate combinations. From the four best gelling agents, further biological and quality parameters will be measured in Chapter 4.

3.3 Materials and methods

3.3.1 Gelling agents

The following gelling agents were tested for use in the laboratory:

3.3.1.1 Seaweed extracts

Agar agar: it has a gel strength of 600-800 g/cm^2 (1.5% solution at 20°C) and a viscosity of 10 to 100 cps (1.5% solution at 20°C) (C.E. Roeper specification sheet).

Carrageenen E 407/ Gelcarin ® ME 2251 stabiliser: it has a gel strength of 100 - 350 g/cm^2 (1.5% solution at 20°C) and a viscosity of 30 to 300 cps (1.5% solution at 75°C) (FMC Biopolymer product specification sheet).

Aquagel GU-805: this is a food grade refined kappa carrageenen forming thermoreversible gels at sufficient concentrations (Marcel Carrageenan product specification sheet). It has a water viscosity of 20-70 cps.

3.3.1.2 Microbial extracts

Kelcogel®F Gelluan gum: this is a hydrocolloid produced by the microorganism, *Sphingomonas elodea.* The set temperature is 70°C - 80°C and it is thermo-reversible (Kelcogel product data sheet).

3.3.1.3 Plant extracts

Elastigel ™ 1000J: this modified starch is produced from sago and heat is required to cause the starch granules to hydrate and swell. It is low in viscosity when hot, and sets quickly to elastic gels when cooling (National Starch Company technical service bulletin).

Pectin CF 025 -D: it is a low methylester citrus pectin with a viscosity of 177 cps (5% solution in distilled water) (Herbstreith & Fox KG data specification sheet).

Guar gum: it is soluble in hot and cold water (Jain et al. 2005) and has a viscosity of 5400 cps (1% solution) (Willy Benecke certificate of analysis).

Kiccolate F170 (Sodium carboxymethylcellulose): viscosity is 620 cps (1% solution) (Nichirin Chemical specification sheet).

Cekol ® Cellulose gum 2000: it is a highly purified carboxymethylcellulose with a viscosity of 1 000 – 3 000 cps (2% solution) (CP Kelco product data sheet).

3.3.2 Diet preparation

The diet of Guennelon et al. (1981) was used as a basis for the different gelling agents tested. The concentrations of the gelling agents used are given in Table 3.1. The gelling agent concentrations were used according to the supplier's recommendations and initial trials, except

for the agar agar. Guennelon et al. (1981) used a 2.6% agar agar concentration. This was reduced to a 1% concentration in these experiments (Table 3.1) to save costs. The maize meal, wheat germ and wood shavings were heated before use in a microwave oven. Whole wheat flour was also used to substitute maize meal.

Table 3.1: Gelling agent concentrations used in the artificial diet for codling moth, *Cydia pomonella*.

Ingredient	Amount
Agar agar	1%
Carrageenen	2%
Kelcogel®	1%
Aquagel	2%
Elastigel™	10%
Pectin	2%
Guar Gum	5%
Kiccolate	5%
Cekol®	2%

Procedure:

- Agar agar, carrageenen, Kelcogel and Aquagel were mixed with boiling water, boiled again and cooled down to 65°C before adding the rest of the ingredients (Table 3.2) as described by Guennelon et al. (1981). No wood shavings were used.
- Elastigel was mixed with cold water before bringing it to boil, cooling it to 65°C and adding the rest of the ingredients with wood shavings added last.
- Citrus pectin was dissolved in boiling water, boiled again and cooled down to 65°C. The rest of the ingredients were added as well as 5% tricalcium dicitrate to thicken the solution and wood shavings were mixed in last.
- Guar gum, Kiccolate and Cekol were mixed with maize meal/whole wheat meal and wheat germ before mixing it with tepid water. The mixture was brought to boil before cooling it down to 65°C and adding the rest of the ingredients with wood shavings added last.

Table 3.2: Diet ingredients used for rearing codling moth, *Cydia pomonella*.

Ingredient	Amount
Water	1.5 L
Ascorbic acid	10 g
Benzoic acid	4.5 g
Methylparaben	3.6 g
Formaldehyde	2.6 ml
Meal (maize/ whole wheat)	282 g
Wheat germ	70 g
Brewer's yeast	75 g
Pine wood shavings	150 g

The ingredients were mixed with a hand blender except for the wood shavings which were mixed in by hand. The mixture (approximately 1.5 kg) was immediately poured into plastic trays with dimensions 28.5 cm x 22.5 cm x 5 cm. The diet was then covered with brown paper bags for humidity control and to reduce the risk of contamination.

After the diet cooled, it was scarified with a plastic comb to facilitate larval penetration into the medium. Wax paper egg sheets were surface sterilized for four minutes in clean water mixed with 1% Sporekill (active ingredient: Didecyldimethylammonium chloride) (ICA International Chemicals), followed by four minutes in 2% sodium hypochlorite solution and rinsed for four minutes in clean water. The egg sheets were air dried in a sterilized plastic box before putting them on a wire rack placed on the diet. An average of 1 000 eggs were put on the diet. The trays were placed in an air conditioned rearing room on purpose made racks for 28 days at 26 ± 2°C and 60 ± 7% relative humidity (RH) with a photoperiod of 18:6 (L:D). Egg sheets were removed after 7 days and percentage hatch calculated. Larvae pupated inside the diet after which the trays were moved to the emergence room for two weeks. The emergence room was kept at 25 ± 2°C, 30 ± 7% RH and photoperiod 0:24 (L:D).

Pupae and adults (n = 20) were selected randomly from each tray and were weighed individually. Five trays per gelling agent were used. Percentage mortality was determined by counting the empty pupal cases after emergence of two weeks and calculating it from the total amount of eggs

placed on the diet. Percentage diet weight loss was determined after 28 days. The diet (excluding the weight of the tray) was weighed on day 1 and weighed again on day 28 before the trays were transferred to the emergence room. Due to the layout of the SIR rearing facility system, trays need to be transferred to the emergence room on day 28 when pupation has already occurred and adult emergence was about to start. Development time (in days) until 2% adult emergence was recorded. Larval development was monitored daily from day 20 until the start of adult emergence. Day 1 was when the eggs were put onto the diet.

3.3.3 Statistical analyses

The data were analyzed using a split plot analysis of variance design in STATISTICA, version 8 for Windows (StatSoft® 2009) with the source of carbohydrates (whole wheat flour and maize meal) as the main plots and the gelling agents as sub-plots. There were five replicates. Dependent variables were percentage mortality, pupal and adult weight and percentage diet weight loss. In the case of adult weight, gender was included as an additional set of sub-plots. The LSD (least significant difference) for each gelling agent was determined at $P = 0.05$ (Snedecor & Cochran 1967).

3.3.4 Selection of the four best gelling agent and carbohydrate combinations

Scores (1 - 9) were given to the various gelling agents according to the performance of the insects on percentage mortality, adult and pupal weight, development time and diet weight loss. Higher adult and pupal weight, shorter development time, lower diet weight loss and lower percentage mortality will achieve a higher score.

3.4 Results and Discussion

3.4.1 Percentage mortality

There were significant interactions between the source of carbohydrates and the gelling agents (Table 3.3). With most gelling agents there was higher mortality in the maize meal diet than in the whole wheat flour diet. However, when Kiccolate and Elastigel were used as gelling agents the converse was true (Fig. 3.1). There were significant differences between the various gelling agents and between the carbohydrates (maize meal and whole wheat flour) (Table 3.3). Overall, the percentage mortality from egg hatch to adult emergence was higher in the maize meal (66.4%) than in the whole wheat flour (55.8%) (Fig. 3.1). The difference was significant (Table 3.3). Percentage mortality was the lowest for the Kelcogel/wheat combination (38.7%); followed by Aquagel/wheat (45.7%) and carrageenen/wheat (48.2%) combination (Fig. 3.1). The lowest percentage mortality in the maize meal and gelling agent combination were in Elastigel (51.4%), agar agar (60.3%) and carrageenen (61.1%) (Fig. 3.1).

Guennelon et al. (1981) found that 1 000 moths required 2 kg of diet, thus 500 moths/kg. Agar agar, Kelcogel, carrageenen, Aquagel and Elastigel produced on average 372 moths/kg (whole wheat flour), 300 moths/kg for maize meal and on average 190 moths/kg on the pectin, guar gum, Kiccolate and Cekol (maize meal) and 270 moths/kg on the whole wheat flour. The most moths per tray that was achieved, was 792 moths in the Kelcogel/wheat combination. Bloem et al. (1997) estimated yields of about 375 adults per liter of diet. Dyck 2010 suggested that a yield of 200 adults per kilogram or liter of diet is a good estimate for mass-rearing insects.

Table 3.3: Split plot analysis of variance for percentage mortality using diets containing two sources of carbohydrates as main plots and nine gelling agents as sub-plots.

Source of variance	d.f	SS	MS	F	P
Source (S)	1	2538.92	2538.92356	6.823096282	0.031
Residual (S x G)	8	2976.86	372.107245		
Gel (G)	8	7220.51	902.5639294	7.493602243	<0.001
S x G	8	2976.86	372.107245	3.08944729	0.005
Residual	64	7708.45	120.4446006		

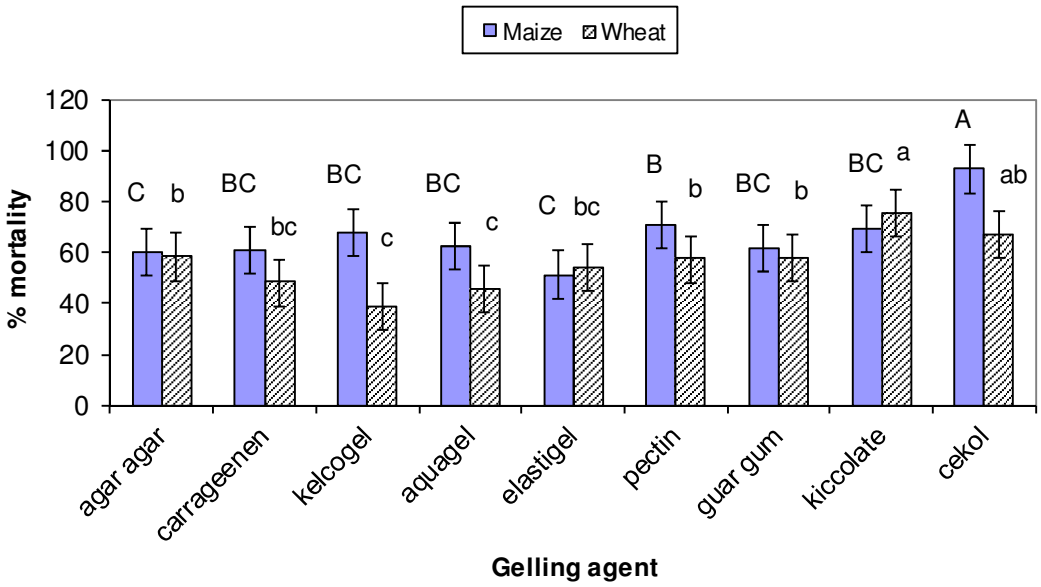

Figure 3.1: Mean percentage mortality of *Cydia pomonella* reared on diets containing different gelling agents, maize meal and whole wheat flour. Gelling agents sharing the same letter within each carbohydrate source are not significantly different ($P > 0.05$).

3.4.2 Pupal weight

There were no significant interactions between the gelling agent and the carbohydrates on pupal weight (Table 3.4). Pupal weight was greater for the insects reared on whole wheat flour (44.3 mg) than those reared on maize meal (41.5 mg). The difference was significant (Table 3.4). There was also a significant difference in the pupal weight between the different gelling agents (Table 3.4). Pectin gave the lowest average pupal weight (Fig. 3.2) (24 mg maize meal and 31 mg with whole wheat flour). Aquagel gave the highest pupal weight on the whole wheat flour (53.2 mg) and agar agar gave the highest pupal weight on the maize meal (46.82 mg), but the difference between these two gelling agents was not significant (Fig. 3.2). Dyck 2010 indicated that the average male pupal weight should be about 31 mg - 35 mg and female pupal weight 39 mg - 43 mg. All of the tested diets reached this target except for the diet containing pectin.

Table 3.4: Split plot analysis of variance for pupal weight using diets containing two sources of carbohydrates as main plots and nine gelling agents as sub-plots.

Source of variance	d.f	SS	MS	F	P
Source (S)	1	0.000173	0.000172917	7.249258191	0.027
Residual (S x G)	8	0.000191	2.38531E-05		
Gel (G)	8	0.003226	0.000403193	25.47642655	<0.001
S x G	8	0.000191	2.38531E-05	1.507198453	0.172
Residual	64	0.001013	1.58261E-05		

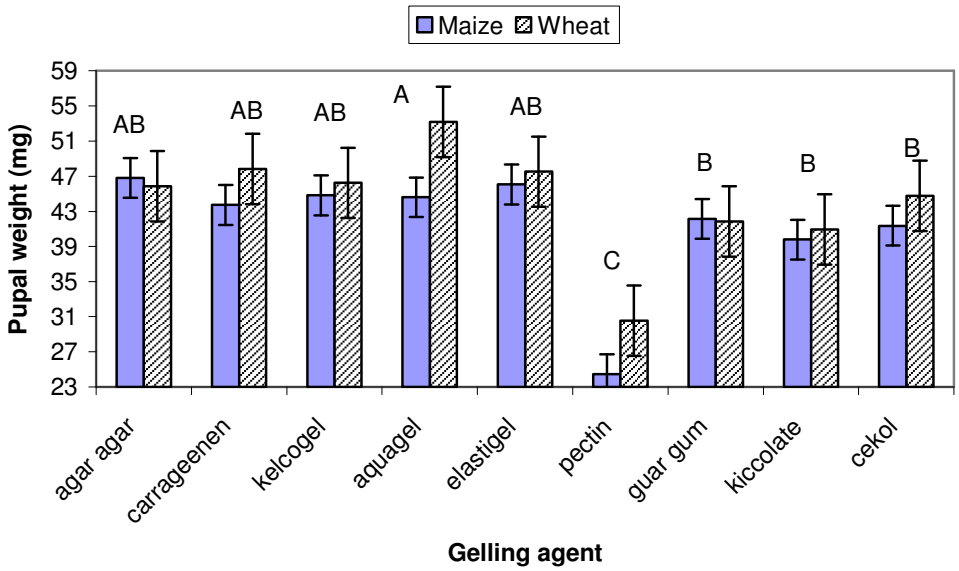

Figure 3.2: Mean pupal weight (mg) of *Cydia pomonella* reared on diets containing different gelling agents, maize meal and whole wheat flour. Gelling agents sharing the same letter are not significantly different ($P > 0.05$).

3.4.3 Adult weight

There were no interactions or differences in adult weight between the two sources of carbohydrate (Table 3.5). However, there were differences between genders (Table 3.5). The females (25.86 mg) were heavier than the males (17.9 mg). In addition there were differences in moth weight between the gelling agents (Table 3.5). The diet containing pectin produced the lowest average adult weight for males (13.2 mg) and females (16.6 mg) (Fig. 3.3a and b). The diet containing carrageenen produced the highest average female adult weight (30.87 mg) and Kiccolate the highest average male adult weight (20.23 mg). The average weight of the male moths should be 18 - 20 mg and for the females 28 - 30 mg (Dyck unpublished 2010).

Table 3.5: Split plot analysis of variance for adult weight using diets containing two sources of carbohydrates as main plots and nine gelling agents and gender as sub-plots.

	SS	Degr. Of	MS	F	P
SOURCE	2.816	1	2.816	0.8180	0.392000
Error	27.545	8	3.443		
GEL	1374.823	8	171.853	8.6750	<0.001
GENDER	3218.297	1	3218.297	162.4566	<0.001
SOURCE*GEL	165.447	8	20.681	1.0440	0.407000
SOURCE*GENDER	0.013	1	0.013	0.0007	0.979000
GEL*GENDER	228.928	8	28.616	1.4445	0.184000
SOURCE*GEL*GENDER	27.545	8	3.443	0.1738	0.994000
Error	2555.516	129	19.810		
TOTAL		172			

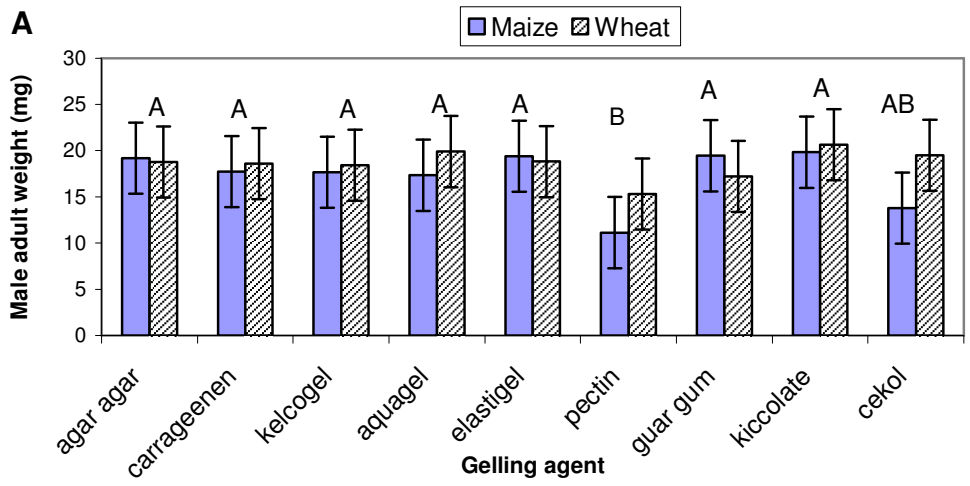

Figure 3.3: Mean male (A) adult weight (mg) of *Cydia pomonella* reared on diets containing different gelling agents and carbohydrate sources (maize or wheat). Gelling agents sharing the same letter are not significantly different (P > 0.05).

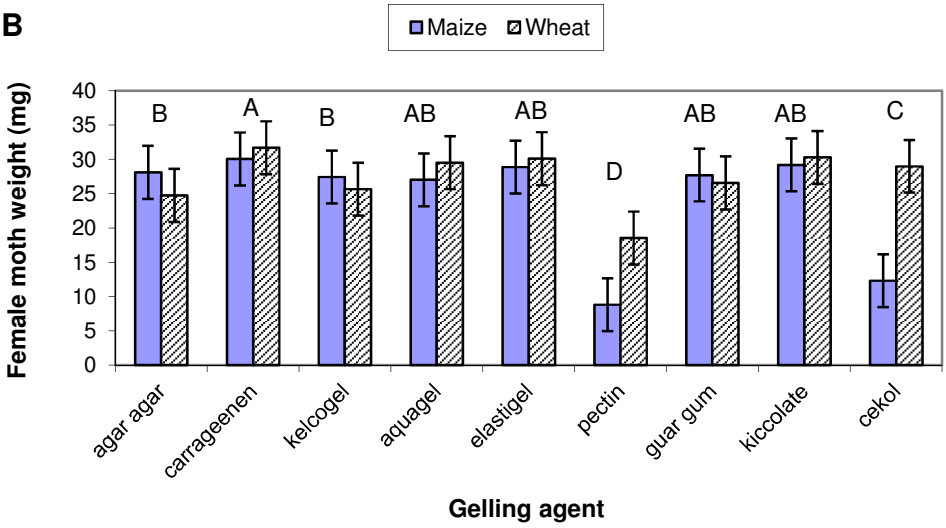

Figure 3.3: Mean female (B) adult weight (mg) of *Cydia pomonella* reared on diets containing different gelling agents and carbohydrate sources (maize or wheat). Gelling agents sharing the same letter are not significantly different ($P > 0.05$).

3.4.4 Percentage diet weight loss

There was no significant interaction between the carbohydrate source and the gelling agents and the percentage diet weight loss stayed constant for the two sources of carbohydrate (Table 3.6). There was a significant difference in diet weight loss between the gelling agents (Table 3.6). The diet containing agar agar, carrageenen, Kelcogel and Aquagel had the highest diet weight loss after 28 days (Fig. 3.4).

Table 3.6: Split plot analysis of variance for percentage diet weight loss using diets containing two sources of carbohydrates as main plots and nine gelling agents as sub-plots.

Source of variance	d.f	SS	MS	F	P
Source (S)	1	29.641	29.64136111	2.973297253	0.123
Residual (S x G)	8	79.754	9.969188611		
Gel (G)	8	1700.678	212.5847303	6.852232591	<0.001
S x G	8	79.754	9.969188611	0.32133634	0.955
Residual	64	1985.546	31.02415563		

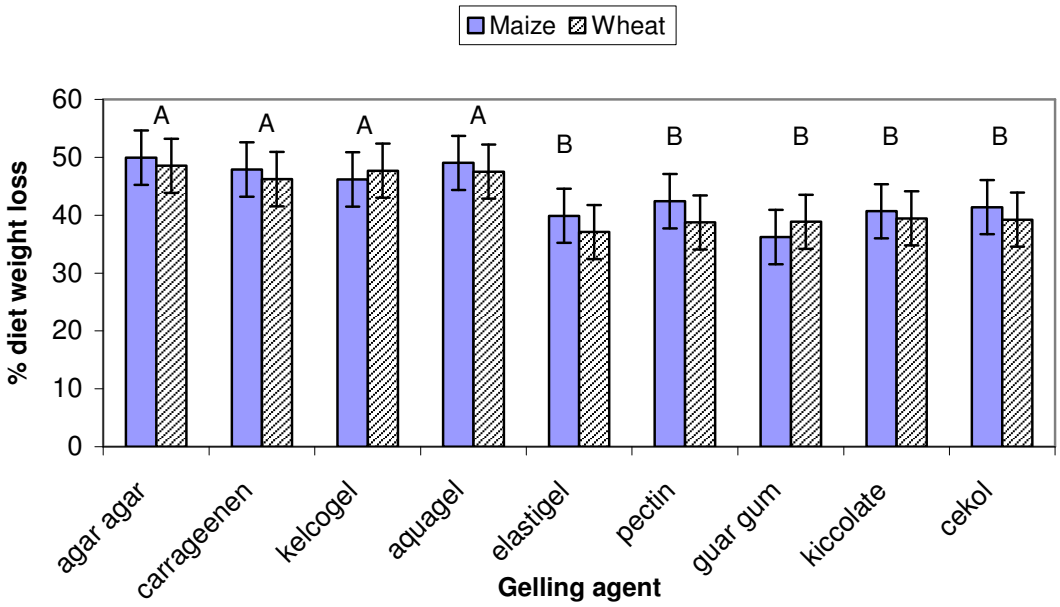

Figure 3.4: Mean percentage diet weight loss of diets containing nine different gelling agents, combined with two carbohydrate sources (maize or wheat). Gelling agents sharing the same letter are not significantly different ($P > 0.05$).

3.4.5 Development time (days) until 2% adult emergence

There were no interactions between the gelling agent and source of carbohydrate and there were no significant differences between the two sources of carbohydrate (Table 3.7). There were significant differences in the time (days) to 2% adult emergence between the gelling agents (Table 3.7). The shortest development time to 2% adult emergence was in diets containing Aquagel (25.3 d), Kelcogel (25.4 d), carrageenen (26.3 d) and agar agar (27.3 d) (Fig 3.5). The insects reared on diets with the rest of the gelling agents (pectin, Kiccolate, Cekol) took on average five days longer to emerge. Bloem et al. (1997) found that moths reared on a modified Brinton diet under standard conditions (27ºC, 16:8 (L:D), 55% RH) started to emerge by day 28. At a temperature of 27ºC, the duration of first instar larvae to adult was about 32 days (Dyck unpublished 2010).

Table 3.7: Split plot analysis of variance for development time using diets containing two sources of carbohydrates as main plots and nine gelling agents as sub-plots.

Source of variance	d.f	SS	MS	F	P
Source (S)	1	15.21111	15.21111	8.064801178	0.218
Residual (S x G)	8	15.08889	1.88611		
Gel (G)	8	786.15556	98.26944	32.89353789	0.0004
S x G	8	15.08889	1.88611	0.631334263	0.749
Residual	64	191.20	2.9875		

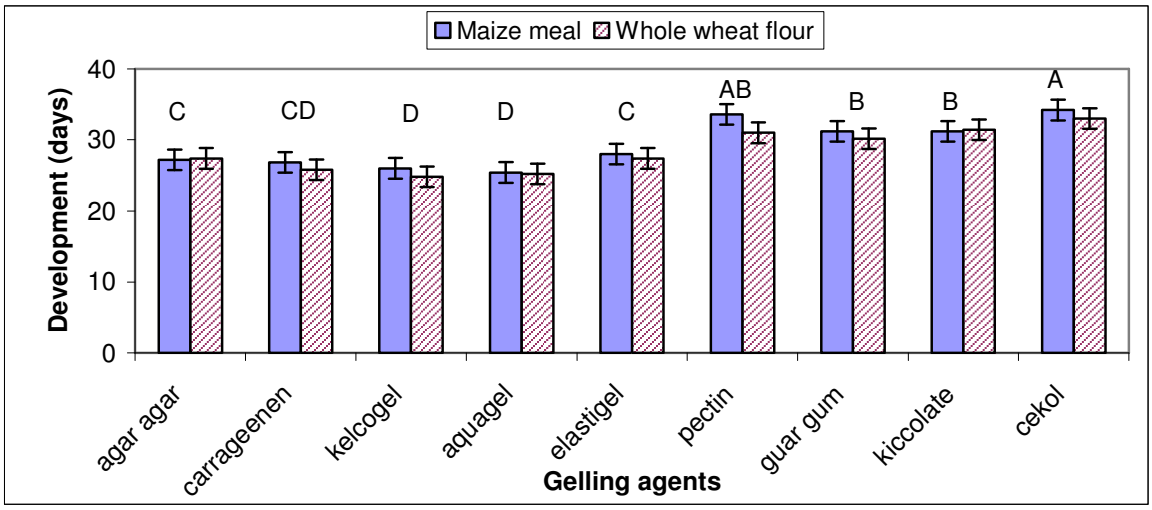

Figure 3.5: Mean development (days) until 2% adult emergence of *Cydia pomonella* reared on diets containing different gelling agents, maize meal and whole wheat flour. Gelling agents sharing the same letter are not significantly different ($P > 0.05$).

3.4.6 Selection of four best gelling agents and carbohydrate combinations

In the absence of information on suitable methods for selecting the best options, the following ranking system was used. When allocating scores the empirical values were used even if the differences were not significant. From the scores in Tables 3.8 & 3.9, the four best gelling agents in combination with the maize meal were agar agar, carrageenen, Aquagel and Elastigel. The best gelling agent in combination with the wheat flour were Aquagel carrageenen, Kelcogel and Elastigel. Aquagel is the best gelling agent with the whole wheat flour, but it is very expensive and not always available. For this reason, Aquagel will not be used in future studies.

Table 3.8: Scores allocated for the selection of the best gelling agent and maize meal combination.

Maize meal	% Mortality	Pupal weight	Adult weight (males)	Adult weight (females)	Moisture loss	Development time	Total
Agar agar	8	9	6	6	9	6	**44**
Carrageenen	7	5	5	9	7	7	**40**
Elastigel	9	8	7	7	2	5	**38**
Aquagel	5	6	3	3	8	9	**34**
Kelcogel	4	7	4	4	6	8	**33**
Kiccolate	3	2	9	8	3	3	28
Guar gum	6	4	8	5	1	4	28
Cekol	1	3	2	2	4	1	13
Pectin	2	1	1	1	5	2	12

Table 3.9: Scores allocated for the selection of the best gelling agent and whole wheat flour combination.

wheat	% Mortality	Pupal weight	Adult weight (males)	Adult weight (females)	Moisture loss	Development time	Total
Aquagel	8	9	8	6	7	8	**46**
Carrageenen	7	8	4	9	6	7	**41**
Kelcogel	9	6	3	3	8	9	**38**
Elastigel	6	7	6	7	1	5	**32**
Agar agar	3	5	5	2	9	6	**30**
Kiccolate	1	2	9	8	5	2	27
Cekol	2	4	7	5	4	1	23
Guar gum	4	3	2	4	3	4	20
Pectin	5	1	1	1	2	3	13

3.5 Conclusion

3.5.1 Source of carbohydrate

Nutrition involves growth, development and reproduction (Rock 1964) and the rate of insect development and reproduction is influenced by the host plant's carbon (digestible carbohydrate) and nutrient (e.g. protein nitrogen) balance (Scriber & Slansky 1981, Bezemer & Mills 2001).

When an insect is allowed unlimited access to food, it will eat at least enough to satisfy its energy requirements (Singh 1984). It is therefore necessary to consider the adequacy of the diets containing different gelling agents and sources of carbohydrates by various tests.

Whole wheat flour contains 59.7 g carbohydrate per 100 g flour and maize meal 72 g carbohydrate per 100 g flour (Sasko, Nutritional information sheet) and carbohydrates are necessary for fat and glycogen synthesis (Sing 1984, Friend 1985). Most insects use proteins as a source of nitrogen (Cohen 2004, Dyck 2010). The proteins are broken down into amino acids and are resynthesized into the proteins that make up the insect's body. The amount of protein required in a diet is influenced by its nutritional quality which is determined by the amino acid composition and also by how efficiently the digested food is absorbed through the gut wall (McGinnis & Kasting 1972, Singh 1984, Cohen 2004). Whole wheat flour contains 12.2 g protein per 100 g flour and maize meal 7.6 g protein per 100 g flour (Sasko, Nutritional information sheet). Sathpathy et al. (2003) found that the biological parameters of the rice grain moth, *Corcyra cephalonica* (Stainton), were positively correlated with the carbohydrate and protein content of the rearing media. Therefore, the efficiency and utilization of diets can be improved by a better choice of nutrient sources.

The results show that whole wheat flour is a suitable replacement for maize meal with larger pupae and lower percentage mortality. The higher pupal weight observed in the insects reared on the diet containing whole wheat flour, suggests a better utilization of the diet and/or better digestibility of the carbohydrate or protein. The use of whole wheat flour minimizes the risk of using a genetically modified product such as maize meal in South Africa and therefore lower percentage mortality. The percentage mortality differed between diets containing the gelling agent/carbohydrate combinations because there was interaction between them and therefore the best gelling agents with the lowest percentage mortality with the maize meal combination was not the same as for the whole wheat flour.

3.5.2 Gelling agents

The texture of the diet can be modified by using gelling agents and some of the gelling agents such as pectin and starches can also act as nutrients that can be utilized (Cohen 2004, Dyck 2010). However, the diet containing pectin gave the lowest pupal and adult weight. The significance of differences in moth weight for sterile moth performance is not yet understood. The gelling agents had an effect on the mortality of the insects, pupal and adult weight, diet weight loss and development. The diet containing the gels (Kelcogel, carrageenen, Aquagel, agar agar and Elastigel), gave the best results and fastest development compared to the gums (pectin, guar gum, Kiccolate, Cekol). Larvae reared on the diet containing pectin, guar gum, Cekol and Kiccolate took on average five days longer to develop than on diets containing agar agar, carrageenen, Kelcogel and Aquagel. This could be due to a lack of sufficient nutrition as food quality affects the development of CM (Hathaway et al. 1971) and the size and number of larval instars (Scriber & Slansky 1981, Blomefield & Gilliomee 2009). The texture of the diets containing pectin, Cekol and Kiccolate was not optimal due to the weaker gel strength resulting in feeding difficulty as these gelling agents did not solidify as well as those containing agar agar, carrageenen, Aquagel and Kelcogel. The highest mortality during the larval stage is experienced during first instar as they need to establish a feeding site soon after hatching (Zalucki et al. 2002). Therefore, a gelling agent with weak gel strength, insufficient structural stability and the presence of free water can result in higher first instar mortality and slower larval development. Also, the diet containing the gelling agents agar agar, carrageenan, Aquagel and Kelcogel had the highest diet weight loss and is significant because it indicates the suitability based on moisture performance.

Wagner et al. (1979) used the quality parameters, survival, development and weight gain of the satin moth, *Leucoma salicis* (Linnaeus), to determine and evaluate the effect of parental and progeny diet. In the present study, the percentage mortality, development time and pupal/adult weights played an important role in deciding which gelling agents and carbohydrates to use in mass-rearing insects on an artificial medium. Choosing a suitable source of carbohydrate and gelling agent combination is important in insect rearing due to their interaction and the effect on

the percentage mortality. It was found that whole wheat flour is a suitable replacement for maize meal. It is a better source of protein and carbohydrate and gives better interaction with the gelling agent carrageenen, Kelcogel and Elastigel and agar agar than maize meal. The diet containing the gelling agents carrageenen, Kelcogel, Elastigel and agar agar conformed to Dyck's (2010) summary of acceptable routine quality parameters (adult and pupal weight, development rate), and these gelling agents were chosen to rear CM over successive generations in Chapter 4.

Further research is needed to assess the four best gelling agents (agar agar, carrageenen, Kelcogel and Elastigel) with the whole wheat flour combination. Suggestions for future study include:

- Mass-rearing the insects over successive generations to ensure that the nutritional and physical requirements of the diet are acceptable for mass-rearing
- Using wood shavings with the gelling agent to reduce moisture loss
- Cost-benefit analyses between the gelling agents need to be compared
- Biological studies of the insects e.g. fecundity and longevity.

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CHAPTER 4

Quality and cost comparison of mass-rearing codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), on diets containing four different gelling agents

4.1 Abstract

The gelling agents, agar agar, carrageenen, Kelcogel and Elastigel were tested for rearing codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in large numbers in a modified diet described by Guennelon et al. (1981). In all the diets, the carbohydrate source was whole wheat flour and wood shavings were used to minimize moisture loss and to act as a filler. Three generations were reared on these diets. There were no interactions between gelling agents and the generations, but there were significant differences between male and female pupal weight, male and female adult weight, male longevity and female fecundity in diets containing the different gelling agents. Diets containing Elastigel had the biggest pupae and adults and the highest female fecundity. The generations played a significant role in development and male longevity. This was due to seasonal temperature differences. Lowering the amount of agar agar to 1% or replacing agar agar with Elastigel resulted in a marked reduction of at least 40.91% in rearing cost. There were no marked differences in the cost of the different gelling agents and cost per 1 000 moths. Overall, the diet containing Elastigel gave the best results.

4.2 Introduction

Codling moth, *Cydia pomonella* (L.) (CM), is an economically important pest on pome fruit in South Africa (Giliomee & Riedl 1998, Pringle et al. 2003, Timm et al. 2006). A pilot Sterile Insect Release (SIR) program in South Africa (Stellenbosch, Western Cape) was started in 2002 (Addison 2005) and is currently in a development phase for commercialization. Economically feasible techniques need to be developed for rearing a large number of insects for a commercial program to be sustainable.

Codling moth has been reared on many different artificial diets for small - large scale and mass-rearing. Choosing a suitable diet is dependent on the type of research and population size needed, availability of the ingredients, experience in rearing, rearing equipment, facilities and cost (Dyck unpublished 2010). Small scale rearing can involve using apple thinnings (Dickson et al. 1952) and plastic cups (Howel 1970, Hathaway et al. 1971, Dyck 2010) whereas large scale rearing can involve plastic containers (Leppla et al. 1982), trays (Howell 1972, Bloem et al. 1997) and boxes (Guennelon et al. 1981). Most of the published information on CM diets deals with laboratory colonies and not mass-rearing systems (Dyck unpublished 2010). Choosing an economically acceptable diet is critical for mass-rearing and should involve methods to reduce labor cost and energy, using an easy-to-prepare diet and produce a large number of insects. In Chapter 3, nine gelling agents were tested for use in the diet described by Guennelon et al. (1981). These diets were tested in small, plastic trays (28.5 cm x 22.5 cm x 5 cm). However, for mass-rearing bigger trays are necessary to reduce labor, handling and rearing cost.

Singh (1983) proposed that the ideal diet for mass-rearing programs should conform to certain specifications:

1: The diet should be inexpensive;

2: Contain all nutrients needed for the insects;

3: The diet should be easily prepared;

4: Diet should have a long storage life and in the case of CM last for at least 35 days;

5: There should be a 75% yield of adults from viable eggs.

Adequate nutrition results in growth, development and reproduction. The suitability of a diet can be quantified using body weight, survival rate, adult longevity and fecundity (Rock 1964). Many stress factors such as degradation of diet and insect quality due to environmental stresses may occur (Cohen 2004). Therefore, a quality control system and diet adequacy determination is important. To establish the nutritional requirements of the insects, they must be reared through several generations on the artificial diet to identify any cumulative effects of long-term deficiencies (Rock 1964, Dyck 2010).

Keeping Singh's (1983) above guidelines in mind, the effect of the four different gelling agents, selected from previous experiments (Chapter 3), on development time, pupal/adult weight, percentage emergence, percentage diet weight loss, fecundity and longevity was studied. This was done for three simultaneously reared consecutive generations on the Guennelon et al. (1981) diet.

4.3 Materials and methods

4.3.1 Egg sheets

Codling moth from the SIR rearing facility were kept in oviposition cages lined with white wax paper (40 g/sm) at 25 ± 1ºC, 50% RH, at a photoperiod of 16:8 (L:D). The egg sheets were removed every 24 hours and incubated until the blackhead stage, just before egg hatch. Egg sheets were cut into 15 cm strips and put in wire mesh booklets. They were surface sterilized in a 1% Sporekill solution for four minutes, 2% sodium hypochlorite solution for four minutes and rinsed for four minutes in clean water. Egg sheets were air dried in a sterilized plastic box before transferring them to the diet. Four sterilized egg sheets were placed on wire racks on the diet. Egg sheets were removed on day 28 and percentage egg hatch calculated. The second generation eggs were collected from moths reared in a diet containing a specific gelling agent from the first generation and used for the same gelling agent for the next generation. Where timing and

weekends were a problem, eggs were stored in a cold room at 2°C (generation 3) before transferring them to the diet.

4.3.2 Rearing conditions

A modified diet from Guennelon et al. (1981) (Table 4.1) was used with different gelling agents. The different gelling agents were:

1: 1% Agar agar;

2: 2% Carrageenen E 407/ Gelcarin ® ME 2251;

3: 0.5% Kelcogel ®F Gelluan gum;

4: 10% Elastigel (modified sago starch) ™ 1000J.

Twenty liters of water were boiled before adding the gelling agent (agar agar, carrageenen or Kelcogel) and then brought to the boil again. This mixture was cooled down to 65ºC before adding the rest of the ingredients (Table 4.1). Elastigel was mixed with 20 liters of cold water and then brought to boil to activate the starch. This mixture was cooled down to 67ºC before adding the rest of the ingredients. Six liters of diet were poured into a stackable tray (77 cm x 40 cm x 3 cm), cooled down and scarified to make larval entry easier. This was replicated 5 times per gelling agent per generation.

The trays were kept in a closed production box with a temperature at 26.04°C and 58.02% RH (first generation), 25.47°C and 49.51% RH (second generation) and 25.3°C and 50.79% RH (third generation) and 18:6 (L:D). These temperature differences were seasonal as the trial took six consecutive months, from autumn to spring. The production box was made out of a thick, clear plastic and could hold 20 stackable trays (as described in Chapter 5). The production box was connected to a filtered air supply via a flexible hose from the main air supply duct (as described in Chapter 5). The trays were transferred to emergence boxes on day 28 (when adult eclosion is about to start) and kept in the emergence boxes for two weeks. The emergence boxes were made out of cardboard with a clear plastic bottle on the side to collect the moths.

Table 4.1: Composition of diet used for rearing *Cydia pomonella.*

Constituents	Amount
Gelling agent	
Water	20 L
Ascorbic acid	133 g
Benzoic acid	66 g
Methylparaben	48 g
Formaldehyde	40 ml
Whole wheat flour	3.76 kg
Wheatgerm	990 g
Brewer's yeast	1.01 kg
Pine wood shavings	10 L

4.3.3 Assessment of moth quality

Samples of moths and pupae from each tray were collected and used to measure different parameters. The parameters were pupal and adult weight for both male and female (n = 50), female fecundity (n=10) and male and female longevity (n = 50). Newly eclosed adults were placed individually in a 25 ml plastic cup with a cotton wool lid (without water) and kept in a room at 25.51°C and 42.91% RH (generation 1), 25.49°C and 42.03% RH (generation 2) and 26.07°C and 52.12% RH (generation 3) and 18:6 (L:D) to measure longevity. The temperature and humidity were monitored inside the room and not inside the cups. The difference in room temperature was seasonal. The insects were examined daily and mortality was recorded. Smaller plastic cups were used (20 ml) for the first replicate of the first generation with a small piece of wet cotton wool inside, but this was unsuitable because mold developed in the containers and on the pupae resulting in mortality.

The oviposition cages for the fecundity studies were made from white wax paper. One newly eclosed female and two newly eclosed males were placed into the oviposition cages and allowed

to mate and oviposit until the female died. The temperature, humidity and day length were the same as the above. Egg hatch and total number of eggs were recorded.

The total number of moths produced was calculated by counting the empty pupal cases per tray after 2 weeks of emergence. The percentage emergence was calculated from the total number of eggs used per tray and the number of moths produced per tray. The number of eggs was determined by counting the number of eggs per sheet including the unhatched eggs. Diet weight loss was determined by weighing the diet (tray weight excluded) on day 1 and again on day 28.

4.3.4 Cost comparison

A comparison of the costs between different gelling agents was made and the number of insects produced was included.

4.3.5 Selection of the four best gelling agent and carbohydrate combinations

Scores (1 - 4) were given to the various gelling agents according to the performance of the insects on percentage mortality, adult and pupal weight, development time, diet weight loss, longevity, fecundity and cost per thousand moths. A higher score suggests better performance of the insects. The scores were allocated according to higher adult and pupal weight, shorter development time, lower diet weight loss, high percentage emergence, high number of moths, longer longevity, higher fecundity and lower cost of rearing.

4.3.6 Statistical analysis

The data were analyzed using a split plot analysis of variance design in STATISTICA, version 8 for Windows. The generations were the main plots and the gelling agents the sub-plots while the dependant variables were percentage mortality, pupal and adult weight, longevity, fecundity and diet weight loss. There were five replicates per generation. The LSD (least significant difference) for each gelling agent was determined at $P = 0.05$. Bartlett's test was used to test for homogeneity of variances.

4.4 Results and discussion

4.4.1 Homogeneity of variances

Most of the cases in which the variances were not homogeneous ($P < 0.05$) were to do with weights of pupae or adults. The exception was percent egg hatch (Table 4.2). Log transformations were used in these instances.

Table 4.2: Bartlett's χ^2 for homogeneity of variance of *Cydia pomonella* reared on an artificial diet containing different gelling agents.

	Bartlett χ^2	df	P
% Diet weight loss	10.999	11	0.443
Total eggs	6.056	11	0.870
%Egg Hatch	30.115	11	0.002
Total Moths	14.282	11	0.218
%Emergence	13.495	11	0.262
Pupal weight M (log mg)	22.542	11	0.020
Pupal weight F (log mg)	37.575	11	<0.001
Ave Moth Weight M (mg)	10.251	11	0.508
Ave Moth Weight F (log mg)	23.374	11	0.016
Development to 50% emergence F	14.419	11	0.211
Development to 50% emergence M	10.202	11	0.512
Longevity F (days)	5.403	10	0.863
Longevity M (days)	4.862	11	0.938
Fecundity	14.136	11	0.226

4.4.2 Interactions between number of generations and gelling agents

There were no interactions between the gelling agents and the number of generations in any of the quality parameters tested (Table 4.3).

Table 4.3: Split plot analysis of variance using the three generations of *Cydia pomonella* as main plots and four gelling agents as sub-plots.

	Generation		Gel		GENERATION*GEL	
Factor	F	P	F	P	F	P
Diet Moisture Loss	2.357	0.106	0.240	0.868	0.434	0.852
Total Eggs	13.865	< 0.001	0.106	0.956	0.248	0.958
%Egg Hatch	13.903	< 0.001	0.145	0.933	0.455	0.838
Total Moths/tray	4.184	0.021	1.836	0.153	1.155	0.346
%Emergence	4.409	0.017	1.586	0.205	1.586	0.172
Pupal Weight M (log mg)	5.044	0.010	5.260	0.003	0.778	0.591
Pupal Weight F (log mg)	0.611	0.547	5.128	0.004	1.526	0.190
Adult Weight M	4.302	0.019	7.909	< 0.001	1.408	0.231
Adult Weight F (log mg)	6.675	0.003	4.483	0.007	0.469	0.828
Development to 50% emergence M	17.030	< 0.001	0.642	0.592	0.279	0.944
Development to 50% emergence F	21.190	< 0.001	0.547	0.653	0.297	0.936
Longevity M	25.697	< 0.001	5.670	0.002	0.588	0.738
Longevity F	2.986	0.061	2.539	0.069	1.203	0.323
Fecundity	5.418	0.008	3.075	0.037	1.436	0.223

4.4.3 Quality parameters

Only in the case of those quality parameters for which there were significant differences ($P < 0.05$) in Table 4.3, were individual means for the three generations (Table 4.4) and four gelling agents (Table 4.5) compared.

4.4.3.1 Number of eggs used per tray

There were significant differences between the numbers of eggs used per tray per generation (Table 4.4). Significantly fewer eggs were used in generation 3 than in generation 1 and 2. The number of eggs used per tray is not a quality parameter, but is important as it influences the percentage emergence of adults per tray.

Table 4.4: A comparison of various quality parameters of *Cydia pomonella* reared over three generations. Generations within each quality parameter sharing the same letter are not significantly different (P > 0.05). Only those quality parameters that were significant (P < 0.05) in Table 4.3 are compared.

	Generation 1	Generation 2	Generation 3
Total Eggs	5048.8 a	4834.8 a	3478.8 b
%Egg Hatch	80.96 a	75.99 a	67.49 b
Total Moths/tray	2051.5 a	1524.7 b	1670.2 b
%Emergence	41.77 ab	34.27 b	47.61 a
Pupal Weight M (log mg)	1.64 a	1.63 ab	1.61 b
Adult Weight M(mg)	21.51 a	20.91 ab	19.56 b
Adult Weight F (log mg)	1.55 a	1.54 a	1.50 b
Development to 50% emergence M	34.30 a	35.95 b	37.05 c
Development to 50% emergence F	34.45 a	35.95 b	37.10 c
Longevity M(days)	9.19 b	9.00 b	10.25 a
Fecundity(eggs/female)	117.15 a	111.41 a	91.73 b

Table 4.5: A comparison of various quality parameters of *Cydia pomonella* differentially affected by diets containing four different gelling agents. Gelling agents within each quality parameter sharing the same letter are not significantly different (P > 0.05). Only those quality parameters that were significant (P < 0.05) are compared.

	Agar agar	Carrageenen	Kelcogel	Elastigel
Pupal Weight M (log)(mg)	1.62 b	1.61 b	1.62 b	1.65 a
Pupal Weight F (log)(mg)	1.70 b	1.69 b	1.71 b	1.74 a
Adult Weight M (mg)	19.90 b	19.70 b	20.05 b	22.99 a
Adult Weight F (log)(mg)	1.52 b	1.53 ab	1.51 b	1.56 a
Longevity M	9.62 ab	9.35 ab	9.03 b	9.92 a
Fecundity	113.45 ab	97.19 b	96.62 b	119.79 a

Table 4.6: A comparison of various quality characters of *Cydia pomonella* reared on an artificial diet containing different gelling agents over three generations.

	Agar			Carrageenen			Kelcogel			Elastigel		
Quality parameter	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3	Generation 1	Generation 2	Generation 3
(Mean ± SE)												
Pupal weight (mg)												
males	42.83 ± 1.33	42.2 ± 1.01	40.7 ± 0.45	43.8 ± 1.24	40.54 ± 0.85	39.17 ± 0.96	41.64 ± 0.66	41.41± 1.03	41.48 ± 0.76	44.64 ± 3.49	44.89 ± 0.54	42.58 ± 0.89
(n)	50	50	50	50	50	50	50	50	50	50	50	50
females	48.85 ± 1.67	52.56 ± 1.06	50.64 ± 0.39	48.77 ± 1.32	50.56 ± 1.31	49.23 ± 1.31	52.3 ± 0.56	51.36 ± 1.59	51.86 ± 0.95	52.25 ± 1.95	54.26 ± 1.32	51.88 ± 0.55
(n)	50	50	50	50	50	50	50	50	50	50	50	50
Adult weight (mg)												
males	21.17 ± 0.61	19.59 ± 1.17	18.93 ± 1.06	19.73 ± 0.51	20.39 ± 0.53	18.99 ± 0.96	19.73 ± 0.51	21.37 ± 1.40	19.06 ± 0.92	25.4 ± 1.34	22.29 ± 1.11	21.27 ± 0.78
(n)	50	50	50	50	50	50	50	50	50	50	50	50
females	33.84 ± 0.24	35.56 ± 2.13	31.06 ± 1.24	34.60 ± 1.10	34.66 ± 1.02	32.60 ± 1.58	33.69 ± 0.85	32.46 ± 1.80	30.93 ± 1.77	39.33 ± 0.29	36.20 ± 1.9	33.4 ± 1.31
(n)	50	50	50	50	50	50	50	50	50	50	50	50
Longevity (days)												
males	9 ± 0.31	9.2 ± 0.20	10.25 ± 0.51	9.2 ± 0.68	8.8 ± 0.20	9.75 ± 0.20	8.2 ± 0.37	8.8 ± 0.20	9.5 ± 0.37	9.2 ± 0.58	9.2 ± 0.20	10.75 ± 0.20
(n)	50	50	50	50	50	50	50	50	50	50	50	50
females	11.6 ± 0.24	11.8 ± 0.37	12 ± 0.32	11 ± 1.05	11.8 ± 0.37	12.25 ± 0.32	10.2 ± 0.58	12.4 ± 0.24	12.5 ± 0.51	11.4 ± 0.93	12.8 ± 0.37	12.25 ± 0.51
(n)	50	50	50	50	50	50	50	50	50	50	50	50
Eggs per female	130.41 ± 8.35	125.69 ± 6.80	84.25 ± 16.02	113.72 ± 6.40	87.78 ± 10.61	90.08 ± 6.68	92.23 ± 14.07	115.58 ± 12.76	82.04 ± 12.96	132.25 ± 8.57	116.58 ± 3.79	110.53 ± 17.77
(n)	50	50	50	50	50	50	50	50	50	50	50	50

4.4.3.2 Percentage egg hatch

There were significant differences in the percentage egg hatch between generations (Table 4.4). Egg hatch in generation 3 was lower than in generation 1 and 2. Diet had no effect on egg hatch (Table 4.3), but temperature, humidity and age of females are important factors affecting egg hatch. Eggs from generation 3 were kept in the cold store for a week before they were used. This was necessary due to a time constraint.

4.4.3.3 Number of moths

There were significant differences in the number of moths produced between generations (Table 4.3). More moths were produced in generation 1 than in generations 2 and 3 (Table 4.4).

The gelling agents had no effect on the number of moths produced per tray, and an average of 1 748 ± 164.47 (95% CI) moths per tray can be expected. However, the highest number of moths achieved per tray was 3 148 moths. This suggests that with a reduction in egg mortality and contamination, this number of moths per tray is possible. Two hundred adults or pupae per liter or kilogram of diet is a good estimate for mass-rearing (Dyck unpublished 2010). Hansen and Anderson (2006) reported that their rearing program at the Yakima Agricultural Research Laboratory in Washington, USA, could produce CM at one adult per 4.5 ml diet. That is equivalent to 222 larvae per liter of diet. With the bigger trays used in the present study an average of 291 moths per liter of diet would be produced at an average of 1 748 moths per tray. Although there were no significant differences between the gelling agents (Table 4.3), the diet containing carrageenen produced the highest number of moths in a tray and the highest percentage emergence. No adult abnormalities such as wing deformation were observed for any generation or gelling agents.

A decision that needs to be taken is whether the egg sheets should be left on the diet trays for 28 days until emergence, or whether they should be taken off after a week. The egg sheets in the

Canadian SIR facility are removed after a week (Bloem et al. 1997). In the current SIR facility, the egg sheets are also removed after a week, but in this study they were left on the diet for 28 days. Leaving the egg sheets on the diet has the advantage of minimizing the risk of contamination from outside air and from the personnel and it is less labor intensive. The disadvantage is that fungal contamination due to less air circulation caused by the egg sheets might develop. This still needs some attention and the amount of air supply in the boxes needs to be quantified.

Microbial contamination is one of the major factors affecting the rearing of insects (Sikorowski & Lawrence 1994). It reduces the nutritional value of the diet, the number of insects and the quality of insects produced (Sikorowski & Lawrence 1994, Cohen 2004). Pupal and larval mortality occurred and was unrelated to the gelling agents. Some trays developed fungal contamination in the production box. This was due to a lack of air circulation supposedly caused by the egg sheets that were left on the diet for 28 days, as discussed above. Fungal contamination was also present inside the cardboard emergence boxes and was responsible for some mortality and a reduction in moth numbers. Fungal contamination increased over time in the emergence boxes because of an increased amount of spores in the boxes.

4.4.3.4 Percentage emergence

There were significant differences in the percentage emergence between the generations (Table 4.3). The percentage emergence in generation 3 was higher than in generation 1 (Table 4.4).

The total percentage emergence was significantly higher for the third generation in diets containing all four gelling agents (Table 4.4) but varied during the three generations, with no significant differences between the gels (Table 4.3). The third generation also had the lowest number of eggs and the lowest percentage egg hatch. This suggested that most of the mortality was in the egg hatch.

Singh (1985) determined the yield of adults from viable eggs and in this study, it was determined from the total number of eggs used per tray. Bloem et al. (1997) stated that the production of the

SIR program in British Columbia increased to 1 270 adults per tray with an average of 3 000 eggs per tray and egg hatch of 81%. That gives a total percentage emergence of 42.33%, determined from the total amount of eggs. Hathaway et al. (1971) tested artificial diets and found that the diet of Redfern (1964) produced 33% adults, Howell's diet (1970) 47% adults and Brinton et al. (1969) 44.4 % adults emerging. These data are very similar to the percentage emergence found in the present study (generation 1: 41.77%; generation 2: 34.27%; generation 3: 47.61% adults emerging).

4.4.3.5 Pupal and adult weight

There were differences in pupal weight between generations for males but not for the females (Table 4.3). Male pupae from generation 3 (40.98 mg) weighed less than those from generation 1 (43.86 mg) (Table 4.4). Female pupae from each generation weighed more (52.52 mg, 52.17 mg and 50.90 mg for generation 1, 2 and 3) than male pupae (43.87 mg, 42.26 mg and 40.98 mg for generation 1, 2 and 3). The diet containing Elastigel produced heavier male (44.89 mg) and female (55.4 mg) pupae than diets containing the other gelling agents (Table 4.6).

There were differences in adult weight between the generations of both genders (Table 4.3). Adult males from generation 3 weighed less than the males from generation 1, while adult females from generation 3 weighed less than the females from both generation 1 and 2. There were significant differences in adult weight between diets containing the different gelling agents for both genders (Table 4.3). The diet containing Elastigel produced heavier males than diets containing the other three gelling agents (Table 4.5). The diet containing Elastigel produced bigger females (39.33 mg) than the diet containing agar agar (33.11 mg) and Kelcogel (32.36 mg) (Table 4.6).

The generations in this study had a significant effect on the male pupal weight and on the adult weights. Blomefield (2003) found that there were differences in the female and male pupal weight between the spring, early and late summer periods. The gelling agents played a significant role in the pupal and adult weight. Agar and carrageenen are nondigestible gelling agents. Starches can act as nutrients that can be utilized (Cohen 2004), and Elastigel is a

modified starch product, which might explain the heavier pupae and adults for both genders (Table 4.5). Hansen et al. (2004) reared CM on diets containing different percentages of cherries. The females showed no differences in weight, but the males did and they hypothesized that males were more sensitive to diet constituents than the females. Diets containing the tested gelling agents gave acceptable adult and pupal weights, but the females reared on the diet containing Elastigel (36.31 mg adult; 55.4 mg pupal weight) were heavier. Dyck 2010 suggested that the pupal weight should be between 31 - 35 mg for males and 39 - 43 mg for females. The mean weight of male adults should be between 18 – 20 mg and 28 – 30 mg for female adults (Dyck unpublished 2010). The average weight of wild CM was 18.4 mg (males) and 26.67 mg (females) (personal observation 2009). The moths in the present study weighed more (20.66 mg male weight; 33.93 mg female weight) than the wild moths, but the male adult weights were similar to Dyck's (unpublished 2010) recommendation. However, the female moths were heavier (Table 4.4, 4.5 & 4.6). Weight is an important quality parameter in insect rearing but there is some controversy about insect weight and sterile release. The release of heavy sterile males could produce better control because the light insects achieve less mating than the wild insects (Jiménez-Pérez et al. 2004). However, there is a negative correlation between body weight and the dispersal capacity of CM (Gu et al. 2006).

4.4.3.6 Development to 50% emergence

The number of days to 50% emergence differed between generations in both genders (Table 4.3). The time to 50% emergence increased significantly in each successive generation (Table 4.4; Fig. 4.1). This appeared to be due to seasonal temperature variations (Fig.4.1).

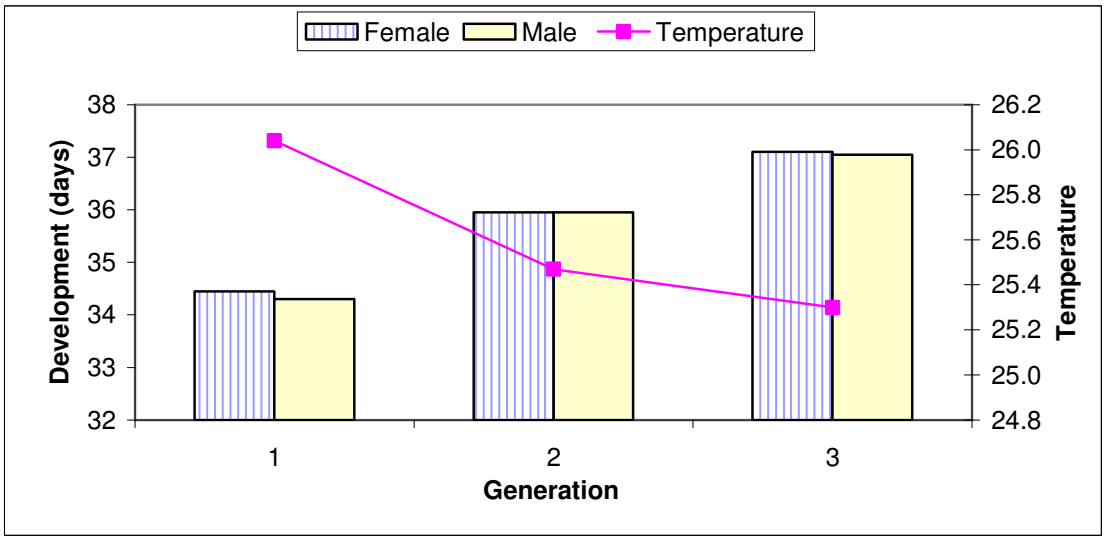

Figure 4.1: Relationship between temperature and insect development to 50% adult emergence of male and female *Cydia pomonella* reared on an artificial diet containing different gelling agents over three generations.

Nutrition, temperature, photoperiod and relative humidity affect the development of CM (Hathaway et al. 1971; Blomefield 2009). The gelling agents had no significant effects on the development of males or females, indicating that the gelling agents were nutritionally equivalent. Generation 3 showed a longer average development period on diets containing all four gelling agents. The reason for the difference in development in the generations was the temperature difference in the production box during the rearing. The average temperature and humidity inside the production box during the first generation until pupation was 26.04°C and 58.02% RH, the second generation was 25.47°C and 49.51% RH and during the third generation 25.3°C and 50.79% RH. This gives a degree day unit (DDU) of 449°D for the first, 433.16°D for the second and 430.92°D for the third generation (based on a 10°C lower threshold and 28 days development). This showed that temperature played an important role in the development rate of the insects and a higher average temperature resulted in a faster development rate. The male and female development was similar supporting findings of Blomefield (2003). Blomefield (2003) found that the development time in days for the larval and pupal stage at 25°C were 21.07 and 18.88 days respectively giving a total of 39 days. Therefore, diets containing all the tested gelling agents gave acceptable CM development rates (34 days – 37 days).

4.4.3.7 Longevity

Humidity in the first replicate affected longevity and moisture condensed in the sides of the small containers because of the wet cotton. Therefore, all tests of the first replicate of the first generation were discarded. The generations had a significant effect on the longevity of the males but not on the females (Table 4.3). Males from generation 3 lived longer than those from generation 1 and 2 (Table 4.4). Although these differences were significant, differences were only one day. The males reared in a diet containing Elastigel lived longer than those reared on a diet containing Kelcogel (Table 4.5). Although differences in longevity for both generation and gelling agents were statistically significant, these differences were only one day. Such small differences have no practical implications.

Longevity of the CM is affected by the presence or absence of food or water, humidity and temperature (Howell 1981). Temperature is an important parameter in the longevity of CM adults as longevity decreases with an increase in temperature (Blomefield 2003). The number of generations and the gelling agents had a significant effect on the longevity of the male moths, with no significant differences between the females. The differences in the gelling agents and the generations in the males varied within a day, which is not significant in practical terms. Male longevity was the longest in the diet containing Elastigel (9.92 days).

Bloem et al. (1997) reared CM on a modified Brinton diet (1969) and found the longevity to be 11.2 days for females and 14.4 days for males at 25°C. Hathaway et al. (1971) reported an adult longevity between 5.4 and 7.0 days for females and between 6.1 and 9.5 days for males reared on different artificial diets or immature apples at 26.7°C. However, in the present study the females (12 days) lived longer than the males (9 days) as was found by Sæthre & Hofsvang (2002). Blomefield (2003) found the average longevity of the spring and summer wild adults in South Africa to be between 14 to 24 days and 7 to 17 days respectively.

4.4.3.8 Fecundity

The number of generations had a significant effect on the fecundity (Table 4.3). The females from the generations 1 and 2 laid more eggs than those from generation 3. The different gelling agents in the diet had a significant effect on the fecundity (Table 4.3). The females reared in a diet containing Elastigel laid more eggs than those reared in diets containing carrageenen and Kelcogel (Table 4.5, Fig. 4.2).

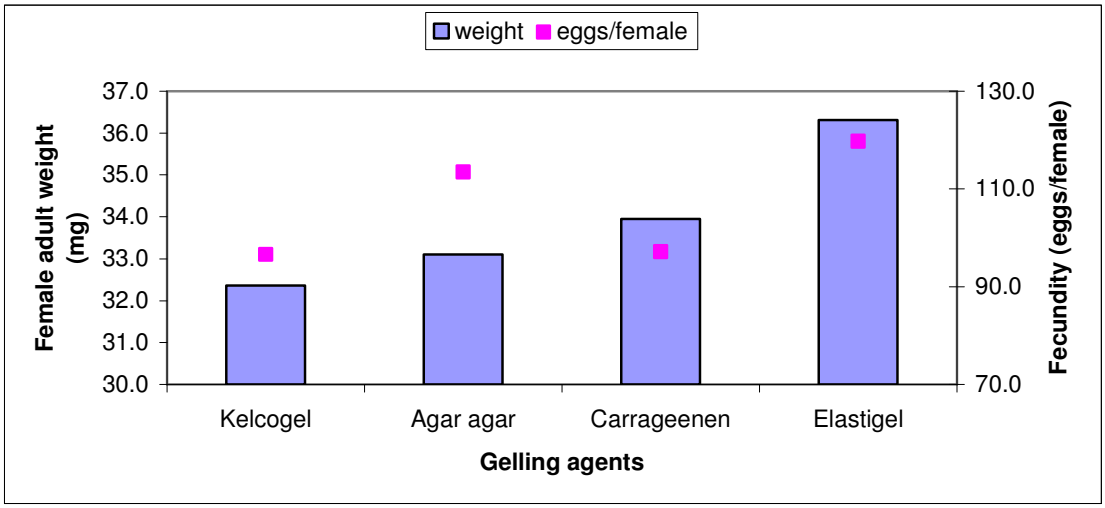

Figure 4.2: Relationship between female adult weight (mg) and female fecundity (eggs/female) of *Cydia pomonella* reared on an artificial diet containing different gelling agents.

The fecundity of female CM is highly variable (Dyck unpublished 2010) as was found in the current study. Data in the literature ranged from 34 – 135 eggs per female (Dyck unpublished 2010). Howell (1981) found that the fecundities ranged from 11 to 145 eggs per female. Blomefield (2003) found that the fecundity of South African wild spring moths to be 90.9 eggs per female. This compares well with the current study of the fecundity of the females reared on diets containing all four gelling agents (106.76 eggs / female).

The fecundity of the females varied significantly between diets containing the different gels. The females from the diet containing Elastigel laid the most eggs (119.79 eggs / female). The weight of female moths reared on the diet containing Elastigel, had a positive effect on fecundity in the case of CM. However, there was a weak correlation (Table 4.6) between the weight of the females reared on diets containing different gelling agents and the fecundity whereas Mansour (2007) found a strong relationship between wild CM female weight and fecundity. This might suggest that not only the female weight had an effect on fecundity, but the gelling agents might also have a physiological effect on the fecundity as some gelling agents can act as nutrients. Moths with a smaller body size had reduced energy reserves available for reproduction as well as a shorter lifespan (Shumacher et al. 1997, Gu et al. 2006). Supporting the findings of previous studies (Gu et al 2006), the heavier female moths reared on a diet containing Elastigel had the highest fecundity and longest lifespan (12.55 days). Jiménez-Pérez et al. (2004) noted that heavy and average female pupal weight of *Cnephasia jactatana* (Walker) (Lepidoptera: Tortricidae) laid significantly more eggs than light females. The female fecundity increased linearly with female weight in light and average females but reached an asymptote when females reached a certain weight (Jiménez-Pérez et al. 2004).

Table 4.6: Analysis of variance for the correlation between *Cydia pomonella* fecundity and female adult weight.

	df	SS	MS	F	P
Regression	1	298.7294766	298.7295	5.920121	0.248248132
Residual	1	50.46002634	50.46003		
Total	2	349.1895029			

There were significant differences in fecundity between the generations. This suggested that the gelling agents had no link with the lower fecundity in generation 3. Room temperature had an effect on the fecundity of the females and the third generation females were held at the highest average temperature (26.07°C). Blomefield (2003) suggested that little oviposition takes place at or below 16°C and above 27°C.

4.4.3.9 Percentage diet weight loss

There were no significant differences in weight loss between the diets containing different gelling agents and the generations (Table 4.3).

Moisture retention can be a problem in insect diets (Howell 1972), but no excessive dehydration or cracking of the diet occurred. The gelling agents and the wood shavings helped to retain some moisture. The wood shavings contributed little to the nutritional value of the diet, but were essential for giving texture and shape to the diet. Diets containing gelling agents need to have some moisture loss to help with pupation. The humidity inside the production box (average 52.77%) was sufficient for pupation to take place within the diet. The average humidity required for most of the diets is 50 – 80% (Dyck unpublished 2010).

4.4.4 Diet cost

The diet cost of the original Guennelon et al. (1981) diet was taken into account (2.6% agar agar) (Table 4.7). The rest of the gelling agents cost did not differ drastically if the costs per 1000 moths are compared (Table 4.7).

Table 4.7: A comparison of costs and moth production between the diets containing different gelling agents.

	Cost per diet tray (R)*	Mean moths/kg diet	Cost per 1000 adults (R)
Agar agar (2.6%)	58.08	300.72	32.19
Agar agar (1%)	34.32	300.72	19.02
Carrageenen	40.51	335.67	20.11
Kelcogel	35.80	256.09	23.30
Elastigel	35.93	273.56	21.89

* Includes cost of diet ingredients

A reduction of 2.6% agar agar to 1% agar agar resulted in a saving of 40.91% in the diet cost. Comparing the 1% agar agar to the other diet ingredients, the cost per 1 000 moths was very similar (Table 4.7). The advantage of the cost of the gelling agents being similar is that a

suitable replacement is always available if there is a shortage of a certain product or a sudden increase in product price. It is difficult to compare the rearing cost with other rearing facilities because of the exchange rate, different diets used and cost over time. The Canadian SIR facility reduced the rearing cost of CM on a non-gelling agent diet to 2.94 USD per 1 000 moths in 2007 (Dyck unpublished 2010) which is R21.25 at the present exchange rate (R:$ = 7.23). This diet cost compares very well with our current diet cost (R19.02/1 000 moths) which makes one think — is using artificial diets containing a gelling agent such as agar agar really expensive or is it only an unsubstantiated perception?

4.4.5 Selection of best gelling agent

From the scores in Table 4.8, the best gelling agent was Elastigel, followed by agar agar, carrageenen and Kelcogel. The empirical values were used even if the differences were not significant.

Table 4.8: Scores allocated for the selection of the best gelling agent.

		Elastigel	Agar agar	Carrageenen	Kelcogel
% Emergence		2	3	4	1
Total moths		2	3	4	1
Pupal weight	(males)	4	3	1	2
Pupal weight	(females)	4	2	1	3
Adult weight	(males)	4	2	1	3
Adult weight	(females)	4	2	3	1
Moisture loss		4	1	2	3
Development time	(males)	4	3	2	1
Development time	(females)	4	3	2	1
Fecundity		4	3	2	1
Longevity	(males)	4	3	2	1
Longevity	(females)	4	1	3	2
Cost		2	4	3	1
Total score		**46**	**33**	**30**	**21**

4.5 Conclusion

Referring to Singh's recommendations (1985), all four diets containing different gelling agents fulfilled the requirements of a suitable diet. Overall, there was no interaction between the gelling agents and the moth generations. This implied that the effects of the gelling agents on moth quality were consistent regardless of the generations. All the diets contained the nutrients needed for the insects according to the quality parameters. Differences between the effects of gelling agents were only apparent for female and male pupal and adult weight, male longevity and fecundity. Gelling agents did not differentially affect the other quality parameters. The diets were easily prepared and had a long storage life until adult emergence.

The size of a rearing tray affects the insects in several ways. The tray must provide enough space to permit unrestrained larval development and pupation. Not all trays are suitable for all diets. The shape of the tray influences diet thickness and moisture retention (Burton & Perkins 1984). Rearing CM in big trays saves time, labor and is more productive in a mass-rearing system. This type of fiberglass, stackable tray (Molded Fiber Glass Tray Company, Linesville PA USA; tray model: 805408) was efficient for mass-rearing CM and for using diets containing gelling agents. The advantages of these trays are that it is stackable, has high durability, strong and light in weight. The disadvantage is the relative high cost.

According to this study and including all the quality parameters and cost factors, Elastigel was the best gelling agent giving bigger pupae and adults, and high female fecundity. The data suggested that food utilization of the larvae reared on the diet containing Elastigel was acceptable and better than for the other gelling agents. The diet containing Elastigel is an adequate food source independent of the presence or absence of the insects' natural food. In addition, it provides for continuous rearing without a decline in fecundity, adult weight or percentage emergence. The cost of the diet containing Elastigel results in a considerable amount of saving of 31.99% from the original Guennelon et al. (1981) diet. A filler or bulking agent is necessary when using a diet containing Elastigel and when using bigger trays for rearing purposes. Studies including different types and size of fillers must be considered in the future.

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CHAPTER 5

A synopsis of codling moth mass-rearing methods: design of an open and closed rearing system

5.1 Abstract

A novel method of a closed mass-rearing system for codling moth, *Cydia pomonella,* is reviewed including diet preparation, egg placement on diet, larval rearing and environmental control. Larval rearing systems available worldwide for codling moth include an open rearing system, a closed large-scale rearing system and closed mass-rearing system using a box ventilated with filtered air. The efficiency of the open rearing system for codling moth is compared with the closed system currently being used locally. The benefits of less building space, less labor requirements and less air handling expenses of rearing codling moth larvae in a closed system are discussed.

5.2 Introduction

The use of Sterile Insect Release (SIR) in an integrated pest management program involves the mass-rearing of insects, irradiating them and releasing the sterile insects. Mass-rearing is an industrial process that maximizes efficiency while maintaining a high quality product (Dowell et al. 2005). Information on methods for small-scale rearing codling moth, *Cydia pomonella* (L.) (CM) is available, but information on mass-rearing systems is limited. The technology is often not documented in the literature because it is too specialized for regular laboratories. In addition, some insectaries regard their technology as trade secrets (Cohen 2004).

Facility design is a critical aspect of the rearing process (Parker 2005) and unfortunately, not much research has been done on how facilities and rearing systems affect insect production (Griffin 1984). Mass-rearing CM requires a sophisticated system including air filtration, controlled temperature, light and relative humidity (Dyck unpublished 2010). Insect diets are held at a temperature suitable for microbial growth (Cohen 2004) and the risks of diseases infecting the larvae and diet are high and usually catastrophic. These risks must be mitigated by incorporating stringent environmental controls and therefore, the design of the facility requires specialized equipment. Insect rearing procedures are being automated or mechanized because demands for mass-reared insects are growing and costs are increasing (Harell & Gantt 1984). Leppla et al. (1982) suggested that for mass-rearing Lepidoptera, larval development should occur in small, controlled rooms rather than large rooms thus reducing the risk of large-scale contamination. When there is contamination in small rooms, one room can be sealed off and be disinfected. Therefore, it is necessary to evaluate the different rearing systems for CM.

5.2.1 Open tray rearing system

Mass-rearing involves programs where the number of progeny produced per day equals 1 million times the number of offspring that can be produced by a single female per day (Chambers 1977, Cohen 2004). The SIR program in British Columbia, Canada, began mass-rearing CM for SIR in 1994 (Bloem et al. 1997). The facility rears between 15 and 16 million moths per week (Bloem & Bloem 2000, Bloem et al. 2010). The system used to mass-rear CM can be classified as an open tray rearing system. In an open tray system, each tray is in direct contact with the air in the room and the risk of contamination is high. Therefore, intensive sanitation is necessary to prevent contamination.

Large-scale rearing is characterized by the employment of artificial diets and higher degrees of automation than in smaller operations (Cohen 2004), but produces fewer moths than mass-rearing systems. A large-scale CM rearing facility was constructed in Argentina in 2006 (400 m^2) and has a maximum production capacity of 200 000 moths per week (Dyck 2010, Taret et al. 2007). The rearing facility in Argentina also uses a stackable, open tray system (Taret et al. 2007).

5.2.2 Closed tray rearing systems

Sreekumar et al (2000) described a closed type mass-rearing system for *Corcyra cephalonica* (Stainton). This involved a closed type cage with drawers to hold rearing trays and an oviposition jar for automatic collection of the moths. Their method reduced labor requirements and exposure of workers to moth scales (Sreekumar et al. 2000) which is very important in a mass-rearing system.

5.2.2.1 Small tray large-scale rearing system

A South African large scale CM rearing facility (100 m^2) was established in 2004 with a capacity of 300 000 moths per week. In this facility, a closed larval rearing system is used with relatively small trays that are placed in brown paper bags to reduce the risk of contamination. This method is not feasible for mass-rearing as the cost of the brown paper bags is high and the method is highly labor intensive

5.2.2.2 Box mass-rearing system

A mass-rearing facility, designed to produce two million moths per week, is currently under construction in South Africa. The reduction of the risk of contamination and the efficient use of labor were regarded as critical elements in the design. A method using relatively small, ventilated, rearing boxes containing large, stackable trays was developed. This method allowed for the simple loading of diet into the trays, simplified air handling and lighting.

The aim of this chapter is to:

1. Develop and describe a larval mass-rearing method suitable for the new South African CM SIR facility.
2. Compare the Canadian open tray mass-rearing CM system with the local closed, small tray large-scale rearing system and the new closed-box mass-rearing system.

5.3 Materials and methods

5.3.1 Open tray rearing system (Canadian SIR facility)

Data for this system were obtained from Bloem et al. 2000. A modified Brinton diet (Brinton et al. 1969), which excludes a gelling agent but uses sawdust and paper pulp, is used in the Canadian SIR facility (Bloem et al. 1997). The diet (3.3 liter per tray) is poured into fiberglass trays (45 x 29 x 2.5 cm) (Photo 1) and to control moisture loss, the diet surface is sprayed with liquid paraffin (Bloem et al. 2000).

The diet-filled trays are placed in custom-made trolleys, 75 trays per trolley (3 x 25 trays/shelf). Egg sheets with 2 500- 3 000 eggs are placed on a wire rack above the diet (Bloem et al. 2000). Diet trolleys (28 trolleys per room) are placed in large, controlled environment rooms (27°C) and 75% relative humidity (RH) (Bloem et al. 2000). Each tray is in direct contact with the air in the room. To minimize the risk of contamination separate rooms, each with independent environmental control equipment and air supply, are used (Dyck unpublished 2010). To increase airflow over the diet surfaces, air is forced through holes in the walls of the rooms and vented through two ducts in the ceiling of each room. The relative humidity is gradually lowered to 55% by day 21. A photoperiod of 16:8 (L:D) is used. Vertically positioned fluorescent tubes are located within an air plenum behind plastic walls (Personal observation 2004, Dyck 2010). At day 22, the trolleys are moved to an emergence room (Bloem et al. 2000).

5.3.2 Small tray large-scale rearing system (South African SIR facility)

Data for this system were obtained experimentally in Chapter 3. Codling moth for the release program were reared on an artificial diet developed by Guennelon et al. (1981). The diet was subsequently modified by using less agar agar, substituting maize meal with whole wheat flour and adding wood shavings. Small, plastic trays (28.5 cm x 22.5 cm x 5 cm) were filled with diet (1.5 liter per tray) and placed into brown paper bags to minimize the risk of contamination and to prevent moisture loss. After the diet cooled, it was scarified with a plastic comb to facilitate larval penetration into the medium. Eggs were sterilized (Chapter 3) and placed on a wire rack on the diet. An average of 1 000 eggs were put on the diet and egg sheets were removed after 7 days. The trays were held in an air-conditioned larval room on custom-made shelves (12 trays per shelf; 18 shelves per trolley) (Photo 2). Outside High Efficiency Particulate Air (HEPA) filtered air was introduced into the room at a rate of 80 l/s. In addition, air was circulated within the room via a pressure fan and ceiling mounted ducting. Temperature and humidity was measured with temperature loggers inside the room and inside the trays. The photoperiod used in the small tray system was 18:6 (L:D). Fluorescent lights were hung on both sides of the trolley. After 28 days, the brown paper bags (40 cm x 30 cm) (SO 25, Ace Packaging, South Africa) were removed and the trays were transferred individually to an emergence room in which moths were allowed to eclose.

5.3.3 Description of box used for closed larval mass-rearing (South African SIR facility)

Data for this system were obtained experimentally in Chapter 4. A modified diet described by Guennelon (1981) was used for rearing the larvae, but the gelling agent, agar agar, was replaced with 10% Elastigel ™ 1000J, a modified starch produced from sago (National Starch Company) (Chapter 4). Elastigel was mixed with 20 liters of cold water in a stainless steel pot and then brought to the boil on a gas ring to activate the starch. This mixture was cooled down to 67ºC before the rest of the ingredients were added as described in Chapter 4. Wood shavings (10 liters) were used as a bulking agent. Six liters of the mixture were poured into a molded, stackable fiberglass tray (77 cm x 40 cm x 3 cm) (Molded Fiber Glass Tray Company, Linesville PA USA; tray model: 805408) (Photo 3). The diet was allowed to cool down to room

temperature and scarified to make larval entry easier. Eggs were sterilized as described in Chapter 4 and put onto wire racks on the diet for 28 days. An average of 5 000 eggs per tray of diet containing Elastigel was used (Chapter 4).

The box (Photo 3) consisted of an aluminium square tubing frame of 1000 mm long, 500 mm wide and 1 800 mm high. The floor was made of laminated wood and two narrow vertical sides and the top side were sealed with 2 mm aluminium sheeting riveted onto the frame. The two large vertical sides were sealed by using two removable sheets of semitransparent multiwall, polycarbonate sheeting. The sheeting was sealed against the aluminium frame using adhesive tape. Three ports were cut into the removable, polycarbonate sides that allowed for visual inspection of the trays during use. The box was ventilated through HEPA filters at the desired temperature and humidity. Filtered air was introduced (270 l/min airflow) into the box via a single 35 mm diameter port in the box floor. A horizontal baffle positioned immediately above the air inlet port distributed the introduced air evenly. Air traveled vertically through the box and exited through two 35 mm diameter exhausts in the roof of the box. The exhausts were screened with fine gauze to prevent insects from entering the box. Temperature and humidity sensors were used to measure the temperature and humidity inside the box and the outlet air temperature**.** The box accommodated 20 stackable trays which were positioned on top of the baffle. The photoperiod used was 18:6 (L:D). Fluorescent lights (Osram L 36W/740) were hung on both sides of the box. The box was kept in a larval room for 28 days and then moved to an emergence room where the sides were removed and moths allowed to eclose.

5.3.4 Production parameters

The total number of moths produced was calculated by counting the empty pupal cases per tray after 2 weeks of emergence (Chapter 3 & 4). The percentage emergence was calculated from the number of eggs used per tray and the number of moths produced per tray (Chapter 3 & 4). The number of eggs was determined by counting the number of eggs per sheet including the unhatched eggs (Chapter 3 & 4).

The number of moths per liter of diet was determined using the average number of moths per tray divided by the amount of diet used per tray. Pupae and adults (n = 20) were weighed individually (Chapter 3) (n = 50; Chapter 4). Diet moisture loss was determined by weighing the diet (tray weight excluded) on day 1 and again on day 28.

A cost comparison was made of costs associated with rearing CM in the Canadian open tray system, closed production using small trays and the box production system. The calculations took into account the cost of the diet ingredients and the brown paper bags for the small tray production.

The production of diet per unit floor area was determined by the floor area of the trolleys (Canadian trolleys and trolleys used for local small trays) and the production box. The amount of diet per trolley was determined by the amount of diet per tray times the amount of trays per trolley or production box. The volume of the air was determined by using the dimension of the local rearing room and the dimension of the production box.

5.4 Results and discussion

5.4.1 Comparison between open tray rearing, closed small tray and box production systems

The rearing efficiency was similar between the different systems. In the large-scale small tray system, an average of 320 moths per liter of diet was produced and in the mass-rearing closed box system, an average of 281 moths per liter of diet was produced (Table 5.1). The open tray mass-rearing system gave an average production of 249 moths per liter of diet. The moth weight was higher for the moths reared in the bigger trays in the box (Table 5.1) than in the open tray and small tray system. This could be due to better utilization of the diet.

The cost (Table 5.1) between the different rearing systems is similar, but it does not include power requirements and labor associated with the rearing processes. Those costs are difficult to

determine but in terms of handling the diet and stacking trays, the big trays in the box were easier and faster to handle than the current small trays. The small trays were handled 2.5 times more than the large trays (Table 5.2). In addition, the production box allows for efficient handling of trays as the box is designed to be lifted using a pallet jack, thus the box is easily moved once loaded.

The use of the production box allows for the efficient handling of trays and allows for increased production of diet per unit floor area compared to the closed, small tray rearing system. The open rearing system used three trays per shelf x 25 shelves in a 1 m x 0.5 m trolley (0.5 m^2). Small tray production allowed for 12 trays per shelf x 18 shelves in a 1.2 m by 1.2 m trolley (1.4 m^2). Using the production box, 20 trays were placed in a 0.5 m x 1.0 m box (0.5 m^2). The open tray rearing system allowed for 489 liter diet per square meter while the use of a production box allowed 240 liter diet per square meter compared to 225 liter diet per square meter using small trays on trolleys. The Canadian tray system (489 liter diet per square meter) is substantially higher.

Table 5.1: A comparison of *Cydia pomonella* reared on small trays (standard operation), large trays (closed production) and the Canadian open rearing system.

Quality parameter	Rearing method		
(Mean +/ 95% CI)	Canadian facility (95% Conf. Int) *	Big trays (95% Conf. Int)	Small trays (95% Conf. Int.)
Moths/l	249	281 (473-1035)	320 (36-603.39)
Moth weight (male)	19.72 (18.78-20.66)	25.40 (21.67-29.13)	18.81 (15.98-21.64)
Moth weight (female)	29.75 (28.32-31.18)	39.33 (38.52-40.15)	30.09 (20.91-39.27)
Cost/1 000 moths	21.25 **	R 21.89	20.79 ***

* Bloem et al. (1997)

** Dyck 2010

*** Including the cost of the brown, paper bags.

Table 5.2: Handling steps for small tray large-scale rearing and box mass-rearing systems

Small tray rearing	Closed box production
Cleaning of trays	Cleaning of trays
Stacking onto racks to dry	Stacking trays in production box
Loading diet into tray	Transferring box to larval room
Bagging of trays	Transferring box to emergence room
Stacking trays onto trolleys to cool	
Unbagging the trays to put egg sheets on	
Bagging the trays	
Loading trolleys in larval room	
Unloading trolleys and transfer to emergence room	
Packing of trays onto racks on emergence room	

5.4.2 Environmental conditions

"Environmental control involves regulation of the external conditions that affect the growth, development and behavior of an organism" (Owens 1984).

Temperatures, 25 - 28°C, are common for rearing CM larvae (Dyck unpublished 2010). Taret et al. (2007) used 28°C for larval rearing and the Canadian facility, 27°C. The average temperature inside the box was 26.03°C (Figure 5.1) and in the larval room for small tray rearing, 26.44°C (Figure 5.1).

There is a relationship between the insects' size to their susceptibility to heat and water loss in their environment (Cohen 2004). The smallest insect (first instar) has the highest surface-to-mass ratio and is the most susceptible to water or heat gain (or loss) (Cohen 2004). Therefore, the removal of excess humidity and heat is necessary. The diet in the small tray rearing lost 37% total weight (Chapter 3) whereas the diet in the closed production box lost 38% moisture (Chapter 4). The diet drying out assists with pupation of the insects inside the diet. With the South African rearing system and diet containing a gelling agent, humidity control is easier than the Canadian system and it is unnecessary to decrease the humidity. The humidity inside the production box (58.02%) was higher than in the room (46.68%) (Fig. 5.1). The temperature and humidity of the room were not necessarily the same as they were in the rearing container. On average, the humidity inside the bag for the small trays was 10% higher than in the room.

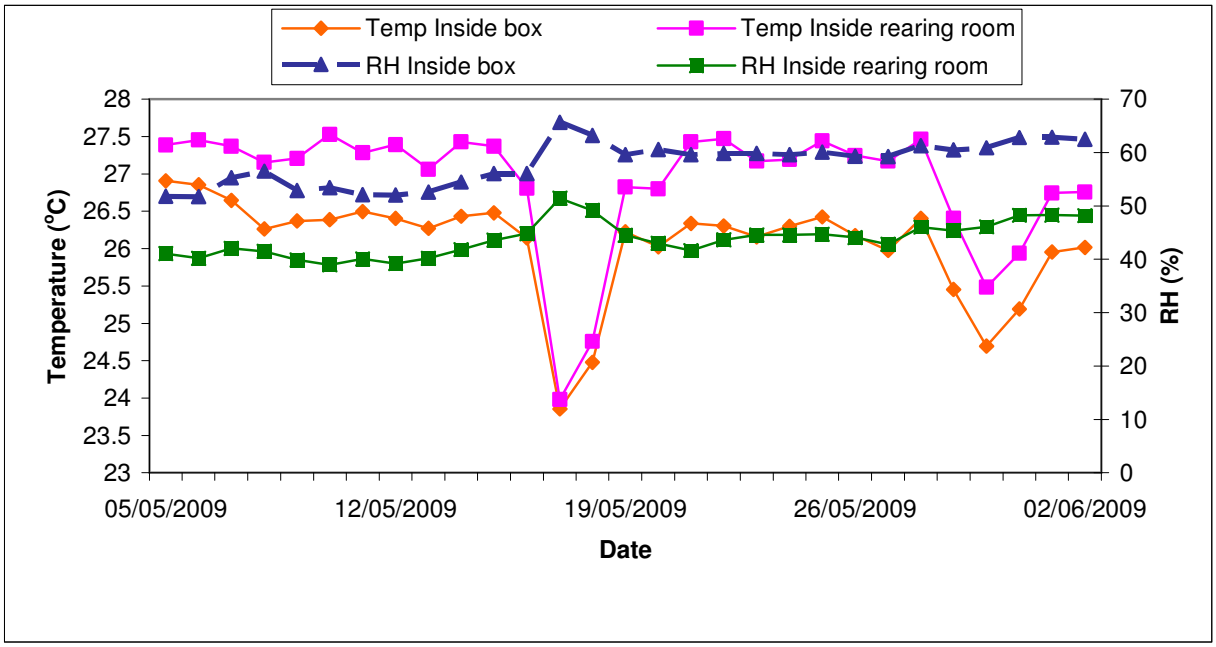

Figure 5.1: Temperature (°C) and humidity (%) for the closed small trays large-scale and box mass-rearing system. Temperature and humidity are measured inside the rearing room for the small trays and inside the production box for the large trays.

The use of large rooms requires the continuous recirculation, filtering and adjustment (temperature and humidity) of the air. The use of production boxes does not require the recirculation of air as the volumes required are relatively low (0.9 m^3) (Table 5.3). Preconditioned air should be introduced continuously into the insectary, not recycled, as it reduces the potential for contamination (Fisher 1984, Wheeler 1984). With the box system, the air is continuously introduced and not recycled.

Table 5.3: Comparison between the amount of airflow in a rearing room and in a box system.

	Small tray rearing room	Box system
Dimensions (m)	10 x 5 x 3	1 x 0.5 x 1.8
Air volume (m^3)	150	54*
Air circulation (2 air changes/min) (m^3)	300	108
Plus 10% fresh air (m^3)	330	118.8

* Air volume determined for 60 boxes per (10 x 5 x 3 m) room.

5.4.3 Advantages and disadvantages of the open tray mass-rearing system, closed small tray large-scale and box mass-rearing system

5.4.3.1 Open tray mass-rearing system

With the Canadian open tray system, one room contains two day's production. Therefore, a big facility is necessary with many separate rearing rooms. The control of relative humidity and air movement are very important because the humidity needs to decrease for the diet to dry out for pupation to occur (Dyck unpublished 2010). The number of rooms necessary and the environmental control equipment for all the rooms are expensive (Dyck unpublished 2010).

The trays of the Canadian open rearing system need to be handled individually until they are filled with diet and placed on racks on the carts. The trays are not stackable making it more labor intensive.

In the Canadian open rearing system, the egg sheets are removed from the diet, which minimizes the risk of contamination from the egg sheets. However, staff members taking the egg sheets off increase the risk of contamination. The advantage of separate rooms is that if one room is contaminated, the other rooms are isolated from the contamination.

The disadvantage of the open tray system is that the level of sanitation and number of cleaning actions necessary to obtain a sterile environment is high. Staff have to shower before starting the day's tasks, excessive cleaning of the rooms and wearing protective, clean clothing, shoes and hairnets are necessary (Personal observation 2003) to reduce the risk of contamination.

5.4.3.2 Closed, small tray large-scale rearing system

The advantage of the smaller trays inside a brown paper bag was that contamination of the diet was minimized and the paper bags increased the humidity. Thus, cheaper and simplified air handling equipment could be used.

Using the smaller trays was very labor intensive as many individual tray-handling steps were necessary (Table 5.2). This system was efficient for large-scale rearing, but in addition, labor intensive for mass-rearing and the cost of the brown paper bags for the trays was high.

The risk of contamination with the closed, small rearing trays was decreased as the trays were placed inside a brown paper bag. If a tray was contaminated, it could be removed without the whole room being at risk. Egg sheet could be removed after a week.

5.4.3.3 Closed, box mass-rearing system

The advantage of the closed box-production system is the elimination of many rooms necessary for rearing, resulting in better utilization of space and energy, cheaper and simplified air handling equipment. Risks associated with large rooms in the case of the Canadian rearing system (loss of two days production if large room is contaminated) is higher than with the closed box system, as single contaminated boxes can be removed. The closed box production system can be manipulated. Airflow, temperature and humidity can be altered within individual boxes relatively easily with the addition of one additional duct. Monitoring of individual boxes is easy as diet temperature and the temperature and humidity of air entering the box and leaving the box can be easily measured. Thus, environmental control in boxes can be more uniform relative to production in large rooms. Seasonal temperature fluctuations (cool in spring and warm in summer) can easily be compensated for in closed production boxes.

The closed box production system is less labor intensive. Stackable trays are used and no racking is required resulting in less handling steps of the trays. A flowable diet is necessary because the trays are stacked before dispensing the diet into the trays. The disadvantage of the

closed box system is the weight of the box loaded with the trays. However, the box is designed to be lifted using a pallet jack, which largely neutralizes this problem.

The ideal facility for maintaining high sanitation levels has no shelves, corners or cracks for dirt accumulations and must be easily cleaned (James et al. 1973). The production box has no shelves, and has easily cleanable walls, floor and ceiling covering. There is no interchange of air between the different production boxes, thus reducing the risk for spread of contaminants. Sanitation is important in a mass-rearing facility, but with the closed box mass-rearing system, the risk of contamination from staff is less during handling. Egg sheets can be a risk of contamination as the idea is not to open the production box during larval development. Egg sheets have to be placed on the diet for 28 days until the box is opened for adult eclosion whereas in the Canadian facility the egg sheets are removed after a week (Bloem et al. 1997).

The closed production system is less expensive because of the infrastructure and a saving in building space. The cost of the proto type production box tested was R2 000. This system is optimal for a smaller mass-rearing facility.

5.5 Conclusion

The cost of SIR can be reduced by mechanizing rearing procedures, improving larval diet and developing better methods to suppress contamination (Proverbs et al. 1982). The performance of the closed production box was satisfactory. The box provides room for the production of approximately 40 000 moths (average 2 000 moths per tray) per box. The closed box rearing system with individual air supply reduces the chance of contamination. This study shows that a closed box-production system can be applied to a CM mass-rearing system. This technique may increase the CM SIR program's efficiency in terms of rearing method, building space, less labor and be more economic due to less air handling expenses.

Based on this study, improvements in the new mass-rearing CM SIR using production boxes can be recommended. These include:

- Automation of diet handling. This includes diet mixing, faster cooling of the gelling agent mixture before adding the other ingredients and dispensing diet into stacked trays
- A larger production box (1 x 1 x 1.8 m) with 40 stackable trays can be used.
- A laminar flow bench should be used for cooling the diet in the stacked trays thus preventing the contamination of the diet during handling and placement of egg sheets.
- Simplified air handling. This will involve the bulk conditioning of outside air in a large plenum and the subsequent ducting of conditioned air to the production boxes. The system is more energy efficient relative to the recirculation of air from large production rooms and is easily controlled and adjusted.

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Photo 1: Trays containing diet in the larval rearing room in the Canadian facility. (Photo credit: D. Stenekamp 2003).

Photo 2: Closed, large-rearing system using small trays covered with brown paper bags. (Photo credit: D. Stenekamp 2010).

Photo 3: Closed, mass-rearing system using a box filled with stackable fiberglass trays. Fluorescent lights are at the back and front of the box. Air enters the box from the bottom of the trays. The bottom tray is upside down for air distribution. (Photo credit: D. Stenekamp 2010).

CHAPTER 6

General conclusion

The aim of this study was to contribute towards knowledge on mass-rearing methods of codling moth, *Cydia pomonella* for the Sterile Insect Release program. Specifically, it was aimed at finding a reason for fluctuating insect numbers in the current mass-rearing facility; finding alternative gelling agents to replace agar agar and to improve the current diet cost used for mass-rearing the insects and improving the larval rearing system. The results from these objectives are outlined below.

6.1. Genetically modified maize meal

The quantification of the effect of genetically modified maize meal containing the *Bacillus thuringiensis* gene is of major importance for all insect rearing facilities using genetically modified maize. This study represented the first report on the quantification of this effect on the development and mortality of *C. pomonella* (Chapter 2). Results indicated that even a small amount of *B. thuringiensis* affected *C. pomonella*, which is unacceptable in a mass-rearing facility. Findings also indicated that whole wheat flour is a suitable replacement for maize meal, giving better production and minimizing the risk of contamination due to *B. thuringiensis* (Chapter 3). As a direct result of these findings the false codling moth *Thaumatotibia leucotreta* (Meyrick) facility (XSit) in Citrusdal, South Africa, is doing tests on using whole wheat flour instead of maize meal in the artificial diet with promising results (Personal communication, S. Groenewald 2010). The results of this study are therefore beneficial for all insect rearing facilities using maize meal as a diet ingredient, as the presence of *B. thuringiensis* in maize meal could directly be related to the fluctuation of insect numbers in the local *C. pomonella* facility.

6.2. Alternative gelling agents and diet cost

The identification of different gelling agents requires the use of local suppliers, must be easy to use and be acceptable for the insects being reared for about 35 days. Other researchers have tested some of the gelling agents (agar agar, carrageenen) previously, but information on mass-rearing *C. pomonella* on different gelling agents such as Elastigel, Kelcogel, Cekol, citrus pectin and Kiccolate are lacking (Chapter 3). Therefore, this study examined and described the use of alternative gelling agents and the effect on the insect quality. This was compared with the quality of the insects reared on an agar-based diet (Chapter 3). Quality control measures and biological studies were used to distinguish between the insects reared from the four best gelling agents (Elastigel, carrageenen, Kelcogel, 1% agar agar) (Chapter 4). Overall, the gelling agents compared well, although Elastigel gave bigger adults and pupae and higher fecundity. Replacing the original 2% agar agar with Elastigel will give a reduction of at least 40.91% in diet cost and will result in a more sustainable, economically viable diet. The prices of the different gelling agents (Kelcogel, 1% agar agar, carrageenen) per 1 000 moths did not differ significantly and should be of value when there is a shortage of Elastigel or a significant price increase.

6.3. Improving the larval rearing system

The use of a closed production system for larval rearing is a first for mass-rearing *C. pomonella*. This study examined and described, with illustrations, the production box and trays used for larval rearing (Chapter 5). This closed production system was compared with other methods used for mass-rearing *C. pomonella* worldwide. The use of this method should contribute to a cleaner larval rearing environment, reducing labor requirements, reducing exposure of workers to allergenic insect contaminants and minimizing the risk of diet and insect contamination. This should result in a more secure and economically viable production system.

In conclusion, the objectives of this study were successfully met. Certain adjustments resulting from information obtained in this thesis have already been implemented with excellent results.

The use of the closed production box system, whole wheat flour, Elastigel and the identification of alternative gelling agents as replacements for agar agar contribute towards more cost effective and productive insect rearing. This is of major economic importance for the South African commercial Sterile Insect Release program starting in 2010 and therefore is a significant contribution in applied integrated pest management.