MODIFYING AN ARTIFICIAL DIET FOR MASS REARING MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE), USING LOCALLY AVAILABLE MAIZE MEAL

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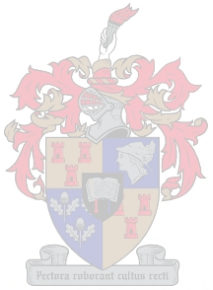

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my original work and has not previously, in its entirely or in part, been submitted at any university for a degree.

ABSTRACT

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is well-known as a destructive pest of fruit worldwide. Various control methods have been used against this insect. The sterile insect technique (SIT) is used as an important and successful technological method for controlling or eradicating this pest in many countries. A key factor to successfully apply SIT is dependent on the availability of efficient and economical rearing methods.

Artificial insect diets with low cost bulking agents have been of interest to many researchers. The present study investigated the use of locally available maize meal as a bulking agent in such diets. Maize meal is used for human consumption (in South Africa) and contains small amounts of protein. This makes the reduction of imported torula yeast as an ingredient of the diet and source of protein possible, thereby reducing the cost of the diet.

The larval development of the Medfly reared on artificial diets was studied in small and large-scale tests. The effect of the diets on larval production was evaluated using pupal recovery, pupal weight, flight ability, sex ratio, fecundity and egg fertility.

The results of the small-scale tests showed that the diet containing maize meal could be used to produce Medfly more economically than the standard Krige diet used by the ARC Infruitec-Nietvoorbij Research Institute at Stellenbosch. However, in large-scale tests the ingredients quantities of the diets used were not the same as those of small scale-tests. The cost of the modified larval diet was not reduced in large-scale tests. This was ascribed to the number of eggs used in the tests to produce one million

of fruit flies. The maize meal with reduced number of eggs require more diet to produce one million flies therefore, making it more expensive and less viable. When similar amounts of eggs were used, the diet appears to be a suitable alternative as the result obtained was almost similar to those of the Krige diet.

OPSOMMING

Die Mediterreens vrugtevlieg ("Medfly"), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is wêreldwyd 'n skadelike plaag. Die steriele insek tegniek (SIT) het in baie lande 'n belangrike en suksesvolle manier geword om die plaag te beheer en uit te roei. Die belangrikste voorvereiste vir die suksesvolle toepassing van SIT is die beskikbaarheid van doeltreffende en ekonomiese teelmetodes.

Meeste navorsers is geïntereseerd in kunsmatige diëte met 'n goedkoop vulstof. Hierdie studie is ontwerp om die gebruik van plaaslik beskikbare mieliemeel as vulstof te ondersoek. In Suid-Afrika word dit vir menslike gebruik aangewend en bevat klein hoeveelhede proteïene wat 'n vermindering van die ingevoerde torula gis moontlik kan maak, en sodoende die koste van die dieët kan verminder.

Die ontwikkeling van Medfly larwes op kunsmatige diëte is bestudeer in kleinskaalse en grootskaalse eksperimente. Die invloed van die diëte op larwale produksie is evalueer deur gebruik te maak van van papie-ontwikkeling, papie-gewig, vliegvermoë, geslagsverhouding, volwasse voortplantingsvermoë en eiervrugbaarheid.

Die resultate van die kleinskaalse toetse het aangetoon dat die mieliemeel dieët gebruik kan word om Medfly meer ekonomies as met die standaard Krige dieët, wat in die ARC Infruitec-Nietvoorbij navorsings instituut by Stellenbosch gebruik word, te teel. By die grootskaalse toetse was die koste nie laer nie. Dit word toegeskryf aan die aantal eiers wat gebruik is om 'n miljoen vlieë te produseer. Die mieliemeel dieët met 'n verminderde aantal eiers benodig meer dieët om 'n miljoen vlieë te produseer, wat dit duurder en minder lewensvatbaar maak. Wanneer soortgelyke hoeveelhede eiers gebruik was, het dit geblyk dat die dieët 'n opsie is, want die resultaat was soortgelyk aan dié van die Krige dieët.

Dedicated to my father, Sithembile and mother Nosiphe who gave me the support and provided me with constant encouragement and unconditional love to complete these studies

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CHAPTER 1

LITERATURE REVIEW OF *CERATITIS CAPITATA*

1.1 INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), often referred to as Medfly, is one of the most destructive fruit pests (Mau & Kessing 1992). Because of its worldwide distribution, its ability to tolerate cooler climates better than most other species of tephritid fruit flies, and its wide host range, it is ranked among the most economically important fruit fly species. It attacks various kinds of fruit, flowers, vegetables and nuts, but prefers thin-skinned, ripe, succulent fruits (Papaj *et al.* 1989). Host preferences vary in different regions and include avocado, bananas, bitter melons, carambola (star fruit), coffee, guavas, mangoes, papayas, peppers, peaches, nectarines, apples, pears and persimmons. It infests more than 250 types of fruit grown commercially in the Mediterranean area. Besides commercial hosts there are also many wild hosts utilized by this fly (Myburgh 1956).

This literature review outlines the distribution of Medfly, and its damage, economic importance and management.

1.2 DISTRIBUTION

C. capitata is native to central Africa and as a result of transport by humans is now found in practically all subtropical and tropical regions of the world except North America (White & Elson-Harris 1992). It occurs throughout most regions of South Africa, but appears to be more abundant in the Cape, KwaZulu-Natal and Gauteng Provinces (Myburgh 1956). It was recorded in the region of the Cape in the late 1890's. Populations of the Medfly increase during spring in orchards on early ripening fruit such as apricots. Fruit flies move to peach

orchards in late summer, then to vineyards and in late autumn and winter to citrus orchards (Myburgh 1963; Annecke & Moran 1982).

1.3 BIOLOGY OF *C. CAPITATA* IN SOUTHERN AFRICA

Although *C. capitata* is an important pest of horticultural crops in Southern Africa, little work on its biology has been done locally. This is partly due to the relative ease of their control by using bait and cover sprays, especially in large orchards. However, in smallholdings, gardens and village communities, control is difficult (Hancock 1989). Repeated and improper use of insecticides as cover sprays is not only costly, but is also environmentally hazardous. The unintentional effects on natural enemies of other pests may create further problems.

An understanding of various aspects of Medfly biology, such as habitat and host preferences, mating and other aspects of behaviour, in addition to the development of efficient population monitoring techniques through the use of attractants, may prove to be of great benefit in reducing insecticide use in integrated pest management systems. Much of the information available on the biology of *C. capitata* in South Africa was reported by Munro (1984) and Myburgh (1956).

1.4 DAMAGE

Damage to crops caused by Mediterranean fruit flies result from oviposition in fruits and soft tissues of vegetative parts of certain plants and by subsequent feeding of the larvae, resulting in tissue decay. The larval feeding stage causes most damage in fruits. Mature fruits, which have been attacked, may develop a water-soaked appearance. Young fruits become

distorted and usually drop. In addition, holes made by female flies during oviposition scar the fruits, which lowers their quality (Mau & Kessing 1992).

1.5 ECONOMIC IMPORTANCE

The economic importance of Medfly cannot be evaluated only from the standpoint of the actual damage to the crops it attacks as many countries have established quarantine measures against this pest and will not permit importation of products from infested areas. In the United States (US), Medfly infestations are due to importation of infested fruit. Thus the US government has strict laws regulating the movement of certain commodities to prevent the establishment of *C. capitata*. The Japanese government also restricts the entry of commodities attacked by this pest into their country (Mau & Kessing 1992).

The deciduous fruit industry in the Western Cape spends approximately R16 million per year on fruit fly control, while a further R3 million is lost due to crop damage (Eyles & Burgess 1999).

1.6 LIFE HISTORY OF *C. CAPITATA*

Adult Medflies are active all the year round in warmer areas. Females oviposit two to 10 eggs under the skin of fruit. After about two days, the eggs hatch into larvae (maggots) which feed inside the fruit pulp and quickly turn the fruit into a rotten mass. Mature larvae fall to the ground and pupate in the soil.

The full-grown fruit fly larva is opaque white in colour, elongated, and pointed at the head. It measures 6.8-8.2mm. Like other muscoid dipteran larvae, it lacks a distinct head or head capsule. Therefore the larvae have the usual characteristics of maggots and are therefore difficult to identify.

The pupal stage lasts approximately 10 days after which they emerge from the soil as adult flies. Adult female flies may live up to 40 days and can each lay approximately 300 eggs. The complete Medfly life cycle, i.e. from egg to egg, takes between three weeks to three months, depending on environmental and host plant conditions (Entomology website, Clemson).

1.7 PEST MANAGEMENT OF *C. CAPITATA*.

Various techniques are used for managing *C. capitata* to prevent or reduce its damaging effect.

1.7.1 Physical control

One of the more effective physical control methods is to bag the fruit to prevent egg laying. This is strongly encouraged in Pacific Island Countries and Territories as an inexpensive, effective method of control against fruit flies (Allwood 2000). Trapping as an alternative method has not been very effective.

1.7.2 Cultural control

Field sanitation is directed toward the destruction of all unmarketable and infested fruits. These should be collected and buried one metre under the soil surface with the addition of sufficient lime to kill the larvae. Weekly harvesting of fruit also reduces food sources from which large populations may develop (Mau & Kessing 1992).

1.7.3 Biological control

Many species of parasitoids of fruit flies were introduced to Hawaii between 1947 and 1952. These parasitoids parasitize the eggs or maggots of fruit flies and emerge during the pupal stage. Only three, *Opius longicaudatus* var. *malaiaensis* (Fullaway), *Opius vandenboschi* (Fullaway) and *Opius oophilus* (Fullaway), have become established. These parasitoids are primarily effective against *Dacus dorsalis* and *C. capitata* in cultivated crops (Christenson & Foote 1960).

The search for new parasitoid species against fruit flies continues (Messing 2000). The egg-larval parasitoid *Fopius arisanus* is utilized for augmentation biological control of tephritid fruit flies and is considered to have great control potential (Harris *et al.* 2000). Augmentation of biological control agents can be considered as a formal alternative for suppressing pest populations and in eradication programmes with the sterile insect technique (Montoya & Liedo 2000).

1.7.4 Chemical control

Chemical sprays have not been effective in protecting fruit from attack by Medflies. Egg laying requires only a few minutes and chemical residues do not kill adults within this time. Proteinaceous liquid attractants mixed with insecticide are recommended as a method of controlling adult Medfly populations in the vicinity of crops. The baited insecticide sprays are applied to broad leaf plants that serve as refuges for Medfly adults. Baits encourage the adults (especially females) to feed on the spray residue and can result in high rates of mortality. To be effective, insecticide bait sprays must be used in combination with good sanitation practices (Mau & Kessing 1992).

1.7.5 Sterile Insect Technique (SIT)

This technique refers to insects reared in large numbers and sterilized by using gamma irradiation. The sterilized insects are then released in the designated areas where they will mate with the wild (non-sterilized) insects. Such matings, however, will not produce any offspring (Enkerlin *et al.* 1996).

In different parts of the world (e.g. North, Central and South America, Japan, Australia) the release of sterile flies proved to be a technology capable of suppressing or eradicating fruit fly populations on an area-wide scale with no ill effects to the environment (Schwarz *et al.* 1989; Hendrichs 1996). In recent years this method of control has become even more cost effective due to improvements in artificial diets used for mass rearing of fruit flies (Hendrichs *et al.* 1995), the development of specific male and female strains, increased precision in sterile fly releases (Enkerlin *et al.* 1996), and by more sensitive monitoring systems (Anonymous 1996).

1.7.5.1 Artificial diets and methods of larval production of fruit flies

An artificial diet usually refers to any diet that is not the natural food of insects (Vanderzant 1966; Mckinley 1971). Singh (1977) refers to an artificial diet as being food that is not the natural diet of the insect, but has been synthesized or processed. The term artificial diet has also been defined as an unfamiliar food, which has been formulated, synthesized, processed and/or concocted for a specific insect, on which an insect can develop through all or part of its life cycle (Singh 1977).

Artificial diets are used for mass rearing of insects. It is obvious that the uses of artificial diets in entomological research programmes are varied. They have been used to rear pest species for the production of parasites, predators, and host material for pathogen

production, attractants to lure pest species into traps, pheromones and hormones (Singh 1977). One highly effective use of mass-reared insects has been in sterile-male release programmes (Singh 1977).

The successful formulation of an artificial diet depends on a basic understanding of nutrition, the chemical composition of the insect and its natural food and knowledge of the habitat and feeding behaviour of the species (Singh 1977).

Artificial diets developed for mass rearing should not only be nutritionally efficient so as to meet the behavioural and physiological requirements of the species, but should also be inexpensive and simple to prepare. Therefore, diet ingredients used to mass-rear insects should ideally be locally available to make diets less expensive.

1.7.5.1.1 Adult colony maintenance

Adult Medflies are housed in rectangular cages, made of sheet metal and an insect screen as described and illustrated by Vargas (1984). Approximately 1 kg (=120 000 pupae) of pupae are placed in a cage 91.4cm long, 182.9cm high and 20.3cm wide (Vargas 1984).

Cages are held in a room maintained at 27±2 °C and 60±10% RH. Fluorescent tubes attached to the ceiling or the sides of the cages provide light. At the Infruitec-Nietvoorbij Rearing Facility, where the study was conducted, cages were held in a room maintained at 24±1 °C and 65±5 % RH. Water was provided to flies as a solution of 1% agar, or by using wet sponges or sprinklers placed on the screen top of the cage or by using a plastic pipe (PVC) fitted with a horizontal strip of filter paper inside the cage. The adult diet for Medfly consists of a 3:1 volumetric mixture of sugar and yeast hydrolysate. A mixture of sugar and protein hydrolysate promoted fecundity in the melon fly, more effectively than when the components were offered separately (Sugimoto 1978).

1.7.5.1.2 Egg collection

The most common egg collection method used for large-scale production of Medfly is oviposition through the screened sides of the cages, as described by Vargas (1984). The eggs drop into a trough containing water or moist blotting paper (Schwarz *et al.* 1985). Oviposition into perforated bottles and their subsequent removal from cages for collection of eggs has also been used (Tanaka 1965; Steiner & Mitchell 1966; Tanaka *et al.* 1970). The screen method is the most commonly used technique and has been successful in the Mediterranean fruit fly mass rearing facility in Metapa, Mexico, where more than one billion eggs a week are collected (Schwarz *et al.* 1985).

1.7.5.1.3 Larval diet

Nutrition of tephritid larvae is very important since nutrients are required to provide energy and building materials for survival, growth, development, and as food storage material to be utilized in the pupal stage (Tsitsipis 1989). Nutrients are required by pupae for metamorphosis, and some nutrients are transferred to the adult stage. The nutritional requirements of adults are largely met by food ingestion, but also by nutrients of the larval stage via the pupa (Singh 1984). In the adult nutrients are required for dispersal, survival and reproduction.

An unsatisfactory nutrient balance may lead to nutritional defects affecting growth, development, reproduction and other life processes (Singh 1984). Proteins supply energy, but are expensive. In Medfly, some eggs were laid without parental protein and these eggs failed to hatch (Tsitsipis 1989). Protein is essential for the reproductive success of both males and females as it greatly enhances fecundity of females and is required by males for mating (Tsitsipis 1989). In mass rearing systems, protein is often supplied in the form of yeast. Yeast products also provide some carbohydrates, lipids, vitamins and mineral salts. The most

commonly used source of carbohydrates is granulated sugar, which fuels both daily activities and lipogenesis. Sugar is needed for flight, foraging and courtship activities of the flies (Warburg & Yuval 1997). Vitamins are indispensable for egg fertility. Water is necessary for the utilization of other nutrients (Tsitsipi 1989) and plays an important role in minimising the effects of metabolic heat build-up during the final stages of larval development (Hooper 1978) and enhances access to nutrients.

Bulking compounds mixed in diets absorb water, swell to provide a uniform distribution of nutrients and serve as a larval feeding substrate. For example, mill feed diet is used in Hawaii as a bulking agent (Tanaka *et al.* 1969; Vargas *et al.* 1983) for the production of Medfly, *D. dorsalis* and *D. cucurbitae*, while sugarbeet bagasse is used (Anonymous 1981) for mass rearing Medfly in Mexico. Sugarcane bagasse is the bulking agent used in Guatemala for Medfly production, while paper is used in a diet for mass production of *D. cucurbitae* (Nakamori & Kakinohana 1980) in Japan. These are examples of various bulking agents used in larval diets.

Local availability of ingredients influences the selection of bulking agents (e.g. bagasse, paper and corn cobs). In some countries, bulking compounds have reduced the cost of the large amounts of diet needed to mass rear millions of fruit flies for SIT eradication programmes (Schwarz *et al.* 1985; Nakamori & Kakinohana 1980).

Different types of mixing apparatus are used to combine ingredients in artificial diets. For example, a concrete mixer is used in Hawaii and an electric mixer in Japan (Vargas 1989).

1.7.5.1.4 Larval production methods

Larval rearing methods differ with respect to temperature, procedure and equipment. In the United States 150,000 Medfly eggs are seeded onto 5 l of diet (Vargas *et al.* 1983) in trays

interlocked inside cabinets. Tray cultures are held at 27°C for four days. On day five, larval cultures are transferred to a cooler room at 20°C to aid the dissipation of larval metabolic heat (Tanaka *et al.* 1972). Mature larvae are collected on six, seven and eight days after egg seeding.

To utilize space more efficiently, in Mexico Medfly eggs are incubated in a room maintained at 26±1 °C for two days in 7 litres polyethylene bottles containing water (Schwarz *et al.* 1985). Here 150,000 eggs are seeded onto 5 kg of diet in trays placed in racks (Anonymous 1981). The tray cultures are incubated at 30°C for 1.5 days and then transferred to a cooler room at 27°C where larvae mature in five to six days.

In Japan, approximately 50,000 *D. cucurbitae* eggs are seeded onto 5 litres of diet (Kakinohana 1982). The trays are placed in monorail frames. Temperatures in individual larval rearing rooms can be controlled over a range of 20 to 27°C to allow for synchronous development of larvae from eggs seeded on the diet on different collection days. At 27°C most flies have completed their larval life stage and can be collected on days six and seven.

1.7.5.1.5 Larval collection methods

Two different larval collection methods are commonly used for mass production of fruit flies. The first method, often referred to as the "popping method", is used in the United States for *C. capitata*, *D. dorsalis* and *D. cucurbitae*, and in Japan for *D. cucurbitae*. Fully-grown larvae leave the trays and drop either into a drawer filled with water or onto a floor flooded with water.

The second method is used for collecting Medfly larvae in Mexico and Guatemala. Bran or bagasse is mixed with the diet in the larval rearing trays to absorb moisture so as to dry out the diet. The contents are then emptied into a larval separating machine or mechanical sieve ("trunkenpolz") to separate larvae from their diet (Schwarz *et al.* 1985).

1.7.5.1.6 Pupation and collecting pupae

Vargas *et al.* (1986) developed two methods for collecting pupae. In the first method, used in the United States and Japan, full-grown larvae collected in water are removed from the water and mixed in vermiculite or sawdust. Larvae are allowed to pupate in boxes placed in a room maintained at 20±1 °C, 60±10 % RH. Two days later, the pupae are separated from the vermiculite, using a soft-action vibrator or mechanical sifter. Separation of Medfly pupae at slow speeds (6 rpm) for two days is important to minimize pupal injury (Ozaki & Kobayashi 1981; Ozaki & Kobayashi 1982). Separation during critical development periods at higher sifter speeds and temperatures has been shown to cause deformities or detachment of wing muscles. This results in poor flight ability of adults. The second method is used in Mexico. Larvae separated from the rearing medium, are placed directly onto screen trays or racks and allowed to pupate in a dark room maintained at 22±1 °C, 70±5 % RH. The racks with pupae remain in this room until the pupae mature (Schwarz *et al.* 1985).

Because of space limitations, the second method was favoured in Mexico. This method has the obvious advantages of not requiring pupation boxes, vermiculite or a mechanical sifter for the separation of pupae. However, although this method is logistically attractive, the procedure apparently reduces pupal quality and adult survival. The first method is initially more expensive, but the production of higher quality flies justifies the expense (Vargas *et al.* 1986).

1.8 CONCLUSION

The Mediterranean fruit fly is established in South Africa and is one of the most destructive fruit pests. The concept of sterile insect technique is therefore being used for protection of South African crops. Control using SIT can be achieved by mass rearing fruit

flies on artificial diets under controlled conditions in a rearing facility and by subsequent sterilization and release. Research has been widely done for the development of diet for the Medfly. The need for such research is to develop an inexpensive artificial diet using locally sourced ingredients for the mass production of Medfly.

CHAPTER 2

ARTIFICIAL DIET FOR REARING *CERATITIS CAPITATA* (WIEDEMANN) (DIPTERA: TEPHRITIDAE): SMALL-SCALE TRIALS

2.1 INTRODUCTION

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann), has been reared in the laboratory in many countries around the world, either on a large scale for sterile insect technique programmes or on small scale for research purposes. In both rearing approaches, artificial diets with low cost bulking agents have been of interest to all researchers.

In some countries use of bulking compounds such as sawdust, sugarcane bagasse and sugarbeet baggase have reduced the cost of the large amounts of diet needed to mass rear fruit flies for SIT eradication programmes (Schwarz *et al.* 1985; Nakamori & Kakinohana 1980). These compounds when mixed in the diets absorb liquid, providing a uniform distribution of nutrients and a suitable substrate for larval feeding and development.

At present, the Krige diet (Fisher 1999) is used by the Agricultural Research Council (ARC) Infruitec-Nietvoorbij Research Institute at Stellenbosch in the Western Cape Province, South Africa, where the present study was also conducted. The Krige diet contains bran as a bulking agent and torula yeast as a source of protein. Torula yeast has to be imported and accounts for about 60% of the cost of the diet.

The current study was designed to investigate the use of locally available maize meal as an alternative bulking agent. Maize meal is used for human consumption in South Africa and contains some protein, which enables the reduced use of torula yeast, thereby reducing the cost of the diet. It also contains some vitamins and trace elements, which could enhance the nutritional value of the diet, possibly resulting in an improvement in the quality of the flies.

2.2 MATERIALS AND METHODS

2.2.1 REARING PROCEDURES

2.2.1.1 Maize meal used

The Iwisa® maize meal that was used in the study contains a number of nutrients (Table 2.1) including proteins, minerals, carbohydrates and vitamins.

2.2.1.2 Diet preparation

The Krige diet used in the ARC Infruitec-Nietvoorbij rearing facility was used as the standard for comparison with the diet containing maize meal as bulking agent. Table 2.2 shows the ingredients used in both diets. Maize meal was added in the experimental diet as a substitute for some of the bran and yeast. Sugar was reduced to meet appropriate carbohydrate amounts needed to rear the insects. Water was also increased in the maize meal diet to provide suitable diet texture. The pH of both diets was throughout the experiments 4.0 to 5.0.

2.2.1.3 Experimental conditions

Flies were reared from the egg to adult stage in a room maintained at 25±1 °C and 80±10% RH. Experiments to compare the diets were replicated five times. The daily maximum and minimum temperature of the diets was recorded, using thermometers placed in one of the replicated tray containing each of the diets. Experiments on fecundity and fertility were conducted at 21±1 °C and 60±10 % RH.

Table 2.1. Nutritional components of Iwisa® maize meal used in the experimental diet (amounts per 100grams maize meal) (Langenhoven *et al.* 1991)

Protein		Fats		Carbohydrates		Minerals		Vitamins	
Moisture (g)	13.0	Total fat (g)	1.2	Total available carbohydrates	83.5	Calcium (mg)	3	Vitamin A in Retinol	0
Kilocalories	366	Saturated fatty acids (g)	0.15	Dietary fibre (g)	2.6	Iron (mg)	0.5	Thiamin (mg)	0.20
Kilojoules	15320	Mono-unsaturated fatty	0.37	Added sugar (g)	0.0	Magnesium (mg)	32	Riboflavin (mg)	0.03
Total protein (g)	8.8	Polyunsaturated fatty acids (g)	0.46			Phosphorus (mg)	82	Niacin (mg)	0.6
Plant protein (g)	8.8	Cholesterol (mg)	0			Sodium (mg)	5	Vitamin B6 (mg)	0.04
Animal protein (g)	0.0					Zinc (mg)	0.50	Folate (µg)	14
						Copper (mg)	0.06	Vitamin B12 (µg)	0.0
						Manganese (mg)	0.10	Pantothenic acid (mg)	0.35
								Biotin (µg)	2.8
								Vitamin C (mg)	0
								Vitamin D (µg)	0
								Vitamin E	0.5

2.2.1.4 Eggs

The eggs used in the study were from the temperature sensitive lethal (*tsl*) strain Vienna 7/97, maintained at the Infruitec rearing facility. These eggs were placed in a 6-litre plastic bottle for incubation and aerated in water at 25°C for 48 hours. Air was filtered to remove microorganisms (Rico 1983). The required number of eggs was placed on moist tissue paper on the surface of 1.5kg of each diet in rectangularly shaped 30 x 20 x 7cm (length x breadth x height) larval rearing containers. A rectangular opening 20cm in length and 14.5cm wide was cut in the lid of each container and covered with laced netting for ventilation.

Table 2.2. Ingredients used in approximately one kilogram of the Iwisa® maize meal and Krige diets.

Ingredients	Maize meal	Krige
Bran	0.2500 kg	0.3090 kg
Sugar	0.1100 kg	0.1350 kg
Maize meal	0.1500 kg	-
Torula yeast	0.0300 kg	0.0590 kg
Sodium benzoate	0.0020 kg	0.0020 kg
HCl	0.0080 l	0.0080 l
Tap water	0.5600 l	0.4910 l

2.2.1.5 Larval and pupal growth

The larval rearing containers were placed in rectangular 42 x 28 x 21cm (length x breadth x height) basins containing 2kg vermiculite. The basins were covered with laced netting to prevent larvae from jumping out of the basins. Mature larvae were collected in the vermiculite on days six, seven and eight after seeding the diet with eggs. After two days, pupae were separated from the vermiculite by sifting (Chapter 1). Pupae were then kept for seven days before they were prepared for quality control tests.

2.2.2 QUALITY CONTROL

Standard methods were used for assessing Medfly quality and competitiveness (Orozco *et al.* 1983; Anonymous 1998). These are described below.

2.2.2.1 Pupal volume

Evaluation of diet suitability for larval development was based on survival from egg to pupae. Pupal production from each replicate was determined volumetrically, and this was used as an indication of the number of larvae produced.

2.2.2.2 Pupal weight

Pupal weight reflects the nutritional conditions during the larval stage and is affected by overcrowding and high temperatures. A sample of 100 pupae from each daily collection from each diet replicate was randomly taken for determination of pupal weight. Mean pupal weight was determined by weighing 100 pupae from each replicate using a loading electronic balance.

2.2.2.3 Sex ratio and percentage emergence

A 5ml sample of pupae from each daily collection from each replicate was used for this test. This sample was separated into white (female) and brown (male) pupae. The numbers of white and brown pupae were determined to estimate the sex ratio. The pupae were then left to emerge in a Petri dish. When all the flies had died, the percentage emergence was determined from the number of unemerged pupae remaining and the total number of pupae counted during sex ratio determination.

2.2.2.4 Flight ability

Flight ability is an important measure of fitness, as the flies need to disperse to find food and mating arenas. It can be affected by pupal holding conditions. From each daily collection of each replicate, 100 pupae were randomly selected for determination of flight ability. They were placed inside a ring of black paper 1cm high and 6cm in diameter, which was centered in the bottom of a Petri dish. A 10cm high black PVC tube 3mm walls thick and with an internal diameter of 8.9cm was placed in the Petri dish. The ring of black paper was inside the PVC tube. When the flies emerged they rested on the sides of the paper ring. The inside of the PVC tube was coated with unscented talcum powder that prevented the flies from walking out. The talcum powder was wiped from the bottom of the tube to provide an additional resting place for newly emerged flies, which left a ring of black paper. The tube was placed in a ventilated 30 x 30 x 40cm (length x breadth x height) cage containing sticky traps inside which caught flies leaving the tube, preventing them from returning to the PVC tube. The bottom of the cage was covered with black plastic. Flight ability was measured by counting the remaining flies (which were in the PVC tube after all flies that emerged left the tube or had died) in the Petri dish.

2.2.2.5 Fecundity and fertility

Determination of fecundity and fertility was based on daily egg collections from 10 pairs of flies held for 22 days after a two day pre-oviposition period. The flies were placed in 10 x 10 x 7cm (length x breadth x height) containers. A square opening of 9 x 9cm on the bottom side was cut in each container and the resulting hole was covered with a piece of laced netting. The adults oviposited through the laced netting. The eggs were collected in a Petri dish placed beneath the laced netting. The bottom of the Petri dish was covered with moist black filter paper on which the eggs were counted. Eggs were collected from five such

containers (replicates) of flies per diet and were spread with a small camelhair brush in the Petri dishes before counting. Percentage eggs hatch was determined after four days. The adults were provided with a 3:1 mixture of sugar and enzymatic yeast hydrolysate (U. S. Biochemica, Cleveland, Ohio) and water. Water was provided by means of a wick, made of cotton wool, inserted through a hole in each of the perforated containers. The end of the wick on the outside of the container was in contact with water in a Petri dish, while the inside end provided water to the flies.

2.2.3 DATA ANALYSIS

All the results were analyzed, using factorial analyses of variance with five replicates. Diet and collection date were the main effects. In the case of the data pertaining to the proportion of females emerging, a logit transformation was used (Cox 1972) to stabilize the variances. Exact probabilities are given.

2.2.4 EXPERIMENTS CONDUCTED IN THE STUDY

There are four experiments conducted in the study to compare the effectiveness of modified diet with the standard Krige diet

Experiment 1. One ml (=25 000 eggs) of eggs was placed on moist tissue paper on the surface of 1.5kg of both the maize meal and Krige diets in the larval rearing containers.

Experiment 2. One ml of eggs was placed on moist tissue paper on the surface of 1.5kg of each diet in the larval rearing containers. 100ml of water was added to the maize meal and Krige diets during larval production on day 8 or 9 depending on the dryness of the diet, this was done after experiment one resulted in diet dryness.

Experiment 3. In both diets 0.7ml of eggs was placed on moist tissue paper on the surface of 1.5kg of each diet in larval rearing containers to determine the amount of eggs that can be used to produce Medflies. This experiment was conducted in order to increase pupal weight which was lower in experiment two.

Experiment 4. In this experiment 0.6ml of eggs was placed on moist tissue paper on the surface of 1.5kg of the maize meal diet, while 1ml of eggs was placed on moist tissue paper on the surface of 1.5kg Krige diet. 0.6ml of eggs was used in maize meal diet to reduce competition of food resources to give rise in good fliers after experiment three.

2.3 RESULTS

2.3.1 EXPERIMENT 1

2.3.1.1 Larval feeding behaviour

Most of the larval feeding occurred in the center of the containers where the eggs were placed onto the diet. On day five (post egg set), aggregations of nearly mature larvae appeared as the diet crust began to bulge. Larvae started to leave both diets on days seven, eight, nine, and 10.

The maximum temperature in the maize meal diet was 1 to 3°C higher than that of the Krige diet on days seven, eight and nine and on days 10, 11 the temperature of the Krige diet was 2.5 to 3°C higher than that of the maize meal diet (Fig. 2.1). The maximum temperature was 32.5°C and 31°C on the surface of the maize meal and Krige diets, respectively. This was slightly higher than the lethal temperature for female larvae.

2.3.1.2 Pupal volume

More pupae were produced from larvae reared in the maize meal diet than in the Krige diet on each of the four collection days (Fig. 2.2). These differences were highly significant ($F_{1:32}$=129.5; P<0.001). Peak larval collection occurred on days eight and nine after placing eggs on the diet. In the case of the Krige diet, the number of larvae collected on days eight and nine were similar, while in the case of the maize meal diet more larvae were collected on day nine than on day eight (Fig. 2.2). This discrepancy gave rise to significant interactions between the diet and collection day ($F_{3: 32}$=18.5; P<0.001).

2.3.1.3 Pupal weight

The average weight of 100 pupae from the Krige diet was higher than from the maize meal diet (Fig. 2.3; $F_{1:16}$=31.6: P<0.001). This was probably due to increased competition between larvae in the maize meal diet resulting from the higher numbers (reflected by the greater pupal volume) in this diet than in the Krige diet.

2.3.1.4 Proportion of flies emerging

The proportion of flies emerging from pupae produced in the maize meal diet was higher than in the Krige diet (Fig. 2.4; $F_{1:24}$=35.9; P<0.001). In addition there were differences between collection days ($F_{2:24}$=203.1: P<0.001) with a lower proportion of flies emerging on each successive day (Fig. 2.4).

2.3.1.5 Proportion of females

A higher proportion of females was produced from the Krige diet than from the maize meal diet (Fig. 2.5). These differences were significant ($F_{1:24}$=13.5; P=0.001), particularly on

the first two collection days. A higher proportion of females was produced on each successive collection day. Medfly females are temperature sensitive. Therefore the survival of females produced in the maize meal diet may have been lower than those produced in the Krige diet because of the higher temperatures in the maize meal diet.

2.3.1.6 Flight ability

More good fliers were produced using the maize meal diet than the Krige diet (Fig. 2.6; $F_{1:24}$=5.1; P=0.033). There was also a difference in flight ability between collection days (Fig. 2.6; $F_{2:24}$=44.1; P<0.001). The flight ability decreased on each successive collection day. Trends in flight ability of flies produced in both diets were the same.

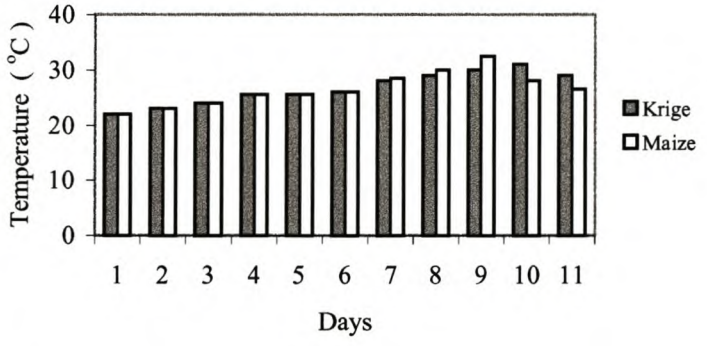

Fig. 2.1. Temperature (°C) of the diets during the Medfly larval production period in the Krige and the maize meal diets.

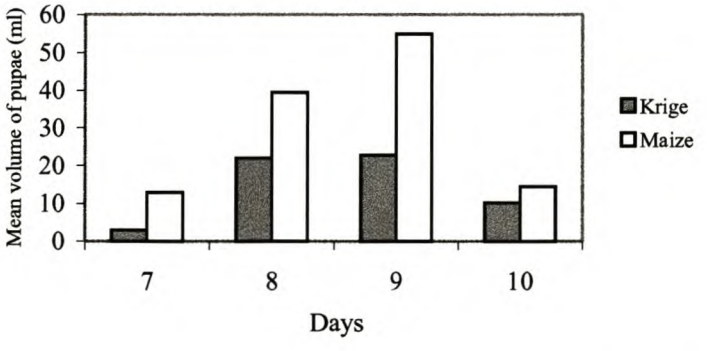

Fig. 2.2. Mean volume of pupae (ml) of Medfly larvae leaving the Krige and the maize meal diets.

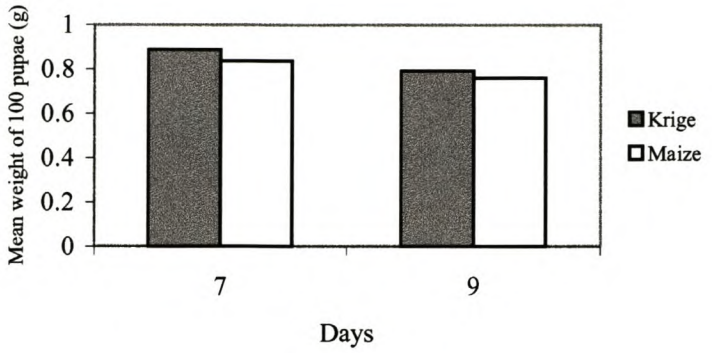

Fig. 2.3. Mean weight (g) of 100 pupae of Medfly by collection day reared in the Krige and in the maize meal diets.

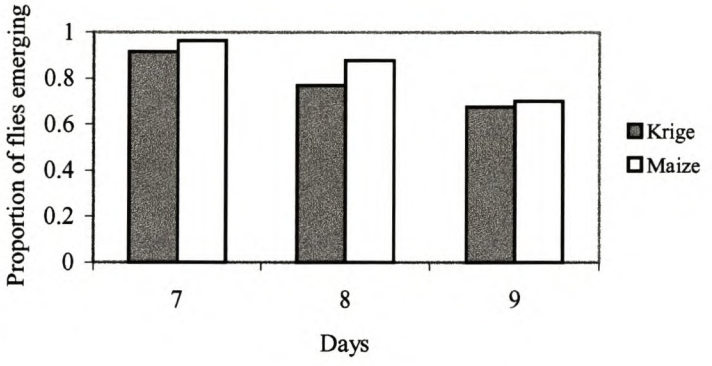

Fig. 2.4. Proportion of Medfly emerging from pupae resulting from larvae collected on three days after being reared in the Krige and in the maize meal diets.

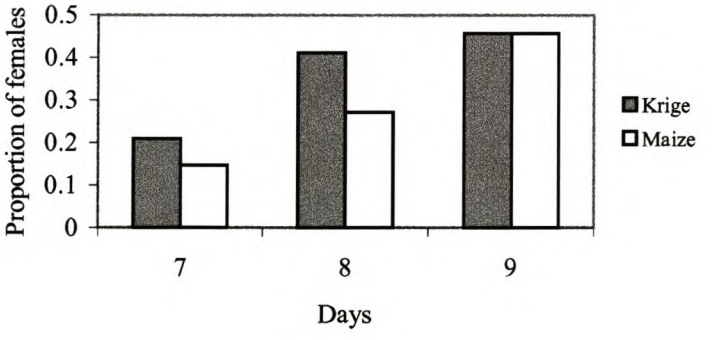

Fig. 2.5. Proportion of females produced in the Krige and the maize meal diets.

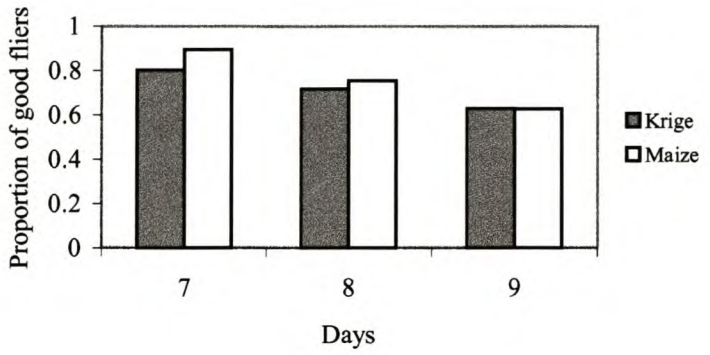

Fig. 2.6. Proportion of good fliers produced in the Krige and the maize diets.

2.3.2 EXPERIMENT 2

2.3.2.1 Larval feeding behaviour

Larval feeding behaviour was similar to that in experiment 1. The maximum temperature was 1 to 2°C (Fig. 2.7) higher in the maize meal diet than in the Krige diet on larval development days eight and nine. The maximum temperature was 28°C and 30°C on the surface of the maize meal and the Krige diets, respectively. This was slightly higher than the lethal temperature for females. Therefore, the addition of water to the maize meal diet resulted in a slight decrease in the temperature.

2.3.2.2 Pupal volume

The maize meal diet produced more pupae than the Krige diet on each of the four days during which larvae were collected (Fig. 2.8). These differences bordered on significance ($F_{1;32}$=3.83; P=0.0591). Most pupae developed from fully-grown larvae collected on day eight and nine after placing eggs on the diet. The number of pupae from larvae collected on days eight and nine were similar in the Krige diet, while in the maize meal diet more pupae came from larvae collected on day nine than on day eight (Fig. 2.8). This discrepancy gave rise to significant interactions between the diet and collection day ($F_{3;32}$=30.01 ; P<0.001).

2.3.2.3 Pupal weight

The weight of 100 pupae from the Krige diet was higher than from the maize meal diet (Fig. 2.9; $F_{1:3}$=202,45 ; P<0.001). This could have been due to greater competition in the maize meal diet resulting from higher numbers of larvae than in the Krige diet. There were also differences in pupal weight between the four collection days (Fig. 2.9; $F_{3:32}$=58.64 ; P<0.001), with smaller pupae produced on each successive collection day.

2.3.2.4 Proportion of flies emerging

The proportion of flies emerging from pupae produced in the Krige diet was higher than in the maize meal diet (Fig. 2.10). These differences were significant ($F_{1:32}$=22.90; P<0.001). In addition there were differences between collection days ($F_{3:32}$=82.04; P<0.001) with a lower proportion of flies emerging on each successive day.

2.3.2.5 Proportion of females

The proportion of females produced from the Krige diet was higher than from the maize meal diet (Fig. 2.11). These differences were significant ($F_{1:32}$=5.68 ; P=0.0232). On the first two collection days, the proportion of females was similar for the two diets (Fig. 2.11). There was a significant difference between the four collection days ($F_{3:32}$=68.68; P<0.001). On the first two collection days (days seven and eight) lower proportion of females were produced than on the last two days (days nine and 10) on both diets.

2.3.2.6 Flight ability

More good fliers were produced by the Krige diet than by the maize meal diet (Fig. 2.12; $F_{1:32}$=29.42; P <0.001). Flight ability decreased with each successive collection day

(Fig. 2.12; $F_{2:32}$=63.59; P< 0.001). The trend in flight ability of flies from both diets was similar. More good fliers were produced during the first and second collection days.

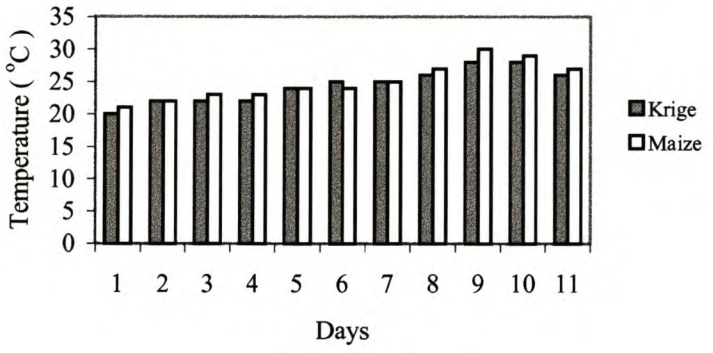

Fig. 2.7. Temperature (°C) of the diets during the Medfly larval production period in the Krige and the maize meal diets.

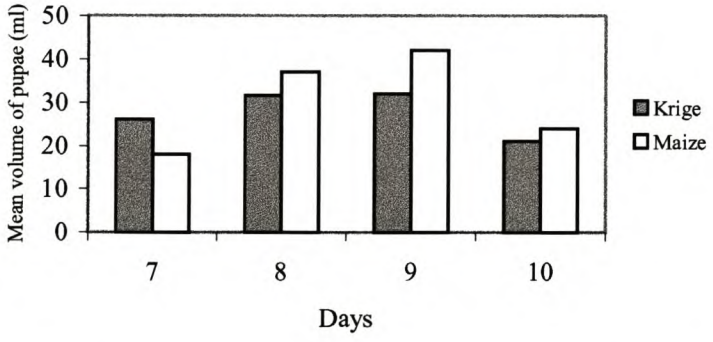

Fig. 2.8. Mean volume of pupae (ml) of Medfly larvae leaving the Krige and the maize meal diets.

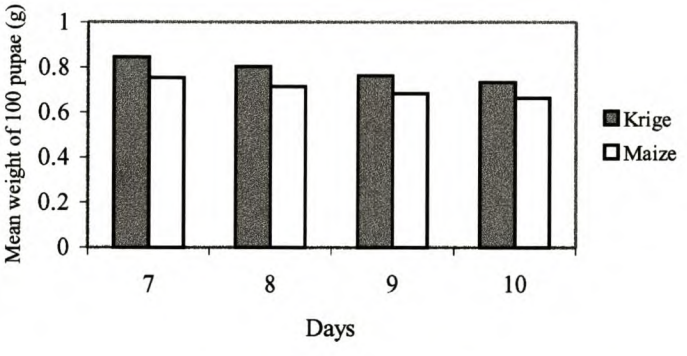

Fig. 2.9. Mean weight (g) of 100 Medfly pupae reared in the Krige and the maize meal diets.

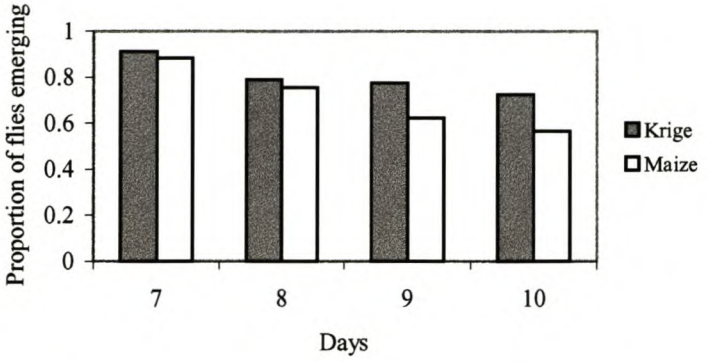

Fig. 2.10. Proportion of Medfly emerging from pupae resulting from larvae collected on four days after being reared on the Krige and on the maize meal diets.

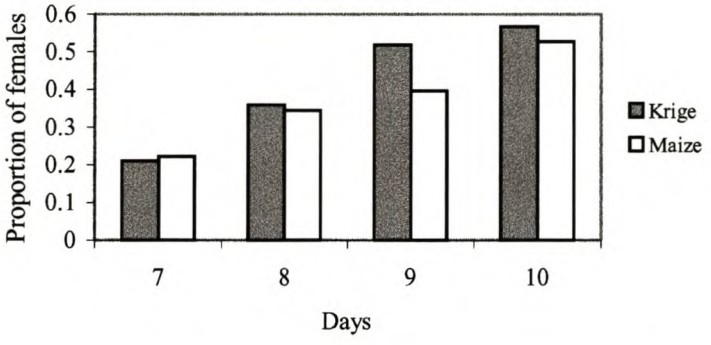

Fig. 2.11. Proportion of females produced from the Krige and the maize meal diets.

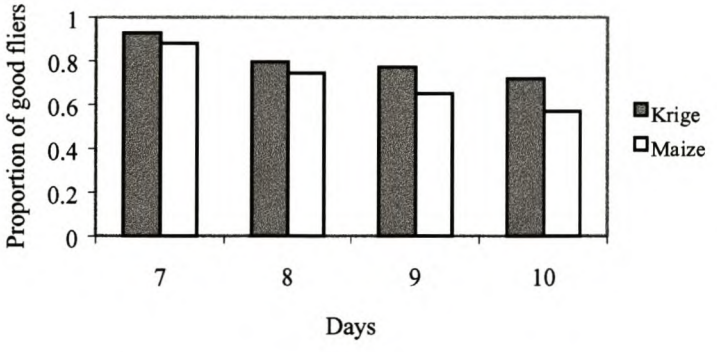

Fig. 2.12. Proportion of good fliers produced from the Krige and the maize diets.

2.3.3 EXPERIMENT 3

2.3.3.1 Larval feeding behaviour

The larval feeding behaviour was similar to that described in experiment 1. The maximum temperature in the Krige diet (Fig. 2.13) was 1 to 2°C higher than in the maize

meal diet on larval development days seven, eight and nine. The maximum temperature was 31°C on the surface of both, the maize meal and the Krige diets respectively. This was slightly higher than the lethal temperature for females.

2.3.3.2 Pupal volume

A greater volume of pupae was produced from larvae reared in the maize meal diet than in the Krige diet on each of the four days during which larvae were collected (Fig. 2.14). These differences were highly significant, ($F_{1:32}$=62.52; P<0.001). On day eight the difference in pupal volume between the two diets was less than on days seven, nine and 10 (Fig. 2.14). This discrepancy gave rise to significant interaction between the diet and the day ($F_{3:32}$=32.30; P<0.001).

2.3.3.3 Pupal weight

There was no difference in the weight of 100 pupae from larvae produced in the two diets ($F_{1:32}$=0.00014; P=0.997). However, there were differences in pupal weight between collection days (Fig. 2.15; $F_{3:32}$=34.35; P<0.001). The weight of 100 pupae from the maize meal diet was higher on the first collection day than those from the Krige diet, while the weight of 100 pupae from both diets was similar on the second collection day (Fig. 2.15).

2.3.3.4 Proportion of flies emerging

The proportion of flies emerging from pupae produced in the Krige diet was higher than from the maize meal diet (Fig. 2.16). This difference was significant ($F_{1:32}$=92.14; P<0.001). There was a difference between collection days ($F_{3:32}$=126.95; P<0.001) with a lower proportion of flies emerging on each successive day (Fig. 2.16).

2.3.3.5 Proportion of females

A lower proportion of females was produced from the Krige diet than from the maize meal diet (Fig. 2.17). These differences were significant ($F_{1:32}$=19.56; P<0.001). A higher proportion of females was produced on each successive day ($F_{3:32}$=379.74; P<0.001). There were interactions between diets and day ($F_{7:32}$=5.6309; P=0.003), because more females were produced from the Krige diet than from the maize meal diet on day eight, while this trend was reversed on days nine and 10.

2.3.3.6 Flight ability

More good fliers were produced using the Krige diet than the maize meal diet (Fig. 2.18; $F_{1:32}$=19.56; P = 0.001). Flight ability decreased with each successive collection day (Fig. 2.18), resulting in significant differences in flight ability between days ($F_{3:32}$=28.81; P<0.001).

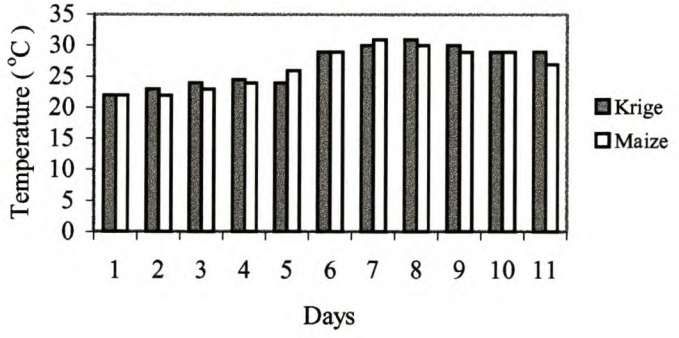

Fig. 2.13. Temperature (°C) of diets during the Medfly larval production period in the Krige and the maize meal diets.

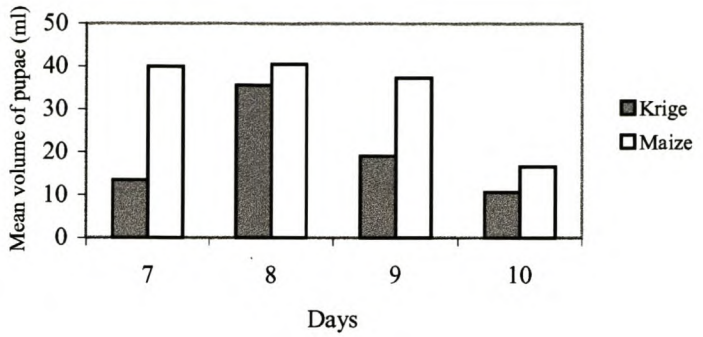

Fig. 2.14. Mean volume of pupae (ml) from Medfly larvae leaving the Krige and the maize meal diets.

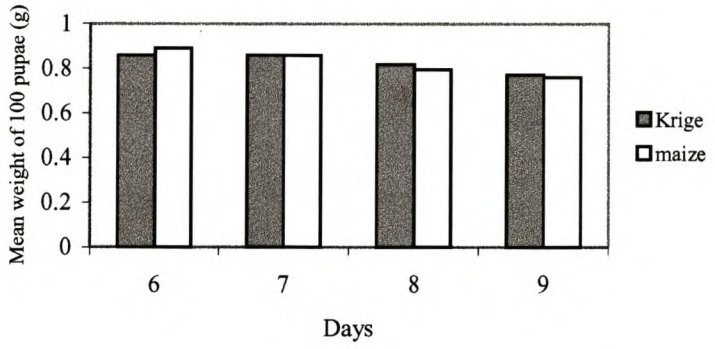

Fig. 2.15. Mean weight (g) of 100 pupae of Medfly by collection day reared in the Krige and the maize meal diet.

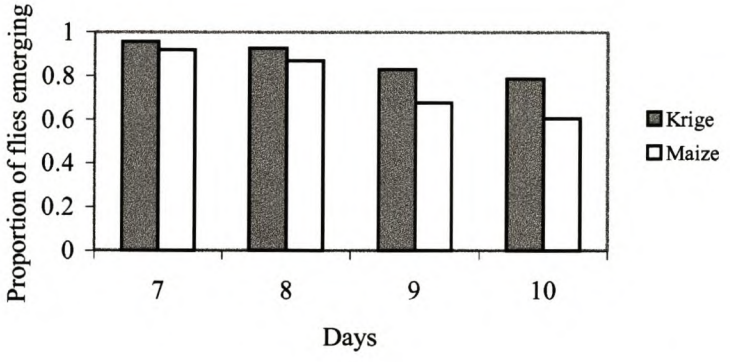

Fig. 2.16. Proportion of Medfly emerging from pupae reared in the Krige and the maize meal diets.

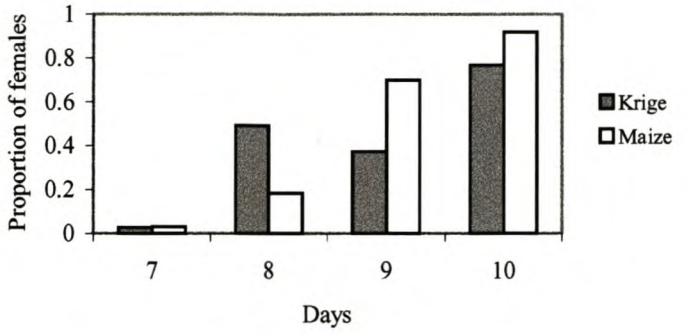

Fig. 2.17. Proportion of females produced in the Krige and the maize meal diets.

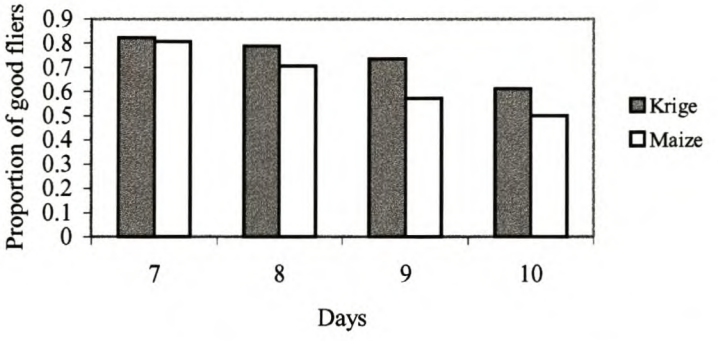

Fig 2.18. Proportion of good fliers produced in the Krige and the maize diets.

2.3.4 EXPERIMENT 4

2.3.4.1 Larval feeding behaviour

The behaviour of larvae on the diet was the same as in experiment 1. Temperatures did not rise above 27°C in maize meal diet, which is the ideal for the temperature sensitive strain (Fig. 2.19). However, the temperatures were slightly higher in the Krige diet than in the maize meal diet. The maximum temperature was 27°C and 30°C on the surface of the maize meal and the Krige diets respectively.

2.3.4.2 Pupal volume

More larvae were produced by the maize meal diet than by the Krige diet on each of the four days during which larvae were collected (Fig. 2.20). However, this difference was not

significant ($F_{1:24}$=2.61; P=0.119). Peak larval collection occurred on days seven and eight after placing eggs on the diet (Fig. 2.20). There was a difference between collection days ($F_{2:24}$=5.27; P=0.012), with more pupae being collected on days seven and eight than on day nine.

2.3.4.3 Pupal weight

The weight of 100 pupae from the Krige diet was higher than from the maize meal diet (Fig. 2.21; $F_{1:16}$=7.17; P=0.016). There was also a difference in pupal weight between collection days ($F_{2:16}$=95.08; P<0.001).

2.3.4.4 Proportion of flies emerging

The proportion of flies emerging from pupae produced from Krige diet was slightly higher than that from the maize meal diet (Fig. 2.22; $F_{1:16}$=5.92; P=0.027). There was also a difference in the proportion of flies emerging between collection days ($F_{2:16}$=95.93; P<0.001) with a higher proportion emerging on days seven than on days eight.

2.3.4.5 Proportion of females

A slightly higher proportion of females was produced from the maize meal diet than from the Krige diet (Fig. 2.23). These differences bordered on significance ($F_{1:16}$=3.98; P=0.063). There was a difference in the proportion of females produced between collection days (Fig. 2.23; $F_{2:16}$=46.21; P<0.001). Both diets exhibited similar trend with more females produced from maize meal diet on day eight than on days seven.

2.3.4.6 Flight ability

More good fliers were produced from larvae reared on the Krige diet than from those reared on the maize meal diet (Fig. 2.24; $F_{1:16}$=5.99; P=0.026). Flight ability decreased with each successive collection day (Fig. 2.24; $F_{2:16}$=162.57; P< 0.001).

2.3.4.7 Eggs laid

There was a significant difference in the number of eggs laid by adult flies produced from the maize meal diet and the Krige diet (Fig. 2.25; $F_{1:176}$=4.81; P=0.0295). More eggs were produced by flies reared on the maize meal diet than on the Krige diet, particularly during the first 14 days. During this period the difference was highly significant ($F_{1:112}$=11.573; P<0.001). There was also a difference in egg production between collection days ($F_{2:176}$=6.85; P<0.001), with peak numbers being laid on day four.

2.3.4.8 Eggs not hatched

More of the eggs produced by flies reared on the Krige diet did not hatch than those produced by flies reared on the maize meal diet (Fig. 2.26). However, this difference was not significant ($F_{1:176}$=3,04; P = 0.0829; Fig. 2.26). There was a difference in the number of eggs that did not hatch between collection days ($F_{2:176}$=2.36; P=0.001). The trend was the same for the two diets (Fig. 2.26).

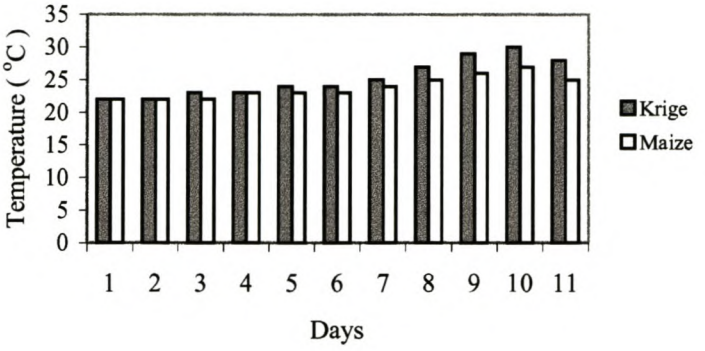

Fig. 2.19. Temperature (°C) of the diets during the Medfly larval production period in the Krige and maize meal diets.

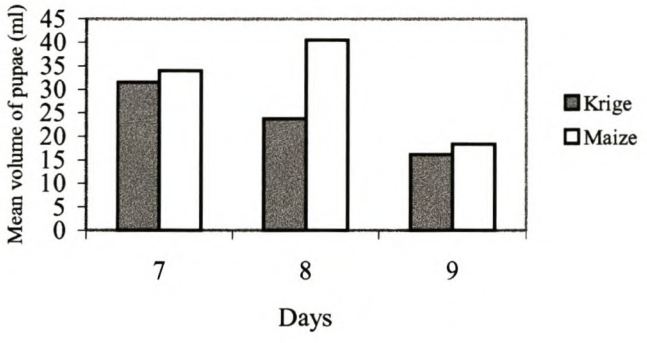

Fig. 2.20. Mean volume of pupae (ml) of Medfly larvae leaving the Krige and maize meal diets.

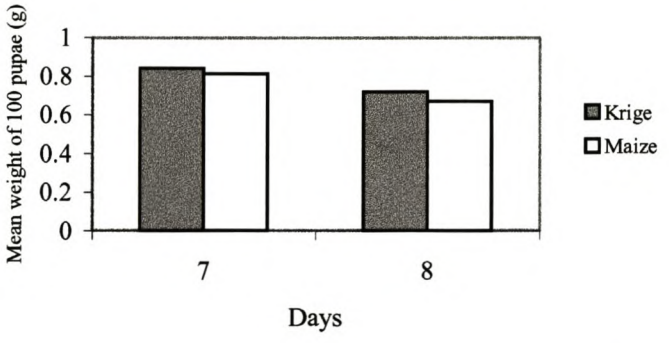

Fig. 2.21. Mean weight (g) of 100 Medfly pupae reared in the Krige and in the maize meal diets.

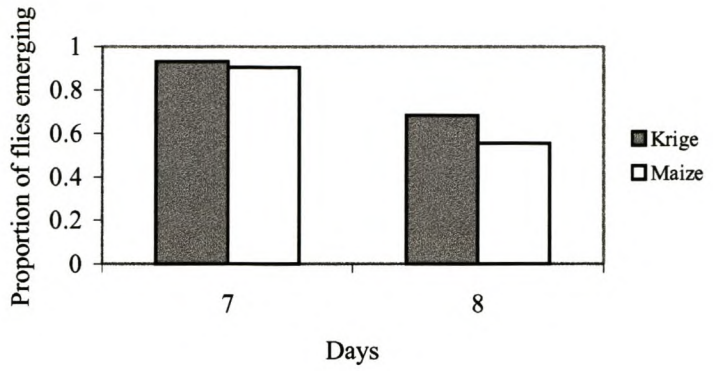

Fig. 2.22. Proportion of Medfly emerging from pupae resulting from larvae collected two days after being reared in the Krige and the maize meal diets.

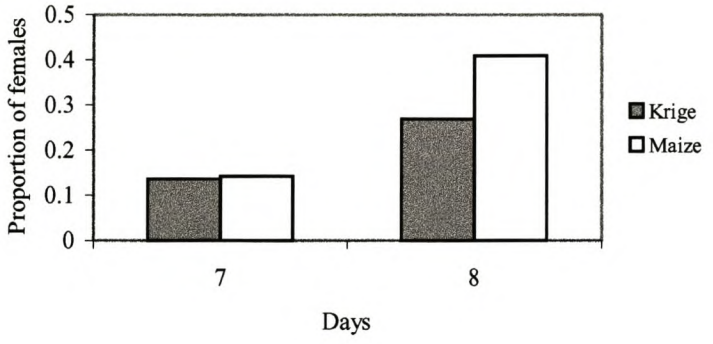

Fig. 2.23. Proportion of females produced from larvae reared in the Krige and the maize meal diets.

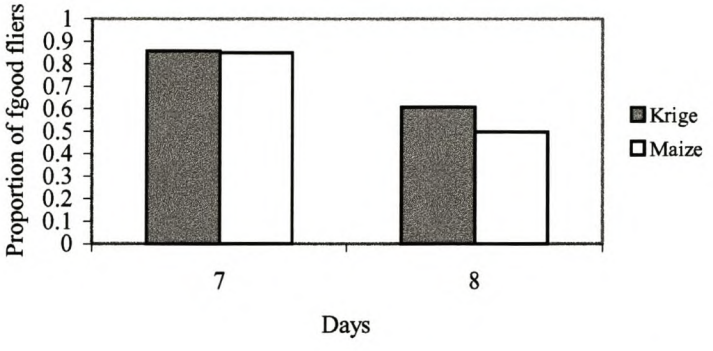

Fig. 2.24. Proportion of good fliers produced from the Krige and the maize meal diets.

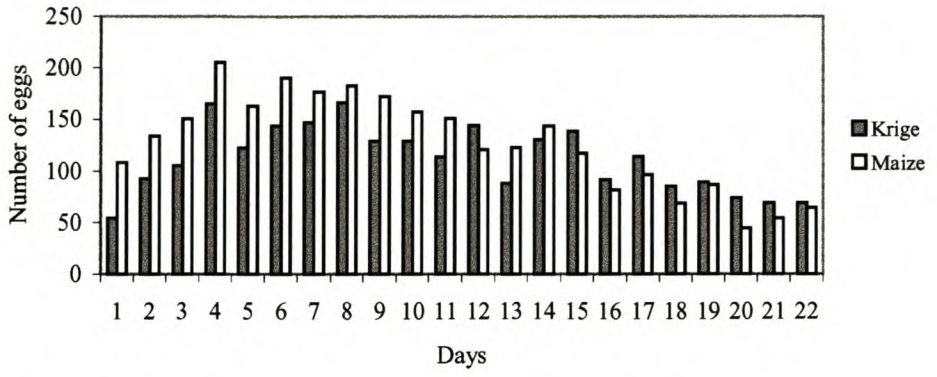

Fig. 2.25. Numbers of eggs laid by Medfly reared in the Krige and the maize meal diets.

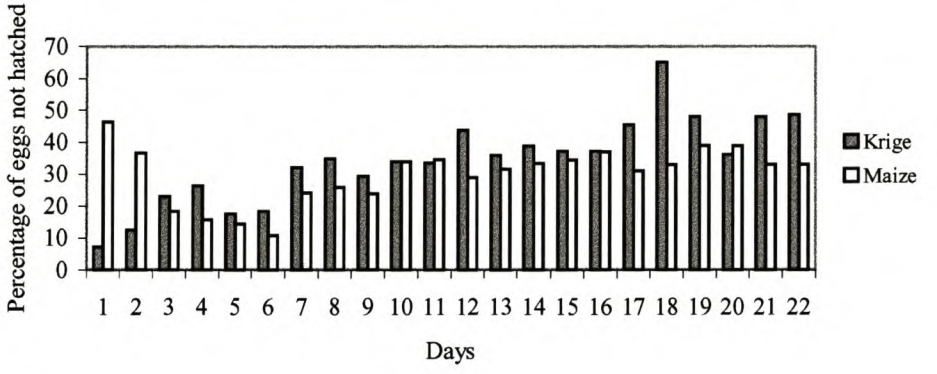

Fig. 2.26. Percentage of eggs not hatched from Medfly reared in the Krige and the maize meal diets.

2.4 DISCUSSION

Suitability of artificial diets for fruit fly larvae depends on both nutritional and physical factors (Chapter 1) (Singh 1977; Vargas *et al.* 1983; Vargas 1989).

In all experiments, the diet temperature increased with time, until the fully-grown larvae started leaving the diet. This was accompanied by a decrease in temperature.

A higher volume of pupae was produced in the maize meal diet than in the Krige diet in all the experiments while the pattern of daily production of pupae from the maize meal diet was similar to that of the Krige diet (Fig. 2.2, 2.8, 2.14, & 2.20), with large volumes of pupae produced on collection days 8 and 9.

High pupal weight is considered to be a desirable characteristic in fruit fly rearing on artificial diets. Churchill-Stanland *et al.* (1986) found that the size of Medfly was important for mating success. Flies emerging from pupae weighing 0.8 to 0.9g had greater mating success than those of smaller pupae. Pupae reared on the maize meal diet ranged from 0.7 to 0.9g during the first two collection days and were within acceptable standards for pupal weight (0.8 to 0.9g; Table 2.3) in Experiment 3. The weight of pupae from the Krige diet ranged from 0.78 to 0.90g in all experiments, which is also within acceptable standards of pupal weight. The pupal weight was affected by overcrowding as reflected by the higher pupal volume from the maize meal diet than from the Krige diet in Experiments 1 and 2. Overcrowding of larvae in the diet resulted in lower pupal weight. In Experiment 4 there was no difference in pupal volume between the two diets, but the weight of pupae produced from the Krige diet was higher than those produced from maize meal diet. However, the difference in pupal weight between the two diets was not as significant in Experiment 4 as in other three experiments.

The proportion of flies emerging from pupae reared in the maize meal diet was lower than those reared in the Krige diet in all experiments except Experiment 1 (Table 2.4). Emergence of pupae from the maize meal diet was 70% to 84%, which was below 85%, the minimum accepted emergence for Medfly reared in the laboratory worldwide (Anonymous 1998). Emergence of pupae from the Krige diet was 78% to 87%. This low pupal emergence indicated that there was a fault in the rearing or handling methods used in the study.

Pupae recovered from the maize meal diet produced a lower proportion of females compared with the Krige diet (Table 2.4) in the first two experiments. This was probably due to higher temperatures in the maize meal diet caused by the greater number of larvae emerging from the diet. After the number of eggs was reduced in the maize meal diet (Experiment 3 and 4), a higher proportion of females was produced from the maize meal diet than from the Krige diet.

Table 2.3. Mean volume of pupae produced (pupal volume), mean weight of 100 pupae (pupal weight), proportion of emerged flies, proportion of females, proportion of good fliers, percentage of eggs laid and eggs not hatched from Medfly produced in the Krige and the maize meal diets.

Experiment	Diet	Pupal Volume (ml)	Pupal Weight (g)	Proportion of Emergence	Proportion of Females	Proportion of Good fliers	Eggs per female	Eggs not hatched (%)
	Minimum requirements	N/A	0.8-0.9g	0.85	0.45-0.55	0.85-0.93	300-400	
	Maize meal	30.40	0.7985	0.8477	0.291	0.7600	-	-
1	Krige	14.50	0.8400	0.7860	0.359	0.7166	-	-
	Maize meal	30.50	0.7033	0.707	0.3700	0.6480	-	-
2	Krige	27.65	0.7854	0.800	0.4100	0.7405	-	-
	Maize meal	33.65	0.8270	0.7685	0.45824	0.6480	-	-
3	Krige	19.65	0.8271	0.8757	0.3038	0.7405	-	-
	Maize meal	31.00	0.7439	0.7296	0.2759	0.6740	466	29.970
4	Krige	23.87	0.7803	0.8081	0.2024	0.732	422	34.284

Table 2.4. Comparison of quality parameters of flies produced in the four experiments.

+ = most favourable value; - = least favourable value.

	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Maize meal diet	Krige diet	Maize meal diet	Krige diet	Maize meal diet	Krige diet	Maize meal diet	Krige diet
	1ml of eggs	1ml of eggs	1ml of eggs + water	1ml of eggs + water	0.7ml of eggs	0.7ml of eggs	0.6ml of eggs	1ml of eggs
Pupal Volume	+	-	+	-	+	-	+	-
Pupal Weight	-	+	-	+	+	+	-	+
Proportion of Emergence	+	-	-	+	-	+	-	+
Proportion of Females	-	+	-	+	+	-	+	-
Proportion of Good Fliers	+	-	-	+	-	+	-	+

Flight ability is important for establishing mating arenas. More good fliers were produced from the Krige diet than from the maize meal diet except experiment one. Acceptable flight ability is from 75% to 85% (Anonymous, 1998) good fliers. This was only achieved by flies produced using the Krige diet in Experiment 1 (Table 2.3).

Females reared on the maize meal diet (experiment 4) produced more eggs than those reared on the Krige diet (466 eggs/female from the maize meal and 422 eggs/female from the Krige diet over 22 day period). Of these, 29.97% from flies reared in the maize meal diet did not hatch, while 34.284% from flies reared in the Krige diet did not hatch. This difference was also significant. Therefore, female flies produced in the maize meal diet had a higher fecundity and fertility than those produced in the Krige diet.

The maize meal used in these studies would appear to be a suitable substitute for torula yeast used in Infruitec-Nietvoorbij rearing facility. This diet should produce more females using fewer eggs (Experiment 4) than is the case with the Krige diet being used in Infruitec-Nietvoorbij rearing facility. The females produced by the maize meal diet were also more fertile and fecund than those produced using the Krige diet.

The Krige and maize meal diets are able to produce Medflies that are comparable to the standard diets used worldwide (Table 2.3).

The cost of production of Medfly on artificial larval diet using the maize meal diet developed in this study could reduce rearing costs used in Infruitec-Nietvoorbij rearing facility (Table 2.5). Total cost of each diet was estimated by adding cost of the mediums calculated according to number of pupae required to produce a million of flies, eggs required to produce required number of pupae, medium to accommodate these eggs, number of females required to produce these eggs, number of pupae to produce these females and the medium to rear flies (Table 2.5). The maize meal diet was less expensive than the Krige diet, the expense was minimized by less amount of diet, eggs, pupae and females used to produce a million of flies in the maize meal diet than those in the Krige diet.

Table 2.5. Cost of producing 1000 000 flies.

	Iwisa® maize meal diet	Krige diet
1. Number of pupae required to produce 1 000 000 flies	1 495 537 pupae 27 038 ml of pupae	1 366 120 pupae 23 800 ml of pupae
2. Number of eggs required to produce required number of pupae	13 083 251 eggs 523 ml of eggs	24 926 689 eggs 997 ml of eggs
3. Amount of medium required to accommodate these eggs	1 307 kg of diet	1 495 kg of diet
4. Costs of this Medium	R 2 209	R 2 527
5. Number of eggs per female per day	22	20
6. Number of fertile eggs per female per day	15	13
7 Number of flies required to produce the eggs in (2)	872 216 females	1 917 437 females
8. Number of pupae required to produce the flies in (7)	1 301 816 pupae	2 619 450pupae
9. Cost of the medium to rear pupae in (8)	R 2 638	R 6 620
10. Total cost of diets (4+9)	R 4 847	R 9 147

CHAPTER 3

ARTIFICIAL DIETS FOR MASS REARING *CERATITIS CAPITATA* (WIEDEMANN) (DIPTERA: TEPHRITIDAE): LARGE-SCALE TEST

3.1 INTRODUCTION

In this study the performance of the artificial diet (maize meal) discussed in Chapter 2 was investigated at mass rearing levels. The quantities of ingredients used in both Krige and maize meal diets differ from those used in Chapter 2. The object was to determine the optimum amount of eggs that could be used in the maize meal diet for producing sufficient quantities of *Ceratitis capitata* (Medflies) of sufficient quality in a mass rearing facility.

3.2 MATERIALS AND METHODS

3.2.1 REARING PROCEDURES

3.2.1.1 Diet preparation

Studies were conducted at the ARC Infruitec-Nietvoorbij Research Institute facility in Stellenbosch. The ingredients and their quantities in the maize meal and Krige diet are given in Table 3.1. These differ from those given in Table 2.2 because the facility altered the diet. Therefore, it was decided to compare the newly modified Krige diet with the maize meal diet. In addition, preliminary results revealed that the maize diet used in Chapter 2 (Table 2.2) produced very poor results when mixed in bulk. This was because the diet decomposed in the large-scale tests, thus reducing production of Medfly. Therefore, the diet was also modified for the large-scale tests. The pH of both diets varied from 4.0 to 5.0 throughout the experiments.

Diets were prepared by first adding the inhibitors, sodium benzoate and concentrated HCl, to the water. The other ingredients were weighed, mixed dry and then added to the mixture of water and inhibitors in a 175-litre concrete mixer, which was operated at 20-25rpm. Table 3.1 shows the ingredients used in the two diets.

In treatment 1, 3.2 ml of eggs were placed on the Krige diet, in treatment 2, 2.0 ml of eggs were placed on the maize meal diet and in treatment 3, 3.2 ml of eggs were placed on the maize meal diet.

Table 3.1. Ingredients of a kilogram of Iwisa® maize meal and Krige diet used in the experiment.

Ingredients	Maize meal	Krige
Bran	0.2500 kg	0.2874 kg
Sugar	0.1100 kg	0.1300 kg
Maize meal	0.1200 kg	-
Torula yeast	0.0300 kg	0.0700 kg
Sodium benzoate	0.0025 kg	0.0025 kg
HCl	0.0150 l	0.0150 l
Hot tap water	0.5000 l	0.4940 l

3.2.1.2 Eggs and incubation rooms

Eggs used in the study were from adults reared in the ARC Infruitec-Nietvoorbij rearing facility on the Krige larval diet. These eggs were placed in a 6-litre plastic bottle for incubation and aerated in water at 25°C. The air was filtered to remove micro-organisms (Rico 1983). After 48 hours eggs were ready for use in the experiments. The eggs used in all treatments were placed on moist toilet paper on the surface of 5kg diet in standard trays (75 x 38 x 4 cm) used for rearing the temperature sensitive lethal (*tsl*) Medfly strain. The trays

were stacked in a trolley. The daily maximum and minimum temperature of the diet was recorded using thermometers placed on the surface of the diet.

3.2.1.3 Larval growth

Three different temperature and humidity rooms were used for larval development. The initiation stage room, where trays were kept covered with hard plastic strips for three days to maintain high humidity, was maintained at 25±1 °C and 90±10 % RH. In the maturation stage room the plastic strips were removed. The trays were kept in this room for two-three days until the larvae started accumulating on the surface of the diet. The temperature and humidity were 22±1 °C and 60±10 % RH respectively. In the larval collection room, PVC pipes cut in half lengthwise and fitted with stoppers at each end were clamped onto trays and filled with water (Chapter 1). The trays were covered with white laced netting to ensure that the larvae leaving the diet fell into the water in the PVC pipes. The temperature was maintained at 20±1 °C and 65-70 % RH.

Sometimes it was necessary to add 250 ml of water to each tray because the metabolic heat produced by the rapidly developing larvae dried and heated the diet (Hooper 1978; Nakamori & Kakinohana 1980; Tanaka *et al.* 1972). Fans were also used to dissipate metabolic heat and to prevent temperature stratification in the room. Larvae were given four days to leave the diets.

3.2.1.4 Pupation

Fully grown larvae which left the diet were collected in the PVC pipes containing water. They were removed from the water, mixed in vermiculite and allowed to pupate in standard pupal boxes (Vargas 1989) in a room maintained at 20±1 °C and 75-80 % RH. The

pupation room was kept dark to promote rapid pupation (Chapter 1). Two days later, pupae were separated from the vermiculite using a mechanical sifter. Separation of Medfly pupae after two days is important to minimise pupal injury (Chapter 1). Pupae were kept at 20±1 °C and 75-80 % RH for seven days before they were prepared for quality control.

3.2.2 QUALITY CONTROL

All the quality control tests that were used were described in Chapter 2.

3.2.3 DATA ANALYSIS

The experimental results were analysed using an analysis of variance procedure, as presented in Chapter 2. Scheffe's method of multiple comparisons was used to separate individual means or groups of means at the 5% level of significance.

3.3 RESULTS

3.3.1 Larval feeding behaviour

Most of the larval feeding occurred in the center of each tray where eggs were seeded. Mature larvae started to leave the diet on day eight in all treatments. The temperature of the diet increased as the larvae started to mature (Fig. 3.1). Temperatures in treatment 2 (maize meal diet, 2 ml eggs) were lower than in treatments 1 (Krige diet, 3.2 ml eggs) and 3 (maize meal diet, 3.2 ml eggs) (Fig. 3.1). Temperatures did not rise above 27°C, which is the ideal for the temperature sensitive strain (Fig. 3.1). The maximum temperature was 27°C, 25°C and 27°C in treatments 1, 2 and 3 respectively.

3.3.2 Pupal volume

There were differences in the volume of pupae produced from the three treatments (Fig. 3.2; $F_{2:48}$=19.4; P<0.001). More pupae were produced in treatment 1 on days two and three than in treatments 2 and 3 (Scheffe's test, P<0.05). There was no significant difference between treatments 1 and 3, while a significant difference was found between treatments 1 and 2. This discrepancy gave rise to significant interactions between the diets and the days ($F_{3:48}$=79.9; P<0.001).

3.3.3 Pupal weight

There was a difference in pupal weight between treatments (Fig. 3.3; $F_{2:48}$=14.5: P<0.001). The pupal weight in treatment 1 was higher than in other treatments (Fig. 3.3; $F_{2:48}$=14.5: P<0.001). The trend in daily production of pupae was similar in all treatments. There was a significant difference between collection days (Fig. 3.3; $F_{3:48}$=125.7; P<0.001), with a decrease in pupal weight on each successive collection day. There was no significant difference in pupal weight among treatments on the first two days, when most of the pupae were produced. There was a significant difference on days three and four (Fig. 3.3; $F_{6:48}$=6.15; P<0.001).

3.3.4 Proportion of flies emerging

The difference between treatments in the proportion of flies emerging from pupae was highly significant (Fig. 3.4; $F_{2:48}$=11.9; P<0.001). More flies emerged in treatments 1 and 3 than in treatment 2 (Fig. 3.4; $F_{2:48}$=11.9; P<0.001). However, there was no significant difference in the proportion of flies emerging between treatments on the first two collection days (Fig. 3.4; $F_{6:48}$=2.86; P=0.018). There was also a difference in proportion of flies

emerging between collection days ($F_{3:48}$=345.0; P<0.001). Emergence of flies decreased on each successive collection day.

3.3.5 Proportion of females

There were differences between treatments in the proportion of females produced (Fig. 3.5; $F_{2:48}$=5.5; P=0.007). A higher proportion of females was produced in the two maize meal diets (treatment 2 and 3) than in the Krige diet (treatment 1). There was a highly significant difference between collection days in the proportion of females produced ($F_{3:48}$=272.5; P<0.001). The proportion of females in all treatments increased on each collection day. The first collection day differed significantly from the last three days ($F_{6:48}$=21.365; P<0.001).

3.3.6 Flight ability

There was a significant difference between the three treatments in percentage of good fliers (Fig. 3.6; $F_{2:48}$=5.5; P=0.006). More good fliers were produced in treatments 1 and 3 than in treatment 2 (Fig. 3.6; $F_{2:48}$=5.5; P=0.006). There was also a difference between collection days in percentage of good fliers ($F_{3:48}$=183.4; P<0.001). The first and second collection days produced more good fliers than third and fourth. Treatments 1 and 3 produced similar trend, while treatment 2 differs to other treatments on day 2 ($F_{6:48}$=10.097; P<0.001).

3.3.7 Eggs laid

There was no difference in the number of eggs laid by adult flies reared in the three treatments (Fig. 3.7; $F_{2:168}$=0.800; P=0.450). However, there was a difference between

collection days (Fig. 3.7; $F_{13:168}$=5.7; P<0.001). More eggs were laid by flies from treatments 1 and 2 than from treatment 3 on days two to four. Similar numbers of eggs were laid on days five, six and seven in all treatments (Fig. 3.7). Flies in treatment 1 and 3 produced more eggs than flies from treatment 2 on days eight to 14.

3.3.8 Eggs not hatched

There was no significant difference in eggs that did not hatch ($F_{2:168}$=2.2; P=0.111) between the three treatments, see figure 3.8. However, there was a difference between collection days ($F_{13:168}$=3.8; P<0.001). On the first three collection days less eggs hatched than on the other collection days (Fig. 3.8).

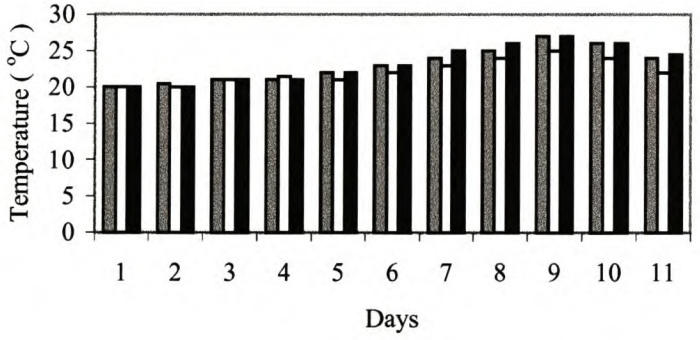

Fig. 3.1. Temperatures (°C) of the treatments during Medfly larval rearing in treatment 1 (shaded bars), treatment 2 (clear bars) and treatment 3 (black bars).

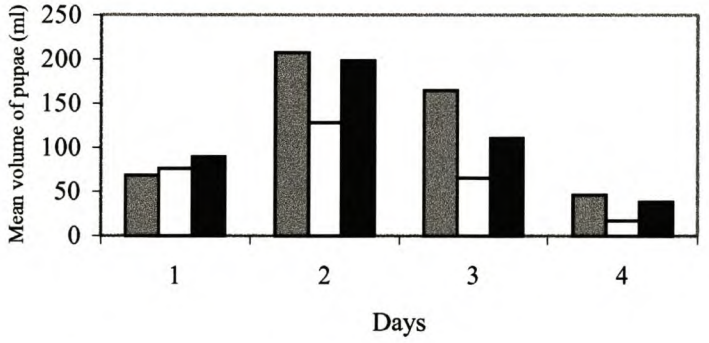

Fig. 3.2. Mean volume of pupae (ml) of Medfly larvae leaving treatment 1 (shaded bars), treatment 2 (clear bars) and treatment 3 (black bars).

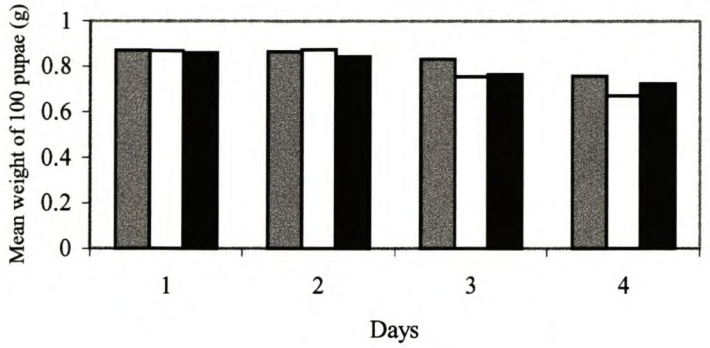

Fig. 3.3. Mean weight (g) of 100 Medfly pupae from larvae reared on treatment 1 (shaded bars), treatment 2 (clear bars) and on treatment 3 (black bars).

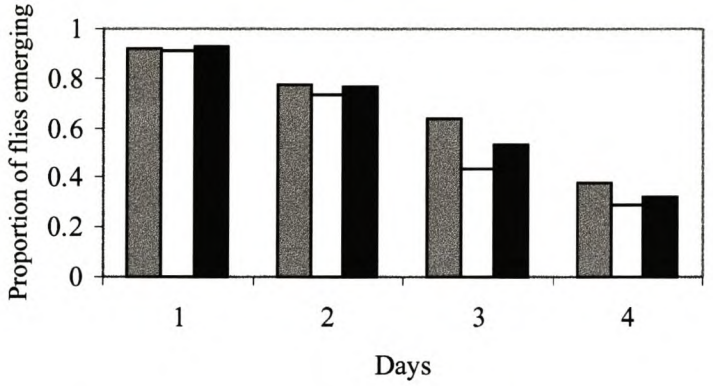

Fig. 3.4. Proportion of Medfly emerging from pupae resulting from larvae collected on four days after being reared on treatment 1 (shaded bars), treatment 2 (clear bars) and treatment 3 (black bars).

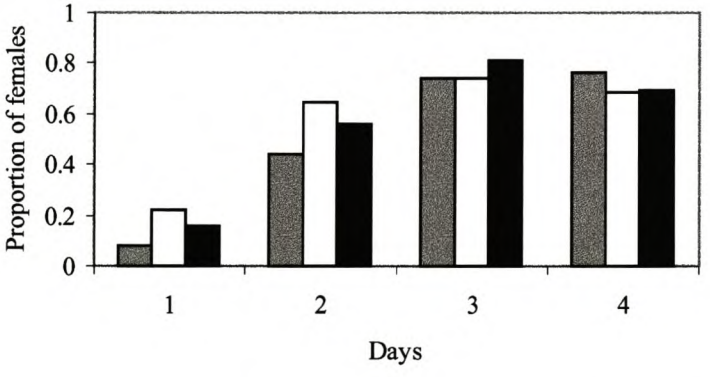

Fig. 3.5. Proportion of Medfly females produced in treatment 1 (shaded bars), treatment 2 (clear bars) and treatment 3 (black bars).

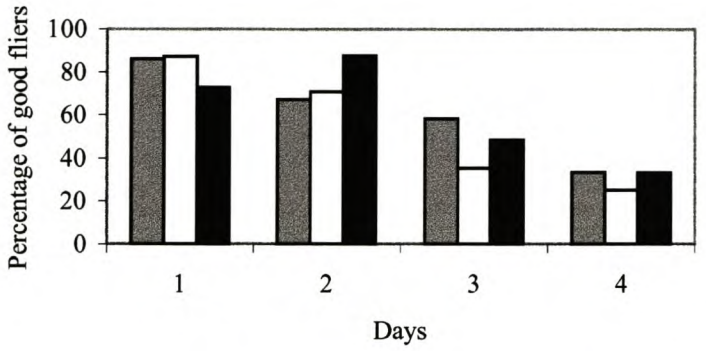

Fig. 3.6. Percentage of Medfly good fliers produced in treatment 1 (shaded bars), treatment 2 (clear bars) and treatment 3 (black bars).

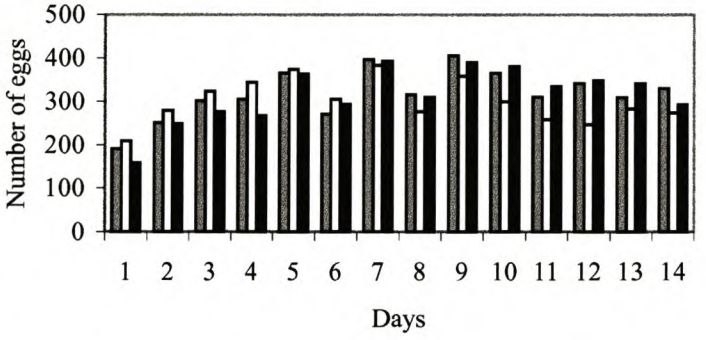

Fig. 3.7. Number of eggs laid by Medfly reared on treatment 1 (shaded bars), treatment 2 (clear bars) and treatment 3 (black bars).

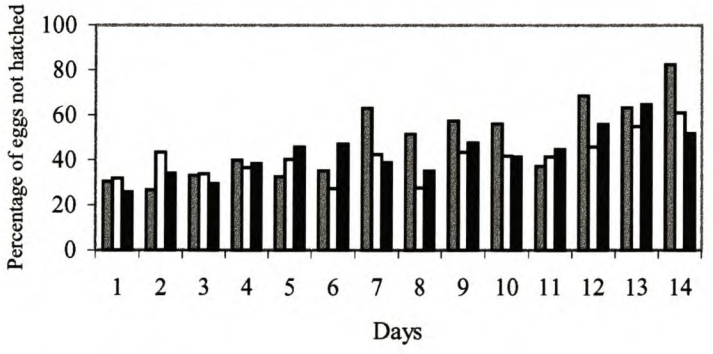

Fig. 3.8. Percentage of Medfly eggs not hatched from flies reared on treatment 1 (shaded bars), treatment 2 (clear bars) and treatment 3 (black bars).

3.4 DISCUSSION

Differences in the quality of larvae reared in treatment 1, 2 and 3 were probably due to diet temperatures. Treatment 2 produced higher proportion of females than treatments 1 and 3

(Table 3.2). Temperatures ranged from 24°C to 27°C for treatments 1 and 3, and from 22°C to 25°C in treatment 2.

A greater pupal volume was produced in treatments 1 and 3 than in treatment 2(Table 3.3), while the pattern of daily production of pupae from treatments 2 was similar to that of treatments 1 and 3 (Fig. 3.2).

The pupal weight from all treatments was of acceptable standard (0.8 to 0.9 g). Treatment 1 produced heavier pupae than other treatments, the smallest pupae were produced in treatment 2 (Table 3.2).

There was no significant difference between treatments 1 and 3 in adult emergence. Both of these treatments produced higher adult emergence than treatment 2. However, in all three treatments emergence was below acceptable standard (Table 3.2). Previous studies of Medfly diets using non-nutritive bulking agents reduced eclosion (Chan *et al.* 1990).

The percentage of females produced in treatments 2 and 3 was higher than that of treatment 1 (Table 3.2). Treatment 2 produced a higher proportion of females on day one, which was higher than the acceptable standard, while fewer eggs were used in this treatment than in the others

Flight ability is important for establishing mating arenas. More good fliers were produced from treatments 1 and 3 than those from treatment 1. None of the treatments produced an acceptable level of good fliers (Table 3.2).

Table 3.2. Mean volume of pupae produced (pupal volume), mean weight of 100 pupae (pupal weight), proportion of emerged flies, proportion of females, proportion of good fliers, percentage of eggs laid and eggs not hatched from Medfly produced in the Krige and the maize meal diets.

Treatments	Pupal Volume (ml)	Pupal Weight (g)	Proportion of Emergence	Proportion of Females	Proportion of Good fliers	Eggs per female	Eggs not hatched (%)
Minimum requirements	N/A	0.8-.0.9g	0.85	0.45-0.55	0.85-0.93	300-400	-
1	121.35	0.8318	0.676	0.506	0.614	318.88	48.44
2	71.5	0.7934	0.593	0.572	0.547	301.44	40.92
3	108.75	0.7993	0.636	0.554	0.605	314.52	43.01

Treatment 1 (Krige diet with 3.2ml of eggs seeded on 5kg diet)

Treatment 2 (Maize meal diet with 2ml of eggs seeded on 5kg diet)

Treatment 3 (Maize meal diet with 3.2ml of eggs seeded on 5kg diet)

Table 3.3. Comparison of quality parameters of flies produced in the three treatments.

+ = most favourable value; - = least favourable value.

	Treatment 1	Treatment 2	Treatment 3
Pupal Volume	+	-	+
Pupal Weight	+	-	-
Proportion of Emergence	+	-	+
Proportion of Females	-	+	+
Proportion of Good fliers	+	-	+
Eggs per female	+	-	+
Eggs not hatched	-	+	-

The number of eggs laid and eggs not hatched was similar in all treatments, suggesting that the maize meal diets (treatments 2 and 3) were as suitable for larval development as the Krige diet.

The cost of production of Medfly on artificial larval diets developed in this study has undergone no reduction as a result of diet ingredients. Treatments 1 and 3 produced almost similar quality of flies and the cost of diet was also similar (Table 3.4). The total cost of the diets was estimated using same method as in Chapter 2. Treatments 1 and 3 had similar number of eggs seeded on the diet while treatment 2 had fewer than the other treatments.

Treatment 2 was the most expensive. This was due to greater amount of diet, eggs, pupae and females used to produce a million of flies in this treatment than in other treatments.

The results of this test did not support the results of the small-scale trials in which the number of eggs used in the diet and the total costs of diet were reduced. This may have been due to the modification of the diet described in the material and methods. The maize meal diet with reduced numbers of eggs in the large-scale experiments required more diet to produce a million flies, making it more expensive. When similar amounts of eggs were used the maize meal diet appeared to be an option as the results obtained were similar to those of the Krige diet.

Table 3.4. Cost of producing 1000 000 flies.

	Krige diet with 3.2ml of eggs	Iwisa maize® meal diet with 2ml of eggs	Iwisa maize® meal diet with 3.2ml of eggs
1. Number of pupae required to produce 1 000 000 flies	1 628 664	1 828 153	1 652 892
2. Number of eggs required to produce required number of pupae	840 ml	1 016 ml	944 ml
3. Amount of medium required to accommodate these eggs	1 312 kg	2 540 kg	1 475 kg
4. Costs of this Medium	R 2 218	R 3 937	R 2 287
5. Number of eggs per female per day	22	21	22
6. Number of fertile eggs per female per day	11	12	12
7 Number of flies required to produce the eggs in (2)	1 909 406 Females	2 117 165 Females	1 967 891 Females
8. Number of pupae required to produce the flies in (7)	3 109 781	3 870 503.205	3 252 713
9. Cost of the medium to rear pupae in (8)	R 6 898	R 15 241	R 7 441
10. Total cost (4+9)	R 9 117	R 19 179	R 9 728

CHAPTER 4

4 CONCLUSIONS

An essential component of a successful sterile insect programme is consistent mass production of competitive insects for field release (Vargas *et al.* 1994). The basic requirement for successfully implementing SIT is the availability of efficient and economically viable rearing methods (Chapter 2). The quantity and quality of dietary nutrients are important for proper development of flies for SIT programmes. However, Zucoloto (1987) found that increased protein levels were the primary determinant in diet selection for larvae of the Mediterranean fruit fly, indicating that protein is a necessary component in the diet. However, proteins are expensive ingredients in the diet.

Reduction of torula yeast, the main source of protein sources in the Krige diet, was the focus of the present study and its partial replacement with maize meal.

In small-scale experiments, a diet containing maize meal and a reduced amount of torula yeast produced more females than the standard Krige diet. In addition fewer eggs could be used than in the Krige diet. The females produced on the maize meal diet were also more fertile and fecund than those produced using the Krige diet. The quality of flies reared in the maize meal diet was similar to those reared in Krige diet.

The results from large-scale trials were not the same as those from the small-scale tests due to differences in ingredients used in the trials. The maize meal diet seeded with reduced number of eggs produced lower quality flies than the maize meal diet with the standard number of eggs and the Krige diet. When the same number of eggs was used, the maize meal diet produced flies of a similar quality to those produced using the Krige diet and the costs were similar.

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