

***Agathis bishopi* (Nixon) (Hymenoptera: Braconidae): its
biology and usefulness as a biological control agent for false
codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick)
(Lepidoptera: Tortricidae), on citrus**

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Abstract

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is one of the major pests of citrus in South Africa, the others being mealybug, Mediterranean fruit fly, bollworm and some mites. Due to problems such as the expense of pesticides, insects evolving pesticide resistance (Hogsette 1999), chemical residue on the skin of export fruit and the negative impact of pesticides on the environment, it became necessary to find alternative methods for pest control (Viggiani 2000). *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae), a larval parasitoid of false codling moth known only from the Sundays River Valley area (Sishuba 2003), offers a means of control for the pest.

A total of 11 389 navel oranges were collected from various orchards in the Addo/Kirkwood area, and false codling moth larvae infested 36.09% of the fruit. A single parasitoid species, *A. bishopi*, was reared from these larvae. In 2006 the highest parasitism rate, 11.43%, was recorded in May and in 2007, the highest parasitism rate, 13.27%, was in April. *Agathis bishopi* parasitizes larvae in instars 2 and 3, possibly due to the accessibility of these younger instars to the female parasitoid and possibly due to the length of the life cycle of this koinobiont. Second instar hosts yielded the highest number of parasitoids, and there was no emergence of parasitoids from fifth instar larvae. Females of *A. bishopi* live for 18.5 days (n = 20; S.E. = 3.1) and males for 8.25 days (n = 20; S.E. = 1.23). Females produce an average of 23 offspring in a lifetime, while female false codling moths produce about 800 eggs each. A high number of parasitoids will be required per hectare to reduce the population of false codling moth. Captive rearing of *A. bishopi* proved difficult due to viral and fungal contamination. *Agathis bishopi* has potential for use in an integrated pest management programme once the hurdle of mass-rearing has been overcome.

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Declaration

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author.

Signed: _____ Date _____

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1

False codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), in citrus in South Africa

1.1. The Significance of False Codling Moth

There are approximately 8.5 million productive citrus trees in seven major growing areas of South Africa: Northern Province; Mpumalanga; North-West; Northern Cape; KwaZulu-Natal, Eastern Cape and Western Cape (Fig 1.1). The Western Cape and Eastern Cape are the areas with the most hectares under Navel orange production (Fig 1.2). Every citrus-producing region has its suite of important pests (Smith & Pena 2002). The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is regarded as one of the major pests of citrus in South Africa, the others being citrus mealybug (*Planococcus citri*), Mediterranean fruit fly (*Ceratitis capitata*), bollworm (*Helicoverpa armigera*) and some mites. It is an important pest of citrus throughout southern Africa and has been considered so for many years (Annecke & Moran 1982; Myburgh 1987). It is indigenous to southern Africa (Stofberg 1954; Carpenter *et al.* 2004) and was first reported by Fuller (1901) as a pest of citrus in KwaZulu-Natal (Gunn 1921; Quayle 1938; Stofberg 1954; Newton 1998).

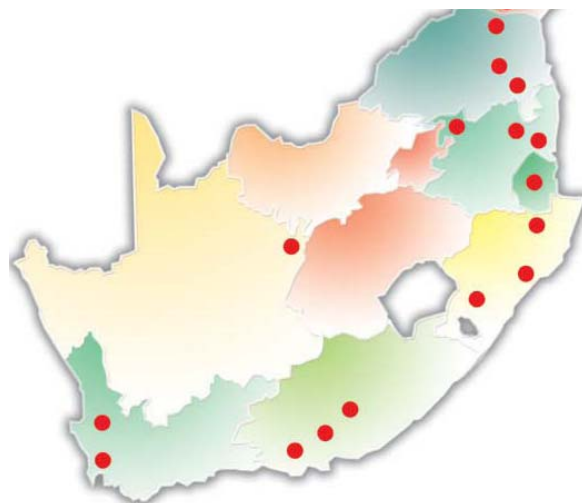


Figure 1.1: A map of South Africa showing the citrus producing areas (red dots): Western Cape, Northern Cape, Eastern Cape, Kwa-Zulu Natal, North West, Mpumalanga and the Limpopo Province (Source: CGA annual report 2007).

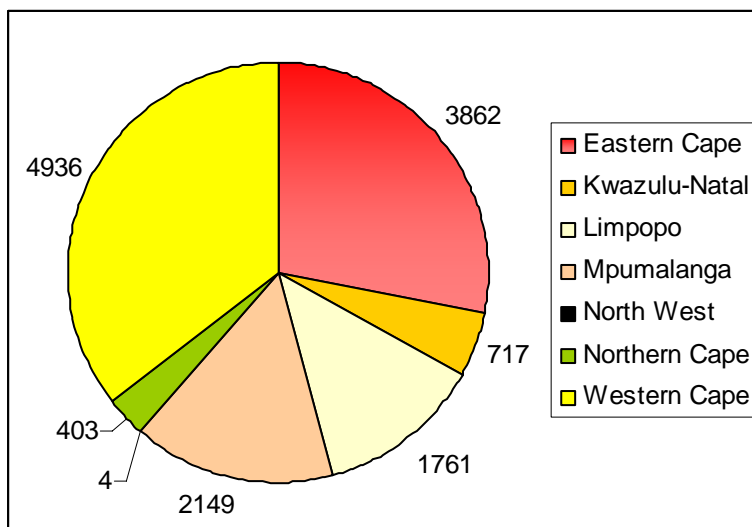


Figure 1.2: The number of hectares under Navel orange production in the seven major citrus producing areas in South Africa (Source: CGA annual report 2007).

The importance of the pest in citrus is based on its being abundant throughout the year and on what part of the tree it attacks (Smith & Pena 2002). The moths infest the fruit, causing the fruit to drop, resulting in a reduction in yield. Second, infestation usually occurs shortly before the fruit is harvested and can result in post-harvest decay of fruit. Finally, it is considered to be a phytosanitary pest; detection of the moth outside

African markets can result in a great loss of export income (Moore 2002). A phytosanitary pest is one that is found within a specific growing area (i.e. country) and not in importing areas where the fruit is also grown. Because false codling moth infests so many crops, its introduction into the USA or Europe would be a major problem to their agricultural industries (Carpenter *et al.* 2004).

1.1.1. Fruit Drop

False codling moth eggs are laid on the surface of the fruit; from experiments it seems that the stimulus to oviposit is greater in prematurely ripening fruit and even greater in physically damaged fruit (Georgala 1969; Newton 1989a). Undamaged citrus fruits in close proximity to damaged fruits were found to be more at risk of attack by false codling moth than fruits that were further away (Newton 1989a). Upon hatching, larvae penetrate the skin and enter the fruit (feeding just below the fruit surface) causing a tiny hole in the skin of the fruit. This penetration hole becomes brightly coloured and readily visible as the surrounding tissue decays and collapses (Fig 1.4). Over a period of time this area is the first to decay due to secondary infection by fungi (Georgala 1969; Newton 1998).

Decay enhances premature ripening and abscission (Newton 1989a). Navel oranges of about half-an-inch in diameter and larger are more susceptible to attack and false codling moth breeds successfully in fruits of this size. This means that it can breed successfully on Navel oranges from November till June (Stofberg 1954). Losses due to false codling moth vary depending on the area and the precise evaluation of the extent of crop damage is difficult due to varying infestations between trees, orchards and seasons (Newton 1998). In the Nelspruit area it is around 20-30% of the total fruit drop and in the Citrusdal area it has been up to 90% (Newton 1998).



Figure 1.3: Decay and collapse of the tissue around the penetration hole (A) caused by the false codling moth larvae. (Photo: K.L. Gendall).

1.1.2. *Post-harvest decay*

Fruit that is damaged close to harvest time can be packed with fruit that is undamaged as it takes time for the penetration hole to become discoloured and visible (Georgala 1969). When the fruit is packed they release heat due to respiration. This creates an environment which is conducive to pathogens and the penetration hole caused by the false codling moth larvae allows the pathogens to enter the fruit. This causes the fruit to decay on the way to the export market where it is rejected and results in a loss of income. By reducing the population of false codling moth, this problem can be reduced (Georgala 1969; Mekbib *et al.* 2006).

1.1.3. *Phytosanitation*

The South African citrus industry has expanded as overseas outlets for the fruit, mainly in Western Europe and the Middle East, have been developed and exploited. In the 1970s, 1980s and 1997-2001, South Africa exported the most citrus of all countries in

the Southern Hemisphere (Fig 1.3). The major cultivars of sweet orange, *Citrus sinensis*, grown in South Africa are Valencia oranges and Naval oranges (CGA annual report 2007). The climates of the different regions of the country where citrus is produced allow for the range of cultivars to be produced (Mather 1999). In the warmer areas i.e. Limpopo, the fruit matures earlier compared to the cooler areas such as the Western Cape. Due to this South Africa is able to provide the overseas market with a steady supply of citrus all year (Mather & Greenberg 2003).

In South Africa the export period is from April to October. At the start of the export period, Argentina, Chile and Australia are the main rivals and during the last few months Israel, Spain, Egypt and the USA are the main rivals (Mabiletsa 2003). When looking at the world market, South Africa is the second largest orange exporter (Sean Moore pers. comm.). In 2007 the number of exported orange cartons amounted to 49.3 million 15kg cartons (Chadwick 2007). The fruit which fails to comply with export regulations, i.e. poor quality or contaminated fruit, is distributed into the local market (Urquhart 1999).

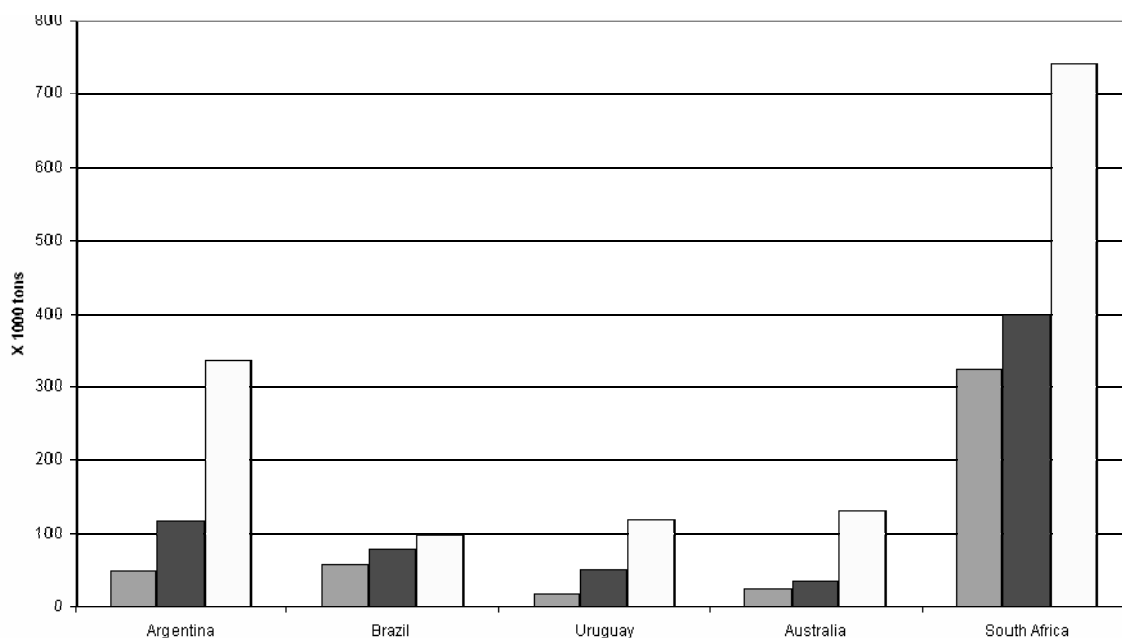


Figure 1.4: Recent Southern Hemisphere fresh citrus exports (Source: www.cga.co.za).

The grey columns represent exports in the 1970s, the black columns represent exports for the 1980s and the white columns represent exports for 1997 – 2001.

1.2. The Biology of False Codling Moth

1.2.1. Taxonomy

False codling moth derived its English name from the resemblance of its infestation symptoms to those of the true codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) due to the adult's habits of depositing eggs singly on or near fruit and the larvae feeding singly on the flesh of fruits (Reed 1974; Annecke & Moran 1982). It was first described by Meyrick (1912) as *Argyroploce leucotreta* (Lepidoptera: Tortricidae). Later, Clark (1958) reassigned it to *Cryptophlebia* Walsingham (1899) (Newton 1998; Razowski 2000), and in 1999, Komai placed it in the genus *Thaumatotibia* Zacher (1915). The latter genus was not originally placed in a family when established, and was later placed as a synonym of *Cryptophlebia*. *Thaumatotibia* and *Cryptophlebia* are similar to each other and there are five characters common to both genera; (1) they have a broad forewing with a blackish triangular pretornal patch with an accessory cell of chorda absent or small; (2) the hind wing has a short discal cell; (3) the eighth tergite has a small patch of long scales; (4) the valva has a patch of long, curled scales on the outer surface of the cucullus and (5) the tenth abdominal segment of the pupa has a pair of strong spines along the anal rise (Stibick 2006). Three characters distinguish *Thaumatotibia* from *Cryptophlebia*: (1) the eighth tergite in males has a broadly sclerotized plate with a convex posterior margin and is laterally produced into curved points; (2) the sterigma is indicated by an ovate or rectangular sclerite connecting posteriorly with a pair of ovate granulations with modified scales; and (3) the corpus bursa has a granular patch at the juncture of the ductus bursae (Venette *et al.* 2003).

In the past, identification of *Thaumatotibia* and *Cryptophlebia* has been restricted to the morphological features of the adults. A recent study by Timm *et al.* (2007) used the technique of DNA markers to study the final instar larvae and pupae of *T. leucotreta* (Meyrick), *T. batrachopa* (Meyrick) and *C. peltastica* (Meyrick). The aim of the study was to use the information to draw up a key for the identification of these species. All three of these species are of economic importance, therefore correct identification is

important. With the use of both morphological and molecular key the identification of species becomes more reliable (Timm *et al.* 2007).

1.2.2. Life History

False codling moth has a holometabolous development. This means that the moth has a complete metamorphosis where the immature stages i.e. larvae and pupae, differ in appearance from the adults (De Villiers 1996). The life stages of the moth occupy different microhabitats, so it is possible to control this pest by using the natural enemies which attack each of the life stages. There are about 5-6 generations of false codling moth per year under favourable conditions and 2-3 generations per year under unfavourable conditions (Daiber 1980).

Egg: The egg is oval, creamy white and translucent with a diameter of about 1mm. As it matures it changes to red and then black when the larva is about to emerge. It is laid singly on the upper surface of leaves or in indentations on the rind (Newton 1989a). The incubation period is 6 to 12 days (Gunn 1921; Hepburn 1947; Georgala 1969; Daiber 1979a; Newton 1998).

Larvae: There are five larval instars and each can be determined by measuring the width of the head capsule (Stofberg 1954; Daiber 1979b). The first instar is white-yellow with a distinct black head, 2-3mm long and the width of the head capsule is about 0.21mm. Mature 5th instar larvae are dark pink on the upper side and creamy below (Gunn 1921; Hepburn 1947; Stofberg 1954; Daiber 1979b), about 15 – 20mm long and the width of the head capsule is about 1.37mm.

These larvae leave the fruit, and drop to the soil to pupate; sometimes the fruit has already fallen to the ground (Daiber 1979c; Newton 1998). The rate of larval development is correlated with temperature. The lower the temperature, the slower the development, with 25°C being the favourable temperature for growth (Daiber 1979b).

Pupae: The fifth instar larvae leave the fruit and drop to the ground, and spin a cocoon using a silky substance secreted from the body, which is used to combine soil particles together. The pre-pupa moults into the pupa and is a creamy colour and soft-

skinned. The pupa forms within 2 weeks once the pre-pupal stage has started. It becomes dark brown due to the hardening of the chitin (Gunn 1921; Daiber 1979c; Newton 1998). Temperature plays an important role in the duration and survival of the pre-pupal and pupal stages (Daiber 1979c).

Adult: The adult moth is small and dark brown to grey. The forewings are mottled while the hind-wings are paler, more evenly coloured and fringed with hairs (Fig 1.5).



Figure 1.5: An adult false codling moth. (Source: www.citrusres.com).

Males are smaller than females and can be distinguished by densely packed, elongated scales on the hind tibia, an anal tuft of scales, and a scent organ near the anal angle of each hind-wing (Gunn 1921; Hepburn 1947; Stofberg 1954; Georgala 1969; Daiber 1980; Newton 1998). Females have a longer life-span compared to the males. The moths live for approximately 2-3 weeks. The pre-oviposition period is 1-2 days with the female moths carrying approximately 100 eggs. At a constant temperature of 25°C, a

single female moth can lay 57.6 eggs in a day. This was noted two days after the emergence of the moths. After this laying declined fairly rapidly (Daiber 1980).

1.2.3. *Host Plants and Distribution*

The false codling moth occurs throughout Africa south of the Sahara (Fig 1.6) (Annecke & Moran 1982), but apparently does not occur in other countries or areas i.e. Botswana, Guinea, Egypt or Europe, which are in close proximity to the countries where false codling moth is present. It is unclear why, but it is possibly due to climate conditions and vegetation i.e. there may only be a few host plants in these areas. Another possible reason could be due to sampling artifacts (Villet pers. comm. 2007).

It attacks a wide range of cultivated crops and indigenous host plants (Table 1.1). It is particularly severe on citrus but also attacks deciduous, subtropical and tropical fruits. Of the citrus, the Navel orange cultivar is the most severely attacked (Newton 1998). The moths may use wild plants growing around orchards as substitute areas to breed when the orchards have been sprayed with chemicals or when there is no fruit (Kirkman pers. comm. 2007).

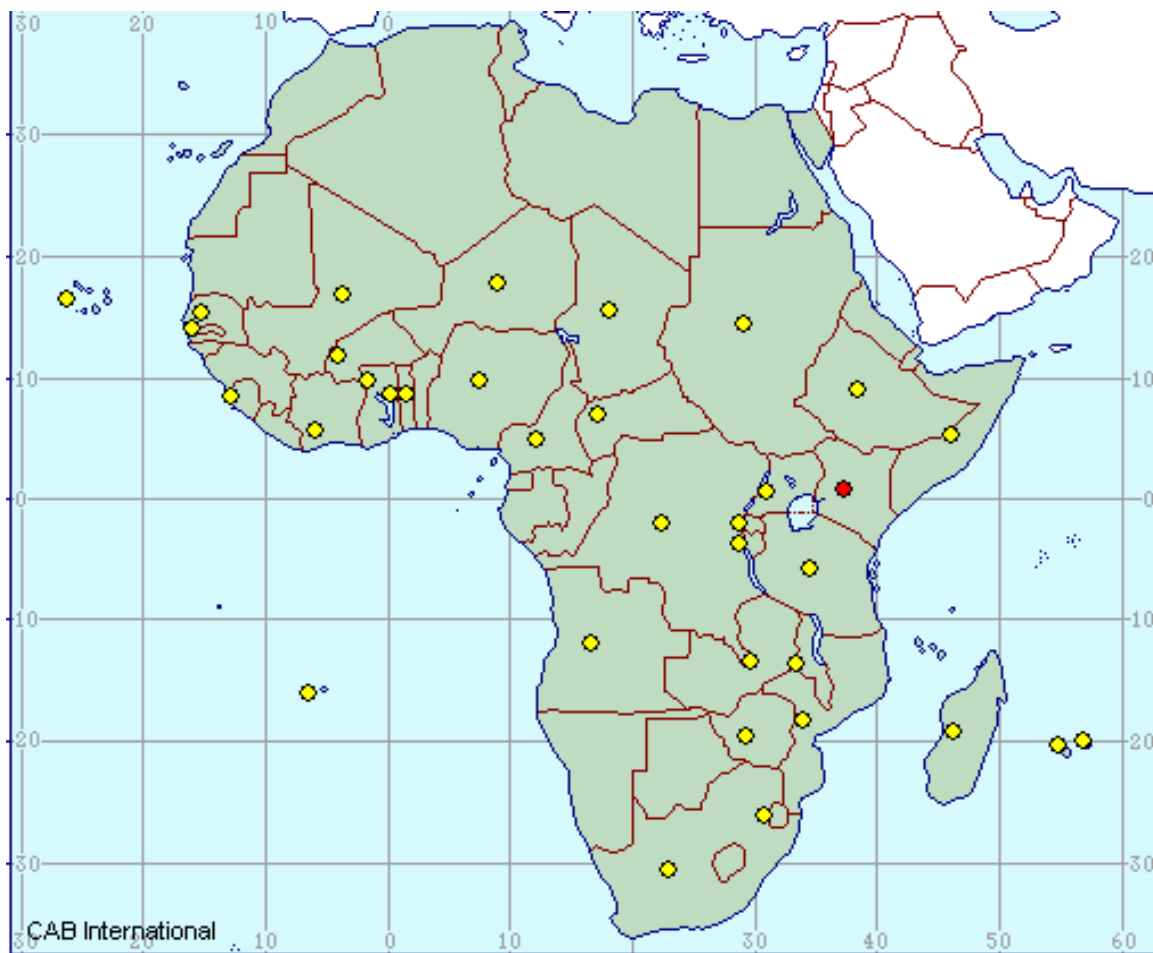


Figure 1.6: The distribution of false codling moth in Africa. The yellow markers indicate the presence of the moth and the red marker indicates that it is widespread in that area. (Source: Stibick 2006).

Table 1.1: Cultivated (in bold) and wild plants which have been recorded as hosts for false codling moth, which attacks the fruit of the plant (Gunn 1921; Hepburn 1947; Stofberg 1954; Pinhey 1985; Schwartz 1981; Venette 2003; Kirkman & Moore 2007).

Common Name	Species name	Family name
Apricot	<i>Prunus armeniciata</i>	Rosaceae
Avocado	<i>Persea americana</i>	Lauraceae
Banana	<i>Musa paradisiacal</i>	Musaceae
Bean	<i>Phaseolus spp.</i>	Fabaceae
Bloubos	<i>Royena pallens</i>	Ebanaceae
Bluebush	<i>Diospyros lycoides</i>	Ebanaceae
Boerboon	<i>Schotia afra</i>	Caesalpiniaceae
Buffalo thorn	<i>Zizyphus mucronata</i>	Rhamnaceae
Bur weed	<i>Triumfeta spp.</i>	Tiliaceae
Cacao	<i>Theobroma cacao</i>	Sterculiaceae
Carambola	<i>Averrhoa carambola</i>	Oxalidaceae
Castor bean	<i>Ricinus communis</i>	Euphorbiaceae
Chayote	<i>Sechium edule</i>	Cucurbitaceae
Citrus	<i>Citrus sinensis, Citrus spp.</i>	Rutaceae
Coffee	<i>Coffea arabica, Coffea spp.</i>	Rubiaceae
Cola	<i>Cola nitida</i>	Malvaceae
Corn	<i>Zea mays</i>	Poaceae
Cotton	<i>Gossypium hirsutum</i>	Malvaceae
Cowpea	<i>Vigna unguiculata, Vigna spp.</i>	Fabaceae
Custard apple	<i>Annona reticulata</i>	Annonaceae
Elephant grass	<i>Pennisetum purpureum</i>	Poaceae
English walnut	<i>Juglans regia</i>	Juglandaceae
Governor's plum	<i>Flacourtia indica</i>	Flacourtiaceae
Grape	<i>Vitis spp.</i>	Vitaceae
Guava	<i>Psidium guyajava</i>	Myrtaceae
Indian mallow	<i>Abutilon hybridium</i>	Malvaceae

Common Name	Species name	Family name
Jakkalsbessie	<i>Diospyros mespiliformis</i>	Ebenaceae
Jujube	<i>Zizyphus jujube</i>	Rhamnaceae
Jute	<i>Abutilon spp.</i>	Tiliaceae
Kapok / copal	<i>Ceiba pentranda</i>	Malvaceae
Kei apple	<i>Dovyalis caffra</i>	Salicaceae
Khat	<i>Catha edulis</i>	Celastraceae
Kudu berry	<i>Psuedolachnostylis maprouneifolia</i>	Phyllanthaceae
Lima bean	<i>Phaseolus lunatus</i>	Fabaceae
Litchi	<i>Litchi chinensis</i>	Sapindaceae
Loquat	<i>Eriobotrya japonica</i>	Rosaceae
Macadamia nut	<i>Macadamia ternifolia</i>	Proteaceae
Mallow	<i>Hibiscus spp.</i>	Malvaceae
Mango	<i>Mangifera indica</i>	Anacardiaceae
Mangosteen	<i>Garcinia mangostana</i>	Clusiaceae
Marula	<i>Sclerocarya birrea ssp. caffra</i>	Anacardiaceae
Monkey pod	<i>Cassia petersiana</i>	Fabaceae
Oak	<i>Quercus spp.</i>	Fagaceae
Okra	<i>Ablemoschus esculentus</i>	Malvaceae
Olive	<i>Olea europaea subsp. europaea</i>	Oleaceae
Peacock flower	<i>Caesalpinia pulcherrima</i>	Fabaceae
Pepper/pimento	<i>Capsicum spp.</i>	Solanaceae
Persimmon	<i>Diospyros spp.</i>	Ebanaceae
Pineapple	<i>Ananas comosus</i>	Bromeliaceae
Plum	<i>Prunus spp.</i>	Rosaceae
Pomegranate	<i>Punica granatum</i>	Lythraceae
Pride of De Kaap	<i>Bauhinia galpini</i>	Fabaceae
Raasblaar	<i>Combretum zeyheri</i>	Combretaceae
Red milkwood	<i>Mimusops zeyheri</i>	Sapotaceae
Rooibos / Bushwillow	<i>Combretum apiculatum</i>	Combretaceae
Sida	<i>Sida spp.</i>	Malvaceae

Common Name	Species name	Family name
Snot apple	<i>Azanza garckeana</i>	Malvaceae
Sodom apple	<i>Calotropis procera</i>	Apocynaceae
Sorghum	<i>Sorghum spp.</i>	Poaceae
Soursop	<i>Annona muricata</i>	Annonaceae
Stemfruit	<i>Englerophytum magaliesmontanum</i>	Sapotaceae
Surinam cherry	<i>Eugenia uniflora</i>	Myrtaceae
Suurpruim / large sour plum	<i>Ximenia caffra</i>	Olacaceae
Tea	<i>Camellia sinensis</i>	Theaceae
Wag'n'bietjie	<i>Capparis tomentosa</i>	Capparaceae
Waterbessie	<i>Syzygium cordatum</i>	Myrtaceae
Weeping boerboon	<i>Schotia brachypetala</i>	Fabaceae
Wild fig	<i>Ficus capensis</i>	Moraceae
Wild medlar	<i>Vangueria infausta</i>	Rubiaceae
Wild plum	<i>Harpephyllum caffum</i>	Anacardiaceae
Wing bean	<i>Xeroderris stuhlmannii</i>	Fabaceae
Yellow-wood	<i>Podocarpus latifolius</i>	Podocarpaceae
Yellow-wood	<i>Podocarpus falcatus</i>	Podocarpaceae

1.3. Control of False Codling Moth

Due to false codling moth having a catholic choice of plants, it occurs all year round and has become a major pest, especially of citrus. Due to its biology and larval behaviour, it is difficult to control (Catling & Aschenborn 1974; Newton 1998). Various experiments have been carried out for the control this pest (Hepburn 1947). These involve orchard sanitation, insecticides and biological control.

1.3.1. Orchard Sanitation

This involves the regular collection and destruction of infested fruit on the trees and the ground. One can destroy the infested fruit in the following ways (Hepburn 1947; Stofberg 1954).

Drowning: This involves placing drums half-filled with water around the orchard. The infested fruit is then placed into the drum and a lid placed on top. Once the fruit has been submerged in the water for a week one can empty the drums, but not near the trees as the products of fermentation may affect the tree through its roots (Newton 1998).

Burying: A pit can be dug big enough to hold a week's collection of fallen fruit, covered with soil and compacted. One should not dig a pit that will take a while to fill, because larvae from the first lot of fruit will have time to escape and breed (Hepburn 1947; Georgala 1969; Newton 1998). Each farmer has their own pit(s) and the number depends upon the amount of infested fruit. This can vary from week to week (Kirkman pers. comm. 2007).

Cutting and pulping: This involves collecting the fruit and cutting it up into tiny pieces and then crushing the fruit, thereby crushing the larvae (Georgala 1969; Newton 1998). This is usually the most common method used by the farmers. Fruit are collected weekly, if there is high infestation of the pest then fruit are collected twice a week, and cut up using a hammer mill. The crushed fruit is then often used as compost (Kirkman pers. comm. 2007).

In the Eastern Cape it was found that sanitation reduced the total weekly loss of fruit from 6.1% to 3.3% (Newton 1998). Sanitation also reduced larval populations by up to 40%, but this only to a small reduction in crop loss from 7.6% to about 6.0%. Sanitation methods can be expensive and labour-intensive as it involves the continuous removal of fruit from the trees and the floor of the orchard at least once a week. Sanitation also cannot guarantee satisfactory results from season to season (Newton 1998).

Another sanitation measure is the removal of native host plants around (\pm 50m) the orchard. This includes wild fruiting trees (Table 1.1) that grow around the orchard area (Gunn 1921). This is not environmentally responsible; therefore there are regulations and guidelines in place to protect the indigenous plants. When looking for an area to start an orchard it would be advisable to look around the area for possible host plants and therefore choose a site where there are less host plants (Kirkman pers. comm. 2007).

1.3.2. *Chemical Control*

A wide range of insecticides have been used for the control of false codling moth. Chemical control has proven difficult due to inaccessibility of the burrowing life stages, development of insecticide resistance, incompatibility with integrated pest management (IPM) and integrated crop management, and its expense (Newton 1998). The chemicals also have a non-target effect, therefore killing beneficial insects along with the pest. This also effects the environment as the spray is not species-specific, therefore it can lead to a decrease in biodiversity. The chemical can also get into water systems and persist for a length of time, resulting in the death of many other animals and human health risks. The use of chemicals is also problematic because of the chemical leaving a residue on the skin of the fruit and residue inside the flesh of the fruit by systemic pesticides (Moore pers. Comm.). The residue on the skin of the fruit is problematic as it is a health risk to humans. It is uneconomical due to the rising costs of the pesticides and the number of times spraying is required (Hepburn 1947; Catling and Aschenborn 1974; Newton 1998; Moore 1999). Chitin synthesis inhibitors have proven useful as they are more specific in their mode of action and they can be used in conjunction with biological control programmes for increased control (Newton 1998). However, it has been found that false codling moth has developed resistance to the chitin synthesis inhibitor, triflumuron (Hofmeyr & Pringle 1998).

1.3.3. *Biological Control*

The success of the biological control programme depends upon the type of natural enemy being exploited. The success of the agent depends upon the biological and population properties of the agent (Strand & Obrycki 1996; Van Driesche & Bellows 1996). Many options have been tried for the natural control of false codling moth in citrus orchards. These include parasitoids, entomopathogens, the sterile insect technique (Bloem *et al.* 2003; Carpenter *et al.* 2004), predators, trapping (Newton & Mastro 1989) and cover crops.

Parasitoids: These are arthropods that are able to kill their host while still completing their development (Eggleton & Belshaw 1992; Van Driesche & Bellows

1996). These agents have been the most commonly utilized natural enemy for the control of insects; especially crop pests (Van Driesche & Bellows 1996; Mattiacci *et al.* 1999). Most of these agents belong to the insect orders Hymenoptera and Diptera (Varley *et al.* 1973). In the Hymenoptera the families Braconidae and Ichneumonidae have had the most species used as biocontrol agents as they are the species most regularly found parasitizing insect pests (Greathead 1986; Van Driesche & Bellows 1996). Four egg parasitoids and two larval parasitoids have been identified as having potential as biological control agents (Moore 1999). Compared to larval and pupal parasitoids, egg parasitoids should be considered better, because if they are effective, they can rapidly reduce the occurrence of commercial damage in the same way as insecticides; whereas the larval and pupal parasitoids will control the pest over time but attack the pest only once the damage has been done (Newton 1998; Moore 1999). However, both egg and larval parasitoids are said to be ineffective in that they are often poorly distributed and are therefore not always present in areas where the pest occurs (Moore 1999).

Parasitic Hymenoptera are the most abundant natural enemies of fruit pests. Many parasitoids are native species and they vary in the host stages which they attack. In citrus orchards, citrus whitefly (*Dialeurodes citri* Ashmead), bayberry whitefly (*Parabemisia myricae* Kuwana) and citrus woolly whitefly (*Aleurothrixus floccosus* Maskell) have all been brought under control by *Encarsia lahorensis* Howard, *Eremocerus* spp. and *Encarsia* spp., respectively (Viggiani 2000). The trichogrammatid egg parasitoids have shown to have some effect. Schwartz (1981) released *Trichogrammatoidea cryptophlebia* (Nagaraja) in the Citrusdal area early in the citrus season, when there was an outbreak of false codling moth in the area, and, with orchard sanitation, there was a decrease in the false codling moth population. This resulted in a programme of mass-rearing and release of *T. cryptophlebia* (Newton 1998). There have been variable control effects in Citrusdal, Nelspruit and the Western Cape, and this could be attributed to the spread of the parasitoid in a citrus orchard (Newton 1998; Moore 1999).

The larval parasitoids recorded appear to be an important mortality factor. There are only two common larval parasitoids, an *Agathis* sp. and the ichneumonid *Glypta leucotreta*

(Wlkn.) (Commonwealth Institute of Biological Control 1984). *Agathis bishopi* is a larval-pupal parasitoid of the false codling moth (Sishuba 2003). This means that *A. bishopi* oviposits in the larval stage of the moth and emerges from the pupal stage. *Agathis bishopi* is a solitary endoparasitoid (Sishuba 2003), which means that it inserts its egg into a host and a single parasitoid develops there. Parasitoids are also classified by how parasitism affects their hosts' physiology. *Agathis bishopi* is classified as a koinobiont as it develops inside the living and mobile host and benefits by the continued feeding of the host (Van Driesche and Bellows 1996). The members of the genus *Agathis* have the following characteristics: simple foreclaw or a foreclaw with a square or round lobe; the forewing vein (RS + M)_a is mostly absent; the notauli are present anteriorly; the frons lacks a carina; there is a strong transverse carina on the mesosoma between the hind coxal insertions and the metasoma insertion is absent; the mandible is dorsoventrally flattened or hidden by the labrum and the pegs at the apex of the hind tibia are thick and conical; the face is elongate; and the third labial palpomere is more than half the length of the fourth (Sharkey 2004).

Agathis bishopi was originally described as *Microdus bishopi* from the Eastern Cape. It closely resembles *Agathis leucotretae* (Nixon) from Zimbabwe, which also parasitises false codling moth. The two species are similar in colour but the notaulal furrows on the mesoscutum are absent in *A. leucotretae*. *Agathis bishopi* is uniformly brownish yellow in colour with the female (Fig 1.7) having a protruding ovipositor and they are 4-5mm in length (excluding the ovipositor). The antennae are long and slender with many segments. Besides not having ovipositors, males (Fig 1.7) can be distinguished from females as they have black markings on their head, thorax, abdomen and legs (Prinsloo 1984).

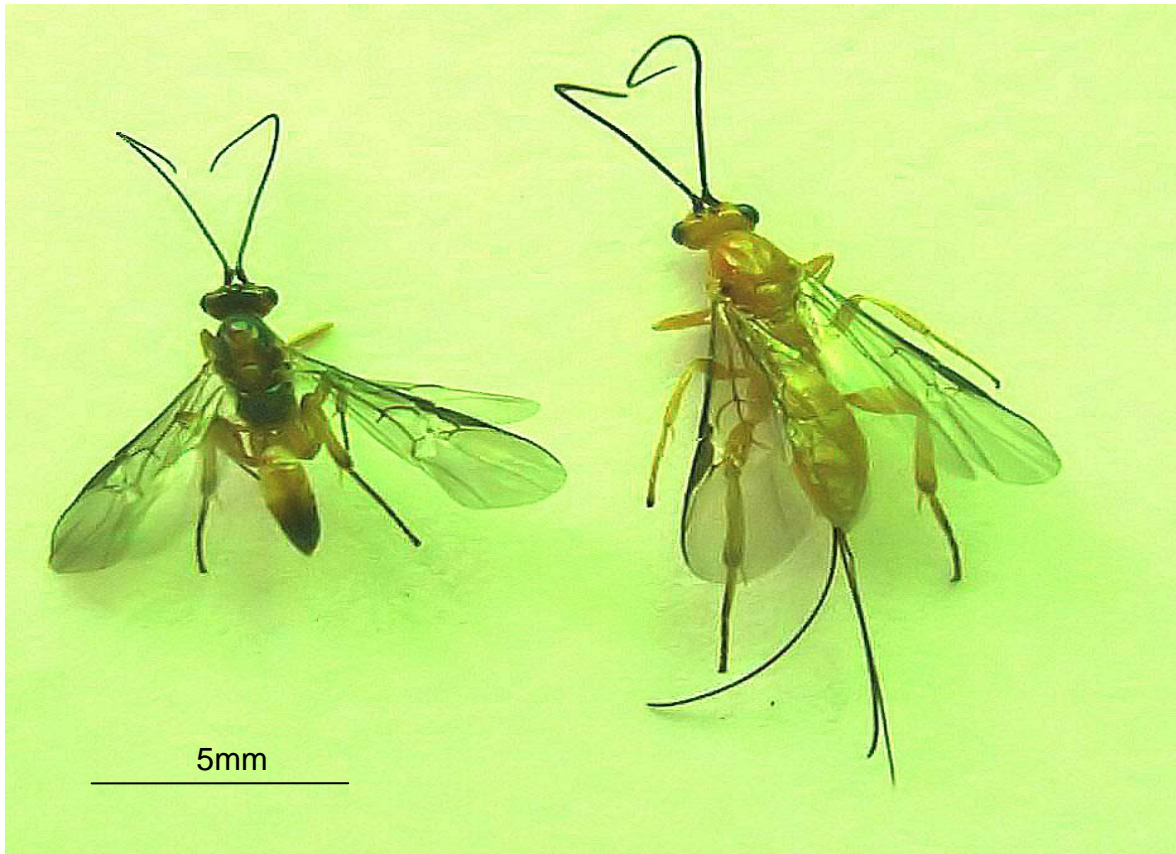


Figure 1.7: Male (left) and female (right) *Agathis bishopi* parasitoids. The black markings can be seen on the thorax and abdomen of the male. The female parasitoid is larger in size and has a protruding ovipositor.

Pathogens: Entomopathogens are naturally occurring and ubiquitous (Van Den Bosch 1971; Lacey *et al.* 2001), therefore they are safe to use in the environment. They are target-specific, so they have no effect on the non-target species and there is no residual effect on the skin of the fruit (Lacey *et al.* 2001). Of the entomopathogens, the bacteria have been the most successful for commercial use. Three species of *Bacillus* are currently being used to control several pests. Bacteria are generally easy to produce on a commercial scale as they can be grown in fermentation media. Entomopathogenic viruses are an alternative method for control of false codling moth (Newton 1989b, 1998). Insect viruses have an advantage over chemical pesticides because they are target-specific and naturally occurring and therefore do not affect the food chain; except that it will deprive

parasitoids of hosts. Viruses in the family Baculoviridae have been the main focus for commercial use. These viruses are known to be lethal and have narrow host ranges; therefore there is no non-target affect (Van Driesche & Bellows 1996; Shapiro 1995; Behle *et al.* 2003). The *Cryptophlebia leucotreta* granulovirus (CrleGV) (Baculoviridae) is used for the control of false codling moth (Moore 2002). The virus particle infects the intestinal cells, spreading throughout the insect and causing appetite loss, morbidity, flaccidity and death (Hendry 2002). The virus is currently being commercially produced in the Sundays River Valley area at River Bioscience under the name 'Cryptogran™'. Four problems need to be overcome for the effective use of pathogens. One of the problems is the speed at which they work. They generally take a while before they kill the pest, therefore in some cases allowing the pest to cause damage before killing it. The virulence of the pathogen and the time it takes to be effective is important. The other problems are formulation, application and persistence in the field of the pathogen (Lacey *et al.* 2001; Behle *et al.* 2003). The main problem posed by persistence in the field as the exposure of the virus to sunlight greatly reduces the effect of the virus (Huber 1990; Shapiro 1995; Behle *et al.* 2003).

Predators: These agents are ubiquitous among the insects and are especially important for biological control in agriculture and forestry. This group also includes vertebrate predators such as birds, lizards and small mammals. Birds and mammals are not really introduced for the control of pests but the indigenous species are known to have some effect on insect pests (Van Driesche & Bellows 1996). These predators are seen as generalists and are therefore not seen as highly effective biological control agents. A problem with generalist predators includes the competition between them as well as predator avoidance behaviour by the pest. A way in which to use the predators in an indirect way is to use the habitat surrounding the field to encourage predators to the field, thereby acquiring some control from the predators on the insect pests (Snyder & Wise 1999).

Bownes (2002) conducted a study on the use of ants for controlling crop pests. Ants can be effective predators and the predacious ants can be used to control crop pests. The

brown house ant, *Pheidole megacephala* (Fabricius), and the pugnacious ant, *Anoplolepis custodiens* (Smith), were found to prey upon pupae of bollworm, false codling moth, fruit flies and citrus thrips. In the study she found that in orchards where the ants were being poisoned there was a higher number of surviving pupae compared to orchards where the ants were active. Ants though also need to be controlled as they can cause pest outbreaks of aphids, scale insects and psyllids. This can be done by using ant bands and preventing the ants from climbing to the top of the trees, while still allowing them to forage on the pupae in the soil (Bownes 2002).

Sterile insect releases: This method involves the mass-rearing and sterilization of the pest. The males are sterilized by gamma radiation. High numbers of sterile males are released into orchards to mate, thereby transferring sterile sperm and reducing the pest population, especially if females mate only once (Dickler *et al.* 2000). The sterile insect technique has been shown to reduce false codling moth populations on navel oranges by up to 50%, but this happened only in the Nelspruit area (Newton 1998).

Trapping: The female false codling moth sex pheromone has been used to trap males for both monitoring and control purposes (Commonwealth Institute of Biological Control 1984). Different types of trap structures have also been evaluated. The wing trap and the delta-type trap have both shown to consistently attract male false codling moths. Both these traps can be used on a large-scale monitoring system (Newton 1998).

Cover crops: Another method is the use of cover crops to manage pests. This involves using resident vegetation, indigenous species and seeded cover crops to provide resources for predators and parasites of generalist pests (Bugg & Waddington 1994).

1.4. Research objective

Insects have become major pests in agriculture due to the monoculture of crops as this allows for resources to be present in abundance all year round (Waage & Mills 1982). Chemical control of crop pests like false codling moth has proven problematic due

to the evolution of pesticide resistance (Hogsette 1999) and residue accumulation in and on the fruits (Newton 1998). Due to the adverse effects of chemicals and insecticides on the environment (Bautista *et al.* 1999) it has become necessary to find alternative ways of controlling crop pests (Greany *et al.* 1984; Urquhart 1999). Resistance to insecticides and stringent export regulations (du Toit 1996) has also led to the need for alternative methods of pest control. Biological control offers a safe and effective method of controlling crop pests (Johns and Whitehouse 2004). In the Eastern Cape, red scale, *Aonidiella aurantii* (Mask.) (Hemiptera: Diaspididae) is a major pest. By augmentative release of thousands of parasitoids, the main species being *Aphytis lingnanensi-coheni*, there was successful control of the pest (du Toit 1996). Parasitoids have been identified as potential biological control agents (Johns and Whitehouse 2004). The larval parasitoid *A. bishopi* is abundant in the Sundays River Valley area (Sishuba 2003), and offers a means of control for false codling moth. Some control is happening on a small scale, but because the parasitoid is indigenous to South Africa; it therefore has its own suite of natural enemies keeping its numbers low.

Sishuba (2003) found that a braconid larval parasitoid, *A. bishopi*, had some promise as a biological control agent. The objective of this study is to examine the usefulness of this agent as a biological control agent and to develop means to mass-rear it for release in citrus orchards in South Africa. This involved collecting the parasitoid from the environment, inventing a mass-rearing technique and then establishing the parasitoid's biology in captivity.

2

Collection of the parasitoid *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae) in the Sundays River Valley

2.1. Introduction

Parasitoids have been known to be important in attempts to control insect pests, especially on crops (Eggleton & Belshaw 1992). Parasitoids aid in control of pest populations as they develop on (ectoparasitoids) or inside (endoparasitoids) the host; thereby killing the host (Strand 1986; Elzinga *et al.* 2002). Essentially no work has been done on the biology of parasitoids that might control *Thaumatotibia leucotreta*, so this chapter reports on the abundance of *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae) and the affects it had on the false codling moth population through the 2006 and 2007 seasons.

2.2 Materials and Methods

2.2.1. Fruit collection

To investigate parasitism of *Agathis bishopi* in the environment; fruits were collected from various orchards on farms in the Sundays River Valley area (Table 2.1) on a weekly basis from January 2006 till the first week of June 2006 and in the same period in 2007.

Table 2.1. Fruit collections were carried out in various orchards on different farms in the Sundays River Valley area.

Orchard	Farm	Area	Province	Variety	Tree age yrs	Co-ordinates
2	Killian	Kirkwood	E. Cape	Lane navels	Late	33°27,15'S 25° 21,50'E
B51	Atmar	Kirkwood	E. Cape	Lane navels	Late 8	33°28,209'S 25° 31,317' E
	Dunbrody estates	Kirkwood	E. Cape	Turkey Valencia	10	33°28,018' S 24° 41,775' E
26	Carden	Addo	E. Cape	Palmer navels	8	33° 28,58' S 25° 41.618' E
27	Carden	Addo	E. Cape	Cara Cara	8	33° 28,58' S 25° 41.618' E
26	Carden	Addo	E. Cape	Autumn Gold	8	33° 28,58' S 25° 41.618' E

Fruit were collected initially from the floor of the orchard and later in the season from the trees. Only fruits which appeared to be infested with false codling moth were collected. Fruits were classified as infested if they had frass-filled penetration holes or if there was some discolouration around the penetration hole or a mark which was suspected to be a penetration hole.

2.2.2. Rearing larvae

Fruit were brought back to the laboratory and dissected to find *T. leucotreta* larvae by cutting thin layers of the orange skin away around the penetration hole. The larvae were placed individually in glass vials containing artificial diet.

The diet was prepared by mixing the following ingredients (Moore 2002): maize meal (2000 g), wheat germ (200 g), Brewer's yeast (100 g), milk powder (36.5 g), Nipagin (15 g) and sorbic acid (6.5 g). A total of 200 g of the diet was placed in a glass dish with 200 ml of double-distilled water (ddH₂O). The dish was then covered with foil

and baked in an oven at 180°C for 25 minutes, and then placed under a laminar flow hood to cool. The medium was transferred to a glass vial by forcing the vial upside down into the diet, forming a plug (40 g) of diet. The plug was then pushed to the bottom of the vial using a sterile finger. A cotton-wool plug was placed in the neck of the vial to serve as a pupation substrate for the larva. The instar of the larvae was determined and written on the vial. The instars (instar 1-5) were estimated (Daiber 1979b) by the length and colour of the larvae. The vials were placed in a constant environment room at 27°C and at approximately 60% relative humidity.

The vials were checked daily for any dead larvae, the emergence of false codling moth and, most importantly, for parasitoids. The instar from which the parasitoid emerged and sex of the parasitoid were recorded and the parasitism rate was calculated using the following formula:

$$\% \text{ Parasitism} = 100(\text{Total number of parasitoids} / \text{Total number of larvae})$$

2.3 Results and Discussion

2.3.1. *Moth collection*

A total of 11 389 navel oranges were collected from various orchards on farms in the Addo/Kirkwood area (Fig 2.1). False codling moth, *Thaumatotibia leucotreta* (Meyrick) larvae infested 36.09% of the fruit collected (Fig 2.2).

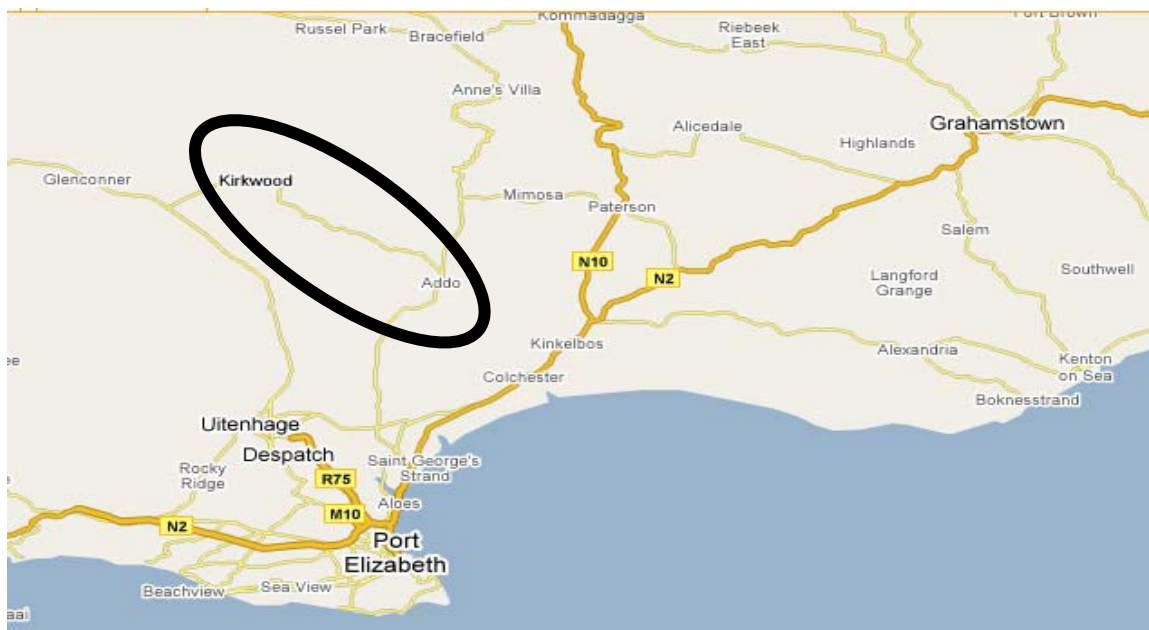


Figure 2.1: A map showing the Addo/Kirkwood area where the fruit collections were done.

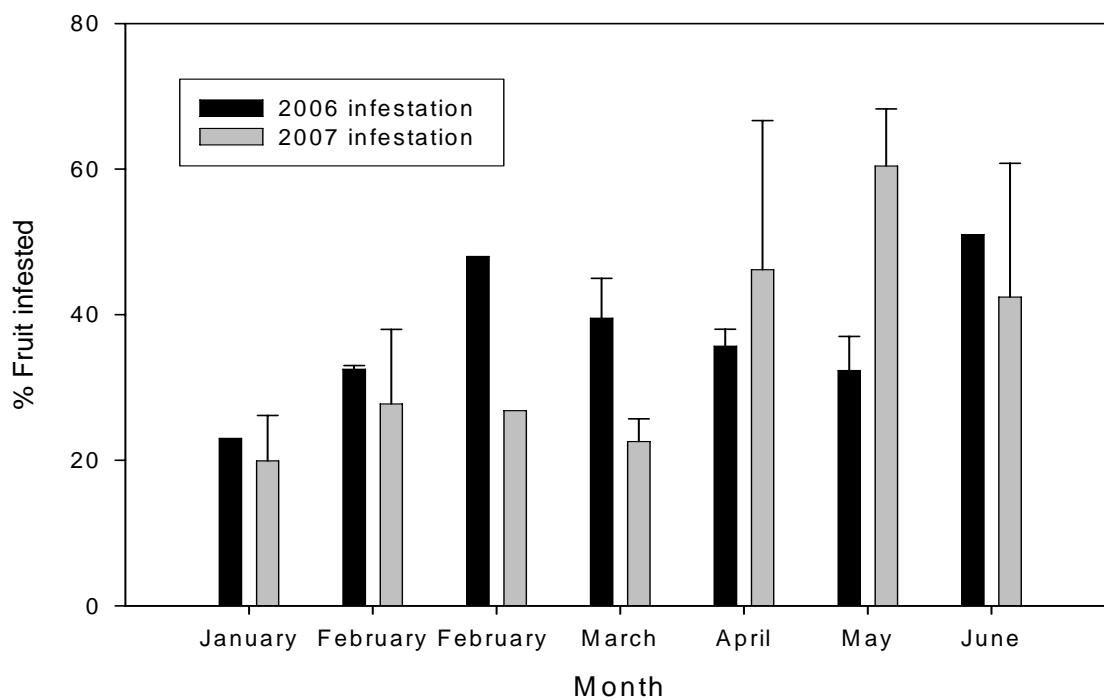


Figure 2.2: The combined average percentage fruit infestation (+ SD) during 2006 and 2007; for the months in which fruit was collected.

The true infestation rate could be under-estimated as only fruit that larvae were found in were considered infested. Many fruits could be seen to have been infested but no larvae were found, and therefore the fruit was not considered infested. The larvae may have left the fruit to pupate or they were too small to be found in the fruit. In 2006 the percentage of infested fruit ranged from 22% infested fruit per month to 51% and in 2007 from 19% infested fruit per month to 65% (Fig 2.2).

During 2006, larvae in their first instar were the most scarce and third instar larvae were the most abundant (Fig 2.3). Larvae in the first instar are very small (2 mm in length), therefore making it difficult to find them. As the larvae get older, they start taking on a pink colour and are larger in size, making them easier to find in the fruit.

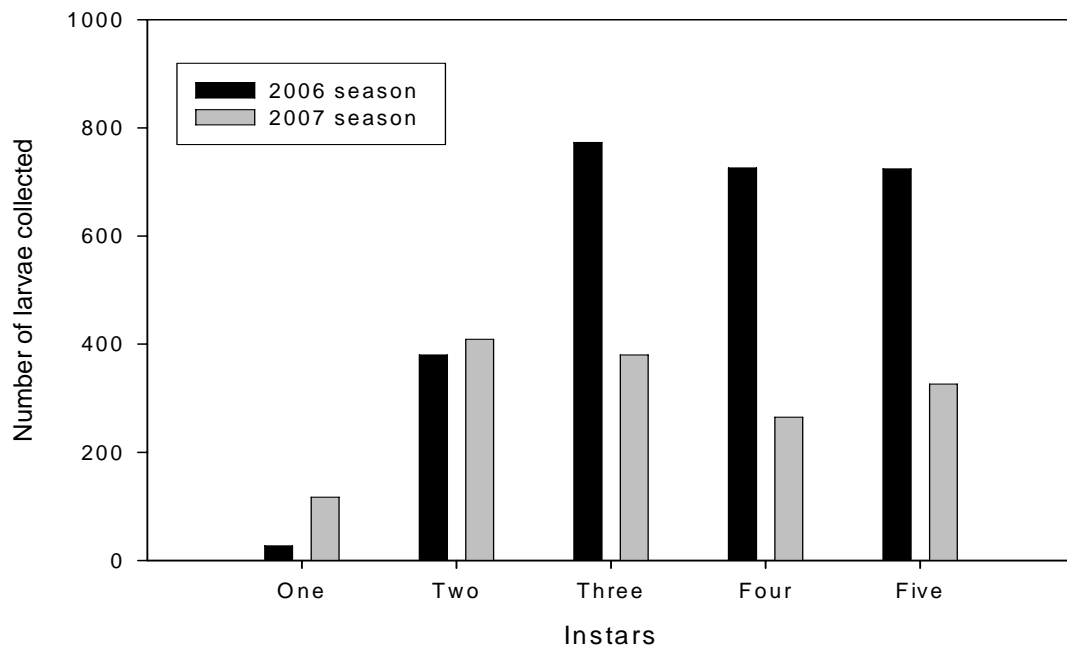


Figure 2.3: The total number of false codling moth larvae collected from the five instars of false codling moth in the 2006 and 2007 seasons.

2.3.2. Parasitoid collection

A single parasitoid (identified by the Biosystematics division of the PPRI) species, *A. bishopi*, was reared from the false codling moth larvae collected from the fruit. In

2006, the highest average parasitism rate was recorded in May, with 11.43% of the false codling moth larvae yielding parasitoids. In 2007, the highest parasitism rate was recorded in April, with 13.27% of the false codling moth larvae yielding parasitoids. In the 2006 season, the parasitism rate was low from January through to April. In the 2007 season the parasitism rate was low in January with a steady increase in February and March. In 2006, the average parasitism rate from January to June was 3.55% and in 2007 it was approximately 1.6 times higher, 5.86%. Comparing the two seasons, it seems like the parasitism rates increased a whole month earlier in 2007. From the two seasons it can be said that the parasitoid is most active in the months of April and May (Fig 2.4). The same results occur when looking at parasitism rates on a weekly basis.

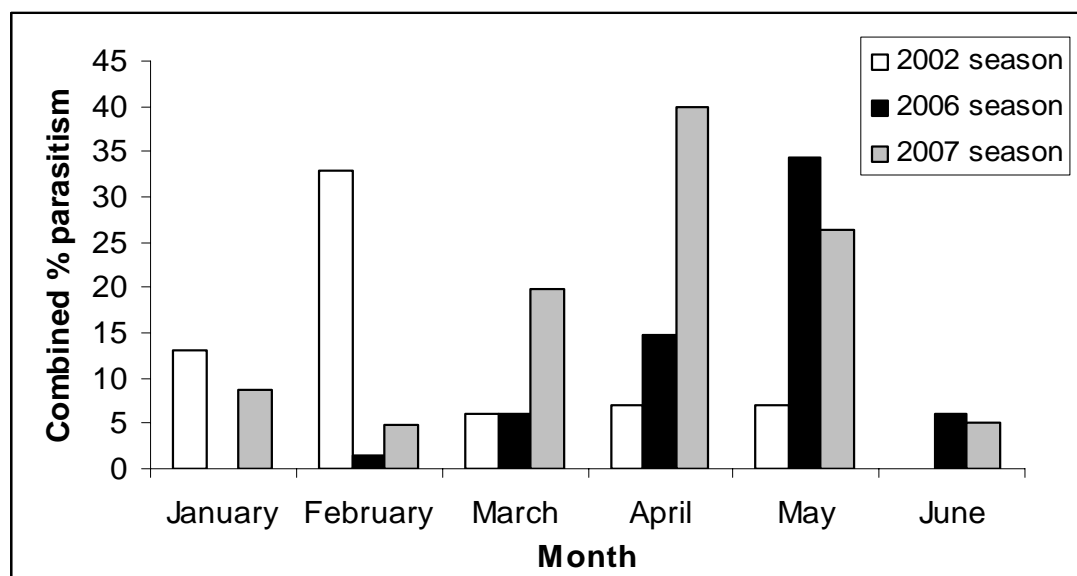


Figure 2.4: The combined percentage parasitism for each month in three seasons. The data for the 2002 season come from Sishuba (2003). The sampling procedure for the 2002 season differed from the 2006 and 2007 seasons as in 2002 sampling only occurred once a month, while in the 2006 and 2007 sampling took place weekly.

Sishuba (2003) found that the highest rate of parasitism by *A. bishopi* in the Sundays River Valley was 34% in December. It was also found that there were irregular parasitism rates through the season, but low levels of parasitism occurred in the later part

of the season, whereas in this study it occurred in the early part of the season. A reason for the 2002 results may be that sampling occurred only once a month. By sampling once a month, one is only getting a sub-sample of the parasitoid and the parasitism rate can be inflated or under-estimated. This does not allow for any variation in the parasitoids activity during the month. The parasitoid activity may change on a weekly basis due to changes in environmental conditions such as temperature and humidity. In 2006, May had a decrease in average temperature (Fig 2.5A) and an increase in average relative humidity (Fig 2.5B) compared to the previous months. In 2007, March and April had a decrease in temperature, averaging 19°C (Fig 2.5C). There was a steady decrease in temperature through May and June. There was a slight increase in relative humidity in March and April, averaging 85% (Fig 2.5D).

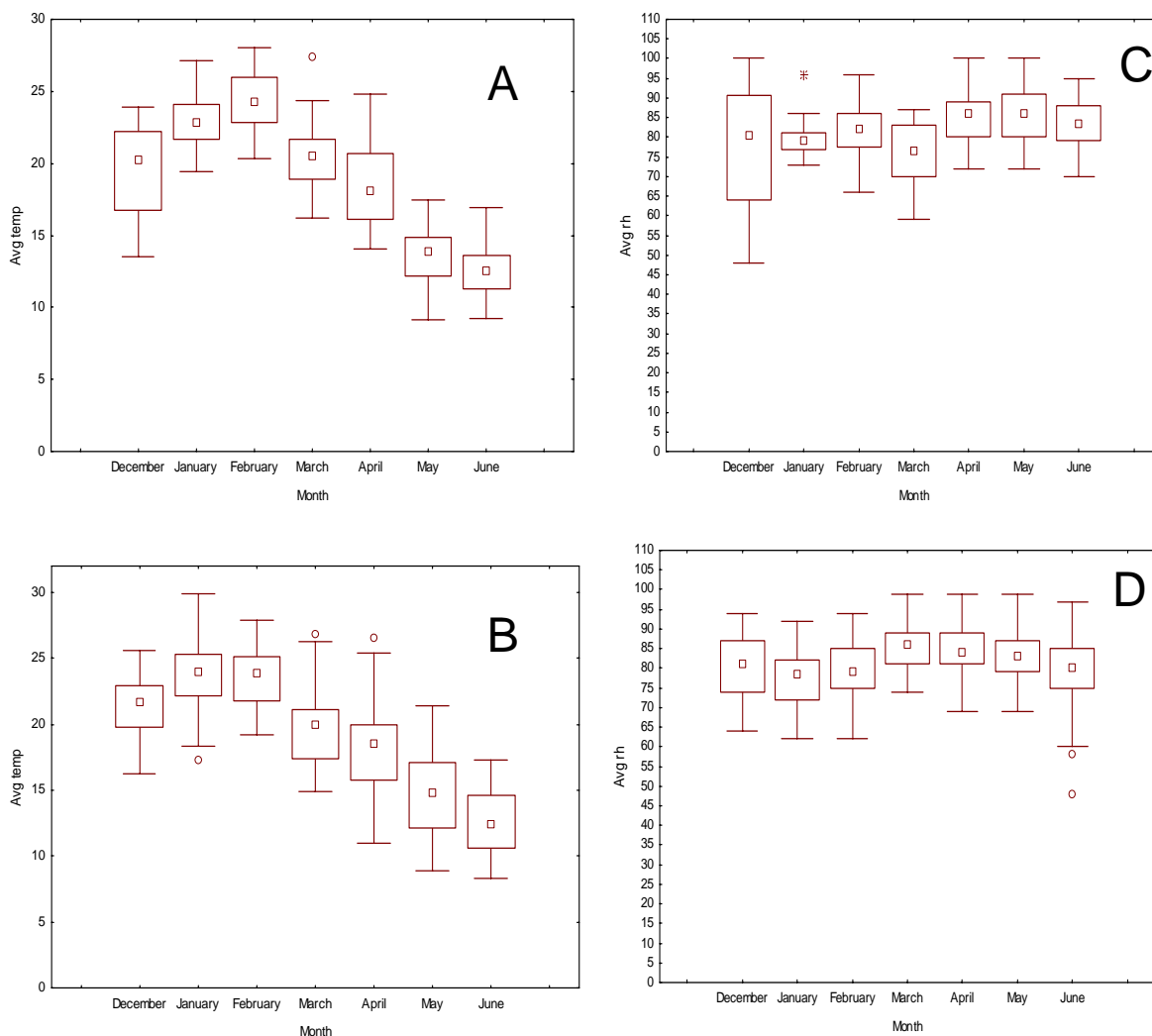


Figure 2.5: The average temperatures for (A) December 2005 to June 2006 and (B) December 2006 to June 2007, and average relative humidity for (C) December 2005 to June 2006 and (D) December 2006 to June 2007. □: Mean, □: Standard deviation; | : 95% confidence interval.

The changes in parasitism rates may be a result of the monthly fluctuations in the false codling moth population and factors such as predation, environmental conditions and farming practices such as orchard sanitation. With orchard sanitation, the removal of fruit from the orchard floor, one could be removing parasitoids too. The false codling moth larvae pupate in the soil (Daiber 1979c), so by removing the fruit from the orchard floor, the larvae are removed before they can leave the fruit to pupate. This could be detrimental to the success of a biological control programme involving the use of *A.*

bishopi. *Agathis bishopi* is a koinobiont, meaning the parasitoid develops inside the host while it is still alive (Hassell *et al.* 1992; Harvey *et al.* 1999; Jervis *et al.* 2001). By removing the fruit early, one is removing the parasitoid along with the fruit, even if not every infested fruit drops. A reason for the low parasitism rates in the early part of the season may be due to the use of chemical sprays in the orchards. These sprays may kill (Moore pers. comm. 2007) both the host in its first instar and the parasitoid. Another reason for this may be the decline of the pest and parasitoid population during the winter months (Moore pers. comm. 2007), and therefore both populations will take a few months to build their numbers up again. The moth is able to increase its numbers rapidly as each female can lay 57.6 eggs a day and approximately 800 in its life time. The parasitism rates may be under-estimated as dead larvae and failed emergences from vials were not taken into account. It is possible that parasitism may have lead to premature death of the host, and therefore no emergence of parasitoids. This though is not usual for koinobionts as they need the host to complete development, but it is possible for hosts to become stressed and result in their premature death.

The second instar yielded the highest number of parasitoids, and there was no emergence of parasitoids from the fifth instar (Fig 2.6). Sishuba (2003) also found that there was no emergence of parasitoids from fifth instar larvae. This is an indication that the parasitoid parasitizes only early instars of the host. Another explanation is that as the parasitoid may retard host development, causing the larvae to appear younger (Fig 2.7) than they really are.

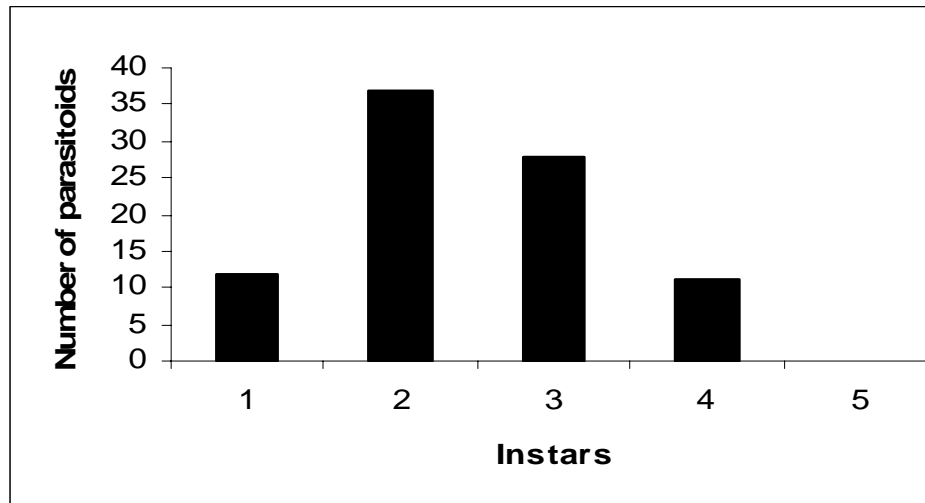


Figure 2.6: The average number of *A. bishopi* parasitoids that emerged from each instar of false codling moth larvae from the 2006 and 2007 seasons.



Figure 2.7: (A) A non-parasitised fifth instar larva of false codling moth. (B) A non-parasitised third instar larva of false codling moth and (C) a parasitised larva of false codling moth appearing to be in the third instar but has the colouring of a fifth instar larva.

From the false codling moth larvae collected from the various host instars, the first instar had the highest percentage parasitism, and the second highest percentage survival (Table 2.2). Although instar 2 yielded a high number of parasitoids the percentage parasitism is low due to the high number of second instar larvae collected from the fruit. Instar 1 had a higher percentage parasitism as the number of first instar larvae collected was low with a high emergence of parasitoids. This is a good indication that the parasitoid prefers to parasitise younger instars. This would make sense as the first and second instars would be the most accessible to the parasitoid. In another study on an *Agathis* species that parasitises the larvae of *Greya subalba* (Lepidoptera: Incurvariidae) it was also found that the parasitoid oviposited in the early instars (Thompson 1986). The instar which is attacked by the parasitoid is often related to the requirements of the parasitoid developing on or within the host and relates to the fitness of the parasitoid progeny (Jenner & Kuhlmann 2004). Early instars are most often attacked if the parasitoid needs to develop within the host, and later instars are attacked mainly by koinobionts with a very short developmental period or idiobionts (Van Alphen & Vet 1986).

The first instar larvae are likely to be the most susceptible to the parasitoids as the adult false codling moths lay their eggs on the skin of fruit or on the underside of leaves. Upon emergence the neonate larvae would have to chew their way through the skin to be able to penetrate the fruit. The larvae penetrate the skin and move into the fruit, the second instar larvae would still be just below the skin, therefore they are still susceptible to the parasitoid as its ovipositor would be able to penetrate through the skin of the fruit, but this would depend on the thickness of the skin of the fruit. The ovipositor of the female is 4-5mm in length; therefore if the skin of the fruit is thicker than this, the female will be unable to reach the host larvae.

Table 2.2: The survival and deaths rates and the parasitism rate of the false codling moth larvae at the five different instars.

Host Instar found	n	% Deaths	% Survival	% Parasitism	Ave. days till emergence
1	117	24.27	75.73	10.68	26.4
2	409	39.07	60.93	7.92	25.47
3	380	48.52	51.48	8.28	28.1
4	265	37.77	62.23	5.33	22.92
5	326	5.69	94.31	0	0

2.4 Conclusion

Only *Agathis bishopi* was reared from the false codling moth larvae collected from the citrus orchards. This species seems to be more abundant in the cooler months of April and May. This may mean that the parasitoids prefer cooler conditions and this should be investigated further for a mass-rearing programme. It may also be that the parasitoids numbers build up late in the season, therefore coinciding with the cooler months. It also seems to parasitise younger larvae, possibly due to the accessibility of these instars to the female parasitoid.

Agathis bishopi shows promising signs in aiding the control of the false codling moth population. It is a promising agent as it attacks the young false codling moth larvae, thereby reducing the moth population. Unfortunately, chemical and biocidal sprays and orchard sanitation may play an important role in reducing the population of the parasitoid. There needs to be continuous monitoring of the parasitoid, by collecting samples of larvae and rearing them for the emergence of any parasitoids, on a monthly basis over a few years. From such data it may be seen which months generally show a decrease in the parasitoid population and when the numbers are low, reduced orchard sanitation may be important to aid in the population build-up of the parasitoid. Orchard sanitation has been shown to reduce the weekly loss of fruit by 3%, but it can also reduce larval populations by 40% (Newton 1998). This 40% reduction in the host larval population could be detrimental to a biological control programme involving the use of a

larval parasitoid. Orchard sanitation can also be costly in both time and money; therefore it will be beneficial if the amount of orchard sanitation can be reduced by the increase in parasitism by the parasitoid. In the initial period after reducing orchard sanitation there could be an increase in pest population but this will be reduced by the parasitoid once its numbers build up.

For this parasitoid to become effective, it is suggested that it be used in an Integrated Pest Management (IPM) programme. Augmentative release of the parasitoid, on a weekly basis during the months of low parasitoid activity and monthly during the months of higher parasitoid activity, will aid in keeping the parasitoid numbers high and increase the number of false codling moth larvae parasitized by *A. bishopi*. Further studies are needed on the affect of pesticide application on the parasitoid. If pesticide application affects the parasitoids then it may not be wise to release parasitoids into the orchard over that period of time. Careful planning of parasitoid release and pesticide application should be taken.

3

Mass-rearing of the parasitoid, *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae) for the biological control of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)

3.1 Introduction

Two areas of pest control rely on mass-rearing. The first is the sterile insect technique, which requires large numbers of sterile pests, and the second is when control relies on the release of large numbers of the control agent (Carey *et al.* 1988). One of the key areas for a successful biological control programme is the production of natural enemies on a large scale for release into the field (Chambers 1977). Parasitoids are known to be an important component of terrestrial communities as they can regulate populations of the species which they parasitize (Cohen *et al.* 2005), and they are therefore becoming an increasingly important part of biological control in integrated pest management systems (Mattiacci *et al.* 1999; Johns and Whitehouse 2004).

It is important that the mass-reared population is as effective in the field as the wild population (Huettel 1976; Nunney 2006). In mass-rearing it is often emphasized that quantity of the natural enemy is of vital importance, but quality of the agent is just as important (Boller 1972; Chambers 1977; Nunney 2006). Some of the problems encountered during mass-rearing programmes include diet, rearing conditions and the population genetics of the captive colony (Greany *et al.* 1984; Carey *et al.* 1988).

One major problem facing mass-rearing is that no artificial diet is available for commercially produced parasitoids, and therefore the host must also be mass-reared (Carey *et al.* 1988). In an attempt to mass-rear the parasitoids *Tamarixia radiate* (Waterson) (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal) (Hymenoptera: Encyrtidae), the host, Asian citrus psyllid, *Diaphoorina citri* (Kuwayama) had to be mass-reared before the attempt could be made to mass-rear the parasitoids (Skelley & Hoy 2004). False codling moth can be successfully reared on an artificial diet (Schwartz 1972), which facilitates the development of a mass-rearing system for *A. bishopi*.

For the successful production of parasitoids it is important to have the balance of hosts to parasitoids correct (Carey 1988). The aim of mass-rearing parasitoids is to produce mainly females; this can pose a problem. Parasitoids are haplodiploid (Waage & Lane 1984; Hassell *et al.* 1992), therefore the female has to mate to produce female progeny (Antolin *et al.* 1995; Bautista *et al.* 1999; Ode & Hunter 2002) and has the ability to store sperm (Hardy 1994; Heimpel and Lundgren 2000) and choose the sex of the off-spring based on various factors such as relative size of the host (Green 1982; Carey *et al.* 1988; Johns and Whitehouse 2004; Cohen *et al.* 2005).

The aim of this study is to rear *A. bishopi* successfully in the laboratory. If this can be done, the techniques can be scaled up to allow augmentative releases to be carried out in citrus orchards for the control of false codling moth.

3.2 Materials and Methods

3.2.1. Host Rearing

A stock of sterilised honey jars (122mm x 65mm) were prepared for the mass-rearing of parasitoids. A hole was cut in the lid of the jar and a cotton-wool plug placed in the hole. The lid was covered with tin foil and autoclaved for 20 min at 127°C, and thereafter allowed to cool under a laminar flow hood. Each jar was prepared with 25g of dry, artificial false codling moth diet (Table 3.1) and 25ml of ddH₂O mixed together.

Table 3.1: The ingredients used in the artificial diet for false codling moth (Moore 2002).

Ingredients	<i>Amount</i>
Maize meal	2000 g
Wheat germ	200 g
Brewer's yeast	100 g
Milk powder	36.5 g
Nipagen	15 g
Sorbic acid	6.5 g
Total	2358 g

False codling moth eggs were obtained weekly from an established culture at Citrus Research International (CRI) in Port Elizabeth, Eastern Cape Province. The moths lay eggs on wax paper provided in their cages. A sheet was removed daily from each cage and cut into squares containing approximately 100 eggs. The egg sheets are then sterilised by placing them in 15% Sporekill (ICA International Chemicals) for 15 min and then dipping them in 25% formalin for 3 seconds to remove or kill fungal spores and any virus particles that may be present.

One egg sheet square was placed in each honey jar. The honey jars were left in a constant environment room at 27°C and approximately 60% relative humidity.

3.2.2. Parasitoid Rearing

A laboratory culture of *A. bishopi* was established from field-collected adult parasitoids obtained by dissecting false codling moth larvae from oranges and rearing them until parasitoid emergence (Chapter 2). The environmental conditions in the constant environment room were maintained at 27°C ± 1°C, 60% relative humidity and under a photoperiod of 12D:12L. Adult parasitoids were placed in the jars when the first instar moth larvae were observed. The same numbers of female parasitoids were used per jar in 2006 and 2007. When the adult parasitoids were removed from the honey jar, a large cotton-wool plug was placed in the neck of the honey jar to serve as a pupation

substrate for the moth larvae. When it was observed that the majority of the moth larvae had pupated (Fig 3.1A), the cotton-wool plug was removed and placed in an emergence jar. A lid was placed on the honey jar in case any larvae had pupated in the diet. The jars were observed daily for the emergence of adult parasitoids (Fig 3.1B), which were then placed in mating jars (Fig 3.1C) for a day, to ensure that the female parasitoids mated before being allowed to parasitise the next generation of moth larvae. The emerged parasitoids were checked for deformations, to ensure the parasitoids were fit for mating. The parasitoids were placed *en mass* in the mating jars, with approximately two males per female to ensure that the females mated. Males were removed from the mating jar when their antennae started to curl, which indicated that the male was going to die and was therefore unfit for mating. The honey and water mixture in the mating jars was replaced daily. When the parasitoid numbers increased, varying numbers of adult parasitoids were placed in the honey jars to test whether this had any effect on the sex ratio of the progeny.

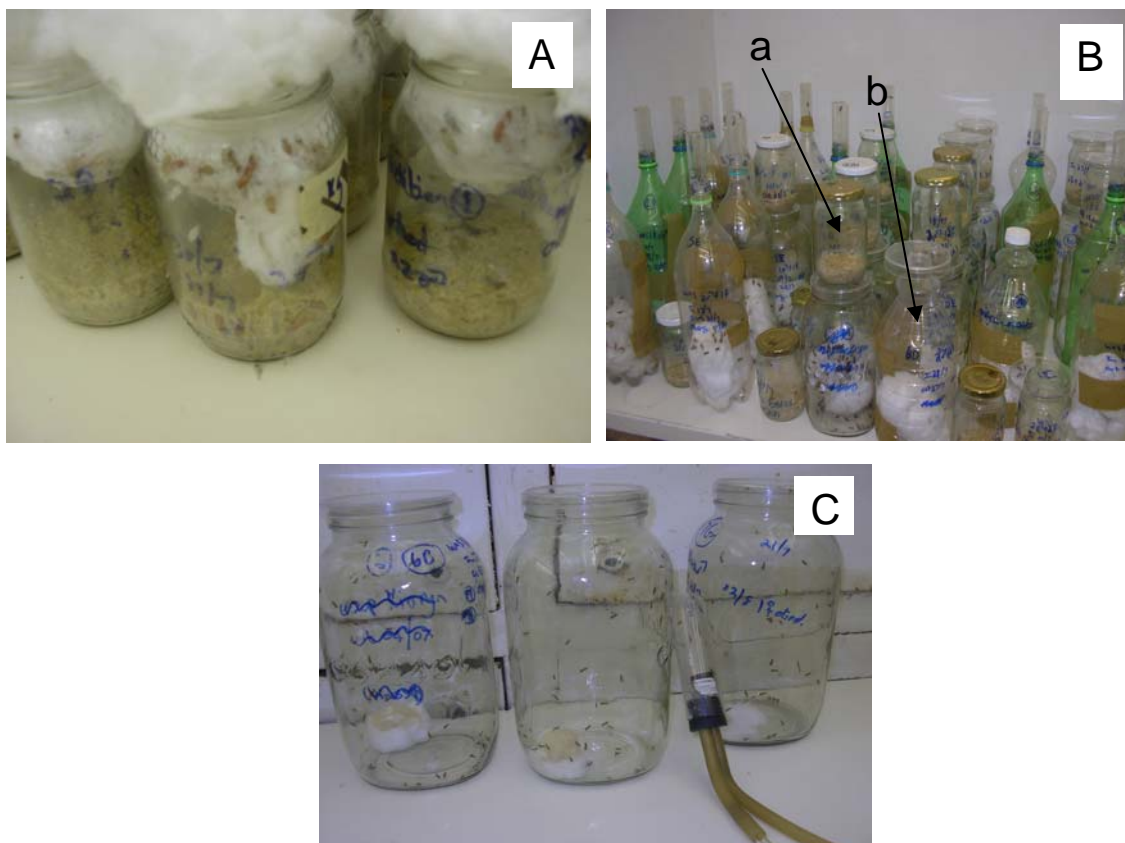


Figure 3.1: False codling moth larvae pupating in cotton-wool plugs placed in the neck of the honey jar (A). The jars (B) into which the cotton-wool plugs were placed (b) when the larvae had pupated. These jars and the jars with the diet (a) were checked daily for the emergence of parasitoids. Females were placed in mating jars (C) for a day after their emergence to ensure that they mated before being placed in the honey jar to parasitize the false codling moth larvae.

Knowing the number of days which was sufficient for the female parasitoids to remain in a single jar to parasitise the larvae before being placed into a new jar would aid in the mass-rearing process by maximising the number of false codling moth larvae available for the female parasitoid to parasitise. The data were analysed using a one-way ANOVA.

The time it took for the male and female parasitoids to emerge from when the parent parasitoids were placed in the honey jar gives an indication of the time it will take for the progeny to emerge and how long it takes for the development of the male and

female parasitoids. It is also important to know after how many days one can expect the highest number of parasitoids to emerge.

3.2.3. Parental sex ratios

The parasitoids were left in each jar for 3-12 days, and then placed in a new jar. Varying numbers and ratios of the parent parasitoid were used; 1♀:1♂, 2♀♀:2♂♂, 2♀♀:1♂ and 1♀:2♂♂. This was repeated until the female parasitoid died, as it is the female that parasitises the larvae and produces progeny.

The number of progeny which emerged per jar from each of the parent numbers was measured to determine which parent numbers would yield more female progeny for the mass-rearing of the parasitoid. The number of progeny which emerged from the diet or the cotton-wool plug; this was to determine which substrate, if any, the male and female parasitoids preferred to pupate in. The jars which had less than ten parasitoids emerging were excluded from the analyses as in these results one individual has a large effect on the precision of the outcome. Where the sample size was above ten, the effect of a single individual on the precision of the data is reduced by about an order of magnitude, therefore allowing for more accurate analysis. The data were analysed using a one-way ANOVA.

3.2.4. Offspring sex ratios

A female-biased sex ratio is important for mass-rearing as it is the females which continue the culture and build up its numbers. The parent parasitoids were left in the honey jars over a varied period of time, from 3 to 14 days. The offspring sex ratio in *A. bishopi*, produced by the varying parent numbers, was investigated. The data were analysed using a one-way ANOVA.

3.2.5. Fungal infestations on the diet in the honey jars

To test the hypothesis that an *Aspergillus* spp. was growing from the bodies of the dead larvae, honey jars were kept where the larvae were dying due to a virus infection. Dead larvae were removed from 30 bottles and not from 30 other bottles. Vials were kept with larvae that had died due to virus from the field-collected fruit. These were used to

determine whether fungus can start in the oranges from the dead larvae inside them. The fungi was identified by the Department of Microbiology.

3.3. Results and Discussion

3.3.1. Parasitoid rearing

Under mass-rearing conditions in 2006, an average of 22.8 (std. dev. = 22.07) parasitoids emerged per jar (n = 73) and in 2007, an average of 4.06 (std. dev. = 9.29) parasitoids emerged per jar (n = 69). A reason for the low number of parasitoid emergences in 2007 could be the increase in the number of virus, *Cryptophlebia leucotreta* granulovirus (CrleGV), infected larvae in the jars. No parasitoid emerges if the virus kills a larva. Studies are being conducted on the possibility of formulating an artificial rearing medium for mass-rearing parasitoids. This will allow for more parasitoids to be mass-reared and therefore an increase in more parasitoid releases into the field (Greany 1986; Thompson 1999).

The emergence of parasitoids from the cotton-wool and from the diet varied. This was determined by counting the number of adult parasitoids emerging from each substrate. More male than female parasitoids emerged from the cotton-wool (Fig 3.2), but there is no statistical difference (One-way ANOVA; $F = 0.77$; $df = 1$; $p = 0.38$), and more females than males emerged from the diet (Fig 3.2), but again there is no statistical difference (One-way ANOVA; $F = 0.15$; $df = 1$; $p = 0.69$). There was also no statistical difference (One-way ANOVA; $F = 1.04$; $df = 1$; $p = 0.31$) with respect to female emergence and male emergence (One-way ANOVA; $F = 0.08$; $df = 1$; $p = 0.77$) from each substrate (Fig 3.2).

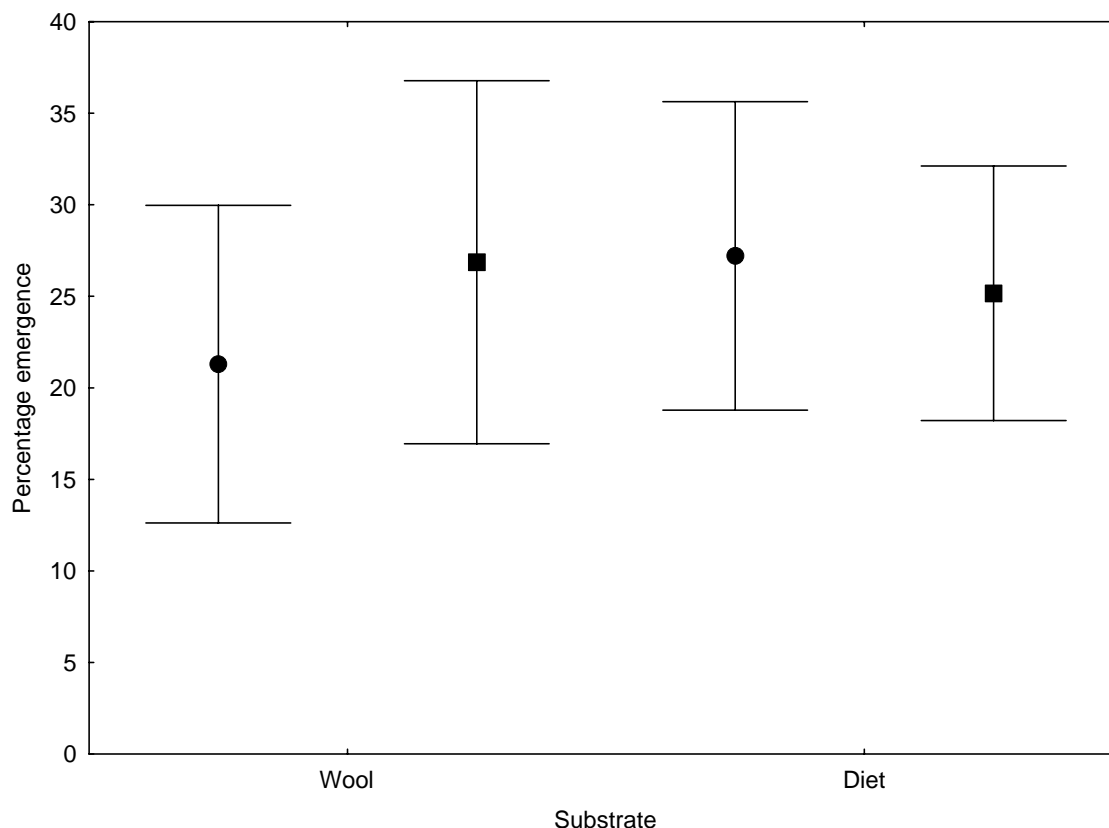


Figure 3.2: The mean percentage (+ SD) emergence of female *A. bishopi* (●) and male *A. bishopi* (■) parasitoids from the wool and diet pupation substrates.

In a mass-rearing situation, both pupation substrates are important for the emergence of parasitoids. If the one substrate is damaged e.g. the diet is infected by fungal growth, half of the emerging parasitoids can be lost. This can be detrimental to a mass-rearing programme.

3.3.2. Parent numbers and sex ratios of the progeny

The sex ratios of the progeny varied. Day 4 had 63.58% females emerge and day 5 had 67.99% females emerge. When mass-rearing, a female-biased sex ratio is important, as it is the female which parasitizes the larvae therefore building the parasitoid population faster. Mass-rearing is usually carried out to build up the numbers of a natural enemy so that mass releases can be carried out in the field to decrease the population of the pest.

The balanced parental numbers of 1♀:1♂ and 2♀♀:2♂♂ produced male-biased sex ratios. The skewed parental numbers 2♀♀:1♂ and 1♀:2♂♂ produced female-biased sex ratios (Fig 3.3).

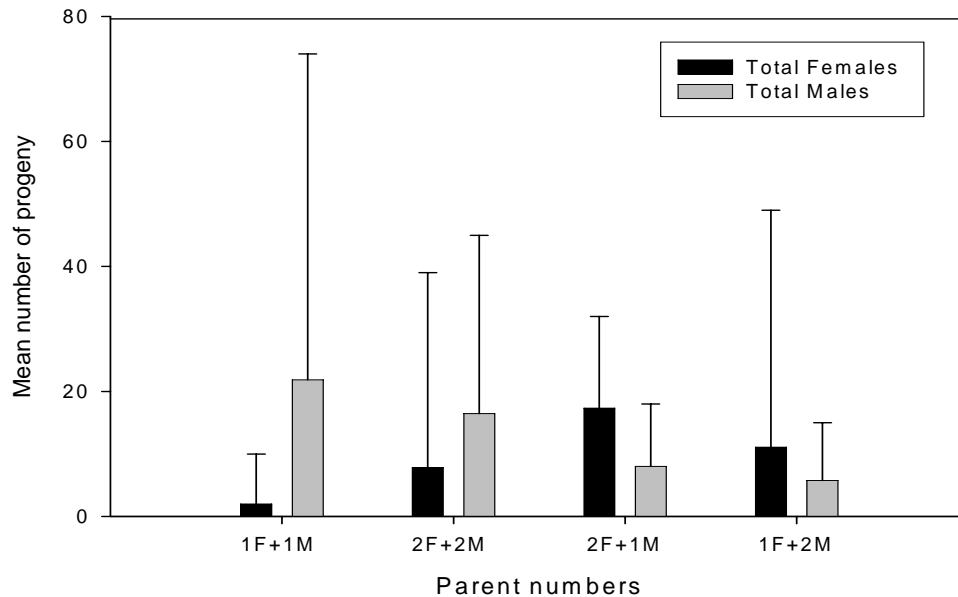


Figure 3.3: The mean number (+ SD) of female and male progeny which emerged from four different parent numbers.

For mass-rearing it is better to use the skewed parental numbers. Sex ratio variation is common among biological control agents, and this can often slow down or prevent the biological control programme from moving forward (Antolin *et al.* 1995). Female-biased sex ratios are often the most beneficial to a biological control programme as it is the females which increase the population growth rates and when using parasitoids it is the female which causes mortality in the host population and not the males (Antolin *et al.* 1995; Heimpel & Lundgren 2000).

3.3.3. Off-spring sex ratios

By 14 days the majority of the larvae had reached their fifth instar and were ready to pupate. There was high variation between the number of progeny produced and the time the parent parasitoids were left in the jar (Table 3.2). Day 13 (n = 4) showed a high

number of parasitoid emergence. It was determined that 3 days was sufficient for the female parasitoid to parasitize larvae, as then an average of 37.8 parasitoids emerged per jar and an average of 9.5 parasitoids emerged per day. There was no statistical difference between the off-spring over the days (Kruskal-Wallis; $H = 14$; $p = 0.36$). Days 8 and 11 were omitted from the table as there was no progeny emergence. When mass-rearing, the aim is to maximise the use of the parents to produce progeny. The more larvae that can be exposed to parasitoids, the higher the chance of more larvae being parasitised by a female parasitoid. To maximise parasitism for mass-rearing of *A. bishopi*, it would be viable to leave the parasitoids in the honey jar for 4-5 days (approximate recovery rate of 30% parasitoid progeny) and then place them in a new jar until the female parasitoid dies.

When the parasitoids were left for 2 days only 3 male parasitoids emerged. This was from 2 females which were exposed to approximately 200 larvae. This is not sufficient for mass-rearing conditions, especially since they were males. Days 4 and 5 showed the highest emergence of female parasitoids (Fig 3.4).

Table 3.2: The average number of parasitoid progeny emerging from jars where the parent parasitoids were left in for a varying number of days. Days 8 and 11 were not included as there was no emergence of parasitoids.

Days parent parasitoids left in honey jars	N	Cumulative Ave. number of parasitoid progeny emerging	<i>Average offspring / day</i>
2	3	11	3.6
3	4	37.8	9.5
4	6	43.5	7.3
5	5	28.8	5.8
6	5	37.6	7.5
7	4	36.0	9
9	3	26.0	8.7
10	3	32.0	10.6
12	4	19.3	4.8
13	4	47.3	11.8
14	2	14.0	7.0

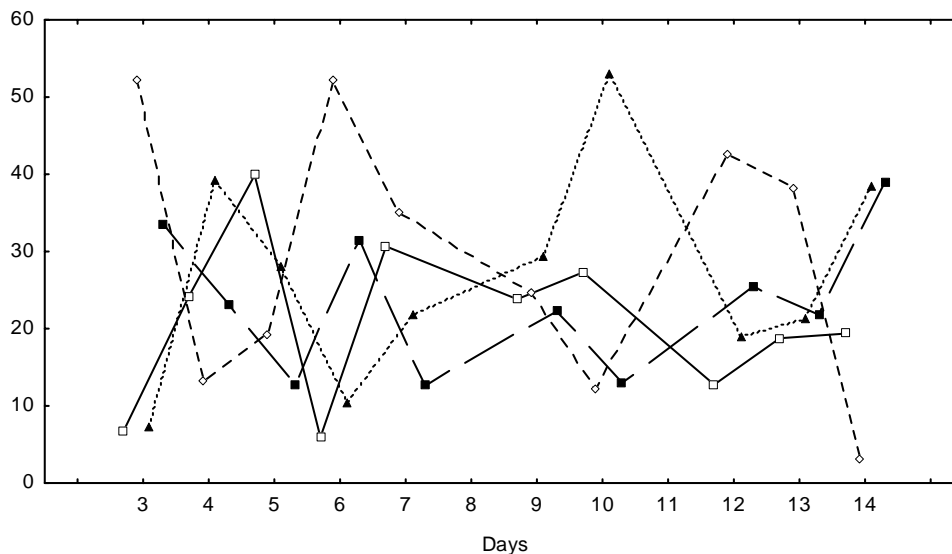


Figure 3.4: The emergence of female and male *A. bishopi* progeny, from the diet and cotton-wool plug, over the number of days the parent parasitoids were exposed to false codling moth larvae. This graph gives an indication of the number of days which is adequate for the female parasitoid to parasitise larvae, and produce both female and male off-spring, favouring female progeny. □; Ave. % female emergence from the cotton-wool; ◇; Ave. % male emergence from the cotton-wool; ▲; Ave. % female emergence from the diet and ■; Ave. % male emergence from the diet.

3.3.4. Fungal infestation on the diet in the honey jars

The third and most important problem was the presence of a fungus, *Aspergillus* sp., in the culture with disastrous effects on the emergence of the parasitoids from the diet. The fungus becomes visible in the late stages of the mass-rearing process, just before the cotton-wool plug was to be removed from the jar. It took approximately 3 days from the first day of seeing the fungus for the entire diet to be affected and the jar had to be discarded. As the majority of the female parasitoids emerged from the diet, this ultimately affected the mass-rearing process, as it is the females that keep the culture going. It was suspected that the fungus was starting from the bodies of larvae dying due to virus infection. There was a significant difference (One-way ANOVA; $F = 4.44$; $df = 1$; $p = 0.039$) between fungus growth in the jars where dead larvae were not removed and

those where they were (Fig 3.5). In jars where there was no removal, 63% yielded fungus and from jars where dead larvae were removed, 36% yielded fungus. One problem was that not all dead larvae could be removed as some of the larvae died within the diet, which made them difficult to find. From the vials, 43% with dead larvae yielded fungus.

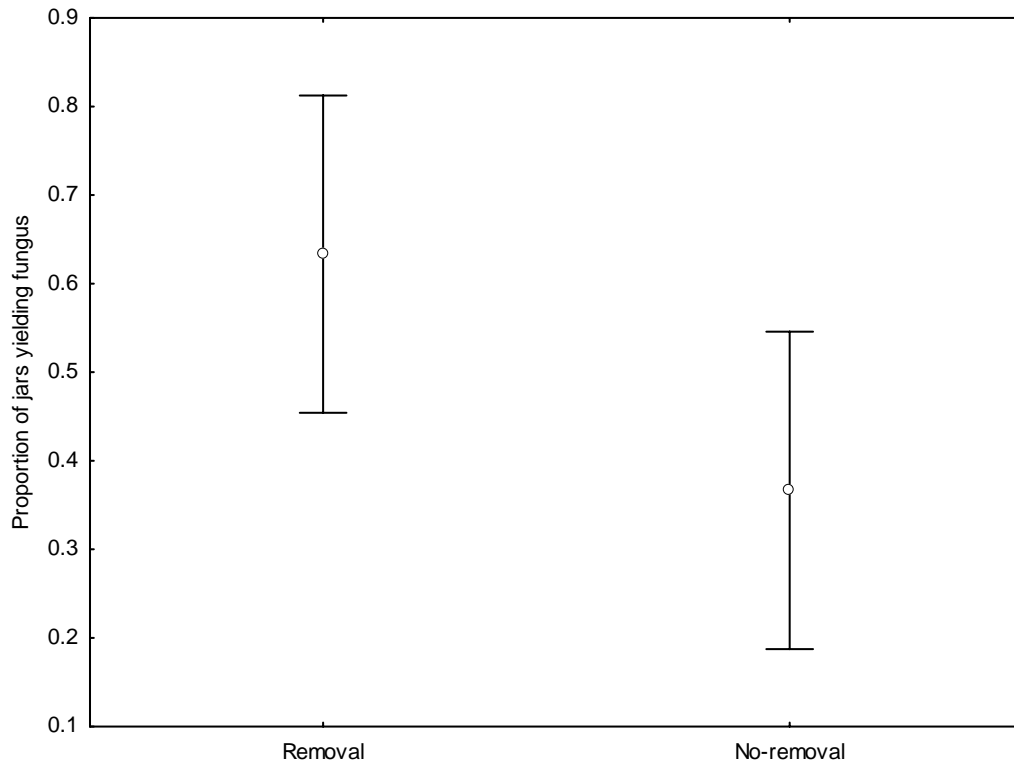


Figure 3.5: The mean proportion of jars (\pm SD) yielding fungal growth on the surface of the diet by the removal and no-removal of dead larvae.

3.3.5. Problems encountered during mass-rearing

The first problem was the asynchronous emergence of male and female parasitoids. At the start of the mass-rearing programme, males would emerge from the collected fruit but not females. When the females emerged there were no males to ensure that mating occurred, so the females produced only males. After a few continuous collections and dissecting of the fruit, males and females started to emerge synchronously and the mass-rearing programme started.

The second problem encountered was the virus, *Cryptophlebia leucotreta* granulovirus (CrleGV), affecting the false codling moth larvae. This virus infects larvae, which become flaccid and die, decreasing the number of larvae that can yield parasitoids, and therefore decreasing the emergence of parasitoids. The virus was present throughout mass-rearing, but it went through periods of high infection when there was still emergence of parasitoids but at a low rate.

The third problem encountered was the emergence of unfit female parasitoids, so termed because they were deformed and unable to parasitize false codling moth larvae. Females emerged with distorted ovipositors that did not allow them to parasitize larvae. Some females emerged with over-sized abdomens and deformed wings, and could not fly or even walk; therefore they were unfit to continue the mass-rearing process. These deformities were attributed to genetic causes like inbreeding. When starting a mass-rearing programme, the size of the original population is important (Prezotti *et al.* 2004), as is adaptation of the agent to laboratory conditions. It is advantageous to start the culture from as many individuals as possible (Prezotti *et al.* 2004). The greater the original genetic structure the more likely the culture's success. Over time the fitness of the individuals in the culture is likely to decrease due to in-breeding (Fellows *et al.* 2005). This can be overcome by introducing new individuals from the wild into the culture at various intervals (Fellows *et al.* 2005; Prezotti *et al.* 2004; Nunny 2006). This assumes that the problem is inbreeding, but the deformed ovipositors may be due to the parasitoid not having enough space for pupation or eclosion, therefore causing the ovipositor of the female to get squashed and curl upwards (Kirkman pers. comm.)

3.4. Conclusion

For the mass-rearing of *A. bishopi*, it is important to keep both the cotton-wool plug and the diet for the emergence of parasitoids as about half of the parasitoids emerge from them. The majority of male parasitoids emerge from the cotton-wool plug with the majority of females emerging from the diet. A reason for this might be that the females take longer to develop and the cotton-wool plug is removed before they can pupate in it;

therefore they pupate in the diet. Although it is the host which ‘makes the decision’ where to pupate, the response can be induced by the stress of being parasitised (Moore pers. comm.) and many koinobionts can regulate this, resulting in larger hosts. Therefore the parasitoid larva developing inside the host may cause the larvae to continue feeding for the development of the parasitoid larva.

It is also important to leave the female parasitoids in a mating jar for approximately 1 day to ensure that they mate before parasitizing the false codling moth larvae. To maximise the use of the parasitoids for mass-rearing, the optimum period the parasitoids should be left in the honey jar is 4-5 days. Taking into account that the female parasitoid lives for approximately 18 days, it would mean that each parent number used can parasitize approximately 3 honey jars (remembering that the rate at which the females lay decreases) with false codling moth larvae. For mass-rearing conditions a female-biased offspring sex ratio is required (Heimpel & Lundgren 2000). For this mass-rearing programme the skewed parental sex ratios of 2 females plus 1 male and 1 female plus 2 males yielded a female-biased sex ratio of the progeny. In the case of 2 females plus 1 male a female-biased sex ratio may be a result of both females having mated and more larvae were parasitised due to 2 females present in the jar. In the case of 1 female and 2 males, a female-biased sex ratio may be the result of ensuring that the female mated, therefore she is able to produce female progeny.

The fungus, *Aspergillus*, is most detrimental to the success of mass-rearing of *A. bishopi*. The fungus started from the bodies of dead false codling moth larvae in the honey jars. The moisture accumulation in the honey jars was also fairly high; therefore creating the perfect environment for fungus. More studies are needed on ways to control or eradicate the fungus, the major problem impeding the successful mass-rearing of *A. bishopi*. The use of honey jars for the rearing of *A. bishopi* has shown to work, but this method is extremely labour-intensive, and therefore impractical. Since a jar yields less than 100 wasps, if one wants to rear 50 000 wasps each month, one must process over 20 jars a working day, besides keeping a breeding stock. Since each jar takes about 10min minutes to handle, mass-rearing using this technique would require over 3 hour and 30

min a day. The use of the honey jars for mass-rearing works on a small scale but a more efficient method is needed for the mass-rearing of *A. bishopi* on a commercial scale. On a commercial scale one would need to devise a method that will reduce the number of times the wasps are handled. For example: all the cotton-wool plugs could be placed in a large single emergence chamber. From this chamber the emerged parasitoids need to be attracted into a separate chamber without the moths. From here the parasitoids can be removed daily and placed in a mating chamber where they are then placed in parasitizing chambers (containing diet and false codling moth larvae), a day later. For emergence from the diet, the jars can be fitted with lids containing pipes all leading into one chamber. Again a method is needed that will attract the parasitoids to fly into the chamber, this would be the major hurdle for this system. This system would minimise the handling of the wasps and therefore reduce the time required in the process.

4

Oviposition preference, development and longevity of *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae), a parasitoid of false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)

4.1. Introduction

The Braconidae is one of the most species-rich families of insects, like its sister group the Ichneumonidae. The majority of braconids are primary parasitoids of other insects (Van Driesche and Bellows 1996), and mainly parasitise the larval stages of Diptera, Coleoptera and Lepidoptera (Whitfield *et al.* 2004). *Agathis bishopi* (Nixon) belongs to the subfamily Agathidinae, which has approximately 1000 described species. Members of this subfamily have a worldwide distribution and they are ubiquitous among terrestrial habitats (Sharkey *et al.* 2006; Sharkey 2006). There is great variation in the life history traits of the parasitoids in this subfamily, but all the species in the subfamily are known to be koinobiont endoparasitoids of Lepidoptera (Sharkey 2006).

Agathis bishopi is currently known only from the Sunday River Valley area, Eastern Cape (Sishuba 2003). The reason for its absence in other citrus-producing areas in South Africa is unknown. The aim of these experiments is to further understand the biology and likely behaviour of *A. bishopi* and use it to guide expectations of the parasitoid's performance as a biological control agent.

4.2. Materials and Methods

A laboratory culture of *Agathis bishopi* was established from field-collected adult parasitoids obtained by dissecting oranges for false codling moth larvae and rearing the larvae until adult parasitoids emerged. The parasitoids were reared at 27°C, 60% relative humidity and under a photoperiod of 12D:12L. The adult parasitoids were then placed in

1159cm³ glass jars containing artificial diet and false codling moth larvae and left for a varying number of days. When the parasitoids were removed and placed in a new jar, the lid was replaced with a cotton-wool plug to serve as a pupation substrate for the moth larvae. Once the larvae had pupated, the cotton-wool plug was removed and placed in an emergence jar that was checked daily for the emergence of adult parasitoids. Some of the parasitoids were used to sustain the culture while others were used in the following experiments.

4.2.1. Pre-oviposition, oviposition periods and developmental rates

Six stages of false codling moth development were used to determine the stage most preferable to *A. bishopi*: eggs only; neonate larvae; and four stages when the surface of the diet was 25%, 50%, 75% or 100% covered by frass, respectively. Each stage contained approximately 100 false codling moth eggs or larvae. An estimate of instar of the false codling moth larvae can be made by the percentage frass covering on the surface of the diet. The 25% frass correlates with the second instar, 50% frass with the third instar, 75% frass with the fourth instar and 100% with fifth instar. The parasitoids (2♀♀:1♂♂) were placed in the jar at the various stages and they were removed from the jar once the next stage began (± 2 days). Once the parasitoids had been removed, the lid of the jar was replaced with a cotton-wool plug. It was determined whether there was any parasitoid emergence. Each stage was replicated 10 times. For the developmental rate, the time from parasitism to the emergence of adult parasitoids was recorded.

4.2.2. Life span

Shortly after the emergence of the adult parasitoids, the males and females were placed individually in jars under four different conditions: no food or water (N), water only (W), food and water (FW), for the female, food, water and a male (FW♂♂) and for the male, food, water and a female (FW♀♀). The number of days each parasitoid survived was recorded. For each condition 20 female and 20 male parasitoids were used. To determine if there was a significant difference between the longevity of the females and males, the data were subjected to a t-test.

4.3. Results and Discussion

The parasitoids mated within a few hours of their emergence from the host. The males tended to mate with many females, while the females mated only once. Only one parasitoid emerged per host. The females are arrhenotokous: mated females produce both male and female progeny (Sishuba 2004; Quicke 1997), while ten unmated females produced 106 male progeny only.

4.3.1. Pre-oviposition, oviposition periods and developmental rates

It was observed that the *A. bishopi* female searched for the host larvae by probing around in the diet with her ovipositor and feeling around on the surface of the diet with her antennae. A number of different methods are used by parasitoids to locate their host (Thompson 1986), including chemical cues, sound, heat, sight and vibrations (Hassell *et al.* 1992; Baaren *et al.* 2005; Fellows *et al.* 2005). The same type of behaviour was observed occurring in an *Agathis* sp. that parasitises *Greya subalba* (Lepidoptera: Incurvariidae) (Thompson 1986).

There was no probing during, or adult parasitoid emergence from, in the egg stage, which is expected as *A. bishopi* is not an egg parasitoid. In the neonate larval stage there was probing by the female parasitoid but there was no emergence of adult parasitoids. The female parasitoid probed around in the diet for approximately 35 seconds at varying intervals. Probing by the female started once the neonate larvae started to burrow into the diet. This stage lasted only 1 day. In the 25% and 50% frass stages there was vigorous probing and 9.7 adult parasitoids emerged per jar from these stages. These two stages coincide with the second and third instar of the false codling moth larvae. It is suggested that female parasitoids prefer second and third instar larvae of false codling moth in which to lay eggs. In the 75% frass stage, the female parasitoid probed around in the diet for approximately 13 seconds at various intervals for about the first 4 minutes when placed in the jar. Only 3.1 adult parasitoids emerged per jar. In the 100% frass stage there was no probing and no emergence of adult parasitoids (Table 4.1).

Table 4.1: Probing behaviour of *A. bishopi* and emergence of adult parasitoids from the six different stages of false codling moth development.

Stage	N	Probing	Adult parasitoid emergence	% Parasitised larvae per jar
Eggs only	10	No	No	0
Neonate larvae	10	Yes	No	0
25% Frass	10	Yes	Yes	10.1
50% Frass	10	Yes	Yes	9.3
75% Frass	10	Little	Yes	3.1
100% Frass	10	No	No	0

In the last two stages the majority of the larvae were in the fourth or fifth instars. It is apparent that female parasitoids do not oviposit in these instars. Although table 4.1 shows that the parasitoids do oviposit in fourth instar larvae, the number of emerged parasitoids was low. A reason could be that these larvae are too far into their development cycle and there will not be enough time for the parasitoid to develop inside the host. The length of the females' ovipositor is approximately 5mm in length. Some of the larvae had burrowed too far into the diet and could not be reached by the parasitoid's ovipositor, but others could be reached as they were just below the surface of the diet. From this study it is estimated that the pre-oviposition stage is approximately 1-2 days and the oviposition period is 3-7 days.

The developmental rate of *A. bishopi* varied between sexes. The males had a faster developmental rate than females; this is based on the fact the males emerged before the females (Fig 4.2). More studies are needed in-order to confirm this assumption. The developmental rate of the parasitoid was in synchrony with the development of false codling moth. The false codling moths that were not parasitised emerged 1 to 2 days earlier than the parasitoids, as would be expected as the parasitoids will need the moths to lay eggs and produce larvae for the parasitoids to parasitise. There were 23 days from the time the parent parasitoids were placed in the honey jar to when the first parasitoid progeny emerged. From Day 23 to approximately Day 34 the emergence of parasitoids

was dominated by males. From Day 35 to approximately Day 45 the emergence of parasitoids was dominated by females (Fig 4.2).

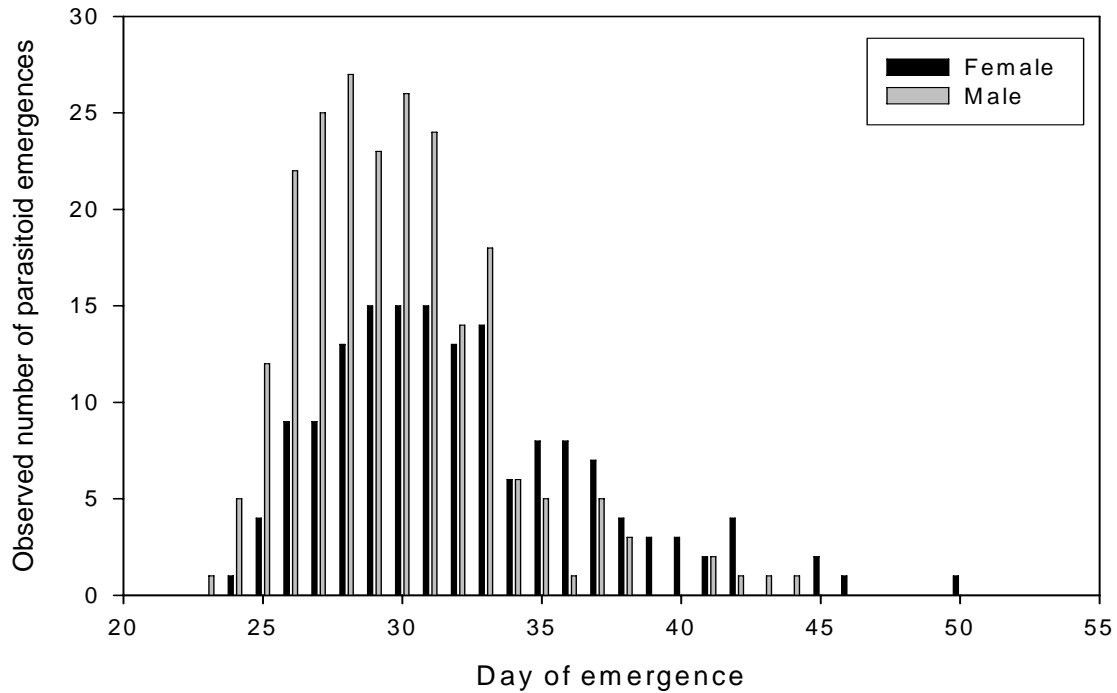


Figure 4.1: The observed number of times parasitoid progeny emergence occurred on the various days after parent parasitoids were placed into the honey jars.

The majority of the parasitoids emerged from Day 26 to Day 33. Even though these days were dominated by the emergence of male parasitoids, a high number of females also emerged. If the female can mate shortly after emerging it will be more beneficial as it will allow her to produce both male and female progeny from the time she starts parasitizing larvae, instead of producing only male progeny if the female has not mated. From an individual point of view it may be because each male wants to be the first to mate, therefore ensuring his genes into the next generation. It is speculated that the females take longer to develop as they are slightly larger in size and because of egg maturation; therefore they emerge over a later and longer period of time compared to the males. Males emerged for up to 44 days and females emerged up to 50 days after the parent parasitoids were placed in the honey jar (Fig 4.2). For the rearing of *A. bishopi*

from the time the parent parasitoids are placed in the jar it takes 23 days for the first parasitoid to emerge and up to 50 days for the last parasitoid to emerge.

4.3.2. Life span

Females of *A. bishopi* can live for 18.5 days and males for 8.25 days under environmental conditions of 27°C, 60% relative humidity and a photoperiod of 12D:12L. When males and females were under conditions of no food or water (N) and water only (W), males lived longer ($p < 0.05$, t-test for independent samples) than females in both cases. When they were under conditions of food and water (FW) or food and water plus the opposite sex (FW♂/♀), females lived longer ($p < 0.05$, t-test for independent samples) than males in both cases (Table 4.2). For males there was a significant difference (Kruskal-Wallis test; $p < 0.05$) in life span under all of the conditions except between FW and FW♀ (Kruskal-Wallis test; $p > 0.05$) (Table 4.3). For females there was a significant difference under all of the conditions except between N and W and between FW and FW♂ (Table 4.4).

Table 4.2: Life spans of male and female *Agathis bishopi* without the host under four different conditions.

Condition	Sex	Sample size	Mean Longevity (days ± S.E.)
no food or water	Males	20	2.45 ± 0.89
	Females	20	1.4 ± 0.59
water only	Males	20	4.2 ± 0.69
	Females	20	3.15 ± 0.58
food and water	Males	20	8.25 ± 1.23
	Females	20	18.45 ± 1.79
food, water and a mate	Males	20	6.55 ± 2.09
	Females	20	18.5 ± 3.1

Table 4.3: Kruskal-Wallis tests of the life span for male parasitoids under different conditions. The numbers in bold show significant differences.

	No food or water	Water only	Food & water
Water only	0.048265		
Food & water	0.000000	0.000046	
Food, water & a female	0.000000	0.027883	0.602050

Table 4.4: Kruskal-Wallis tests of the life span for female parasitoids under different conditions. The numbers in bold show the significant differences.

	No food or water	Water only	Food & water
Water only	0.069550		
Food & water	0.000000	0.000145	
Food, water & a female	0.000000	0.000208	1.000000

For both male and female parasitoids, food and water were important for survival. *Xanthopimpla stemmator* (Thunberg) parasitoids lived significantly longer when provided with food and water (Moore & Kfir 1996). There is no effect of males on females' survival or vice versa. For females, the presence of food and water is possibly more important than for males, as they live longer when both are provided. For females, feeding directly affects reproduction as it determines the amount of eggs matured and the quality of the eggs (Quicke 1997; Rivero & Cass 1999). The female is also slightly bigger than the male, therefore needing more nutrients for survival.

4.4. Conclusion

The rearing of *Agathis bishopi* proved difficult due to viral contamination of the larvae and fungus in the rearing jars, which negatively affected the biological studies because sufficiently large numbers of the parasitoids could not be produced to be able to remove some of them for experiments. This was especially so for female parasitoids as it is the females that sustain the culture.

Other experiments that need to be done include rate and duration of development at various temperatures and host suitability. The rate and duration of development at various temperatures will give an indication of the minimum, optimum and maximum temperatures at which the parasitoid can survive. The host suitability test will give an indication of which other major lepidopteran pests such as *Cryptophlebia batrachopa* (Tortricidae), *C. peltastica* (Tortricidae), *Cydia pomonella* (Tortricidae), can be parasitised by *A. bishopi*. Most parasitoids are host specific, therefore it is best to test other species from the same family or genus.

Agathis bishopi prefers to parasitise false codling moth larvae in instars 2 and 3. This would allow enough time for the parasitoid larvae to develop inside the host before the host pupates. In the citrus orchard, instars 1-3 are most likely the stage of the host most accessible to the parasitoid as they are either on the skin of the fruit or still burrowing through the rind. The other two instars, 4 and 5, would be in the fleshy part of the fruit and the ovipositor of the female parasitoid will be unable to reach the moth larvae. Female parasitoids live longer than males and food and water are important for their survival (Rivero & Cass 1999). It is important for the female to live longer as it is them that find the host larvae and parasitise them before they bore too deeply into the fruit. It is important to release a high number of female *A. bishopi* parasitoids as their life span is short and the shorter the life span of the female, the fewer host larvae she is likely to parasitise compared to a female that lives longer. It is important for the female *A. bishopi* to find food and hosts quickly as her life span is only 14 – 18 days, in which time she needs to parasitise as many larvae as possible to ensure herself progeny in the next generation. Food and water is important for survival of female parasitoids, so it is important that they find these resources shortly after emergence. They will be able to acquire water from what has collected on or in the vegetation and food from nectar-producing flowers.

It is also apparent that the developmental rate of *A. bishopi* is in synchrony with that of the false codling moth, with adult parasitoids emerging 1 or 2 days after the emergence of the moths. Synchrony between the host and its natural enemy is important for the success of biological control. For a parasitoid to be successful, the hosts stage which is to

be parasitised needs to be accessible to the parasitoid. For example, *A. bishopi* being a larval parasitoid, it is important for the larval stage, especially instar 1 – 3, of false codling moth to be present in the field. If this life stage was absent or was present too early or too late in the life cycle of the parasitoid, then it would not be able to parasitise any larvae and would have no effect on the population of false codling moth.

Due to the short life span of female parasitoids and the need for the parasitoid to parasitise the early instars it would be necessary to release the parasitoid early in the season so that it can parasitise larvae at the start of the season to aid in preventing the population of false codling moth from getting out of control. It may be better to release the parasitoids in the evening when it is cool and moist and moths (being nocturnal) are active, since the presence of adult moths may be important for the parasitoid in host location.

The development of false codling moth larvae from neonate to pupal stage was 11-16 days and from pupae to emergence was 15-20 days (Sishuba 2003). The development of parasitised larvae was 12-15 days from parasitism to pupation and 16-20 days for the pupation period (Sishuba 2003). This is an indication that the life cycle of *A. bishopi* is in synchrony with that of the false codling moths; therefore it is potentially a good agent for biological control of false codling moth. If the life cycles of both the parasitoid and the host were not in synchrony it would make survival of the parasitoid difficult as the parasitoids survival depends upon the presence of the life stage of the host that is attacked. A single female moth can live for 2-3 weeks and lay up to 800 eggs in her life time (Stibick 2006). Generally only a few survive as if there are many females laying eggs the larvae will die due to cannibalism and lack of food (Stibick 2006). A female *A. bishopi* parasitoid can also live for 2-3 weeks and under mass-rearing conditions, lay 4 – 23 (Ave. 13.43) eggs per 100 larvae. Based on this and using the figure of 23 eggs per 100 larvae, it can be estimated that 77 larvae per 100 are developing into adult false codling moths. Therefore per 100 false codling moth larvae, approximately 4 parasitoids are required if all the larvae were to be parasitised and over a single female moths life time, approximately 32 wasps would be required to parasitise all the larvae. For a mass-release programme it would be necessary to find out the number of female moths per

hectare in-order to calculate the number of female wasps to release into the field. This also explains the low retrieval rate of the parasitoid from field collected larvae.

5

Biological control and integrated pest management (IPM) of False Codling Moth in citrus

5.1. Biological control

From the beginning of agriculture, insects have been known to reduce production and transmit diseases. A wide variety of methods have been used for the control of insect pests: chemical, cultural, mechanical and biological. The main contemporary method has been the use of chemicals, which began in the 1940s with the production of synthetic pesticides (Rechcigl & Rechcigl 2000). In 1947, experiments were conducted in the Sundays River Valley area with DDT for the control of false codling moth (Hepburn 1948). Later it was discovered that pesticides had a long-term negative effect on the environment, and pests also evolved pesticide resistance. This led to further pest outbreaks and to the need of a more environmentally safe and cost-effective method (Rechcigl & Rechcigl 2000), such as biological control.

Biological control can be defined as ‘the control or regulation of pest populations by natural enemies’. Natural enemies are biological organisms (predators, parasitoids and pathogens) that are able to control the pest population (Rechcigl & Rechcigl 2000; Hoy 2000).

5.1.1. History of biological control

One of the oldest methods of pest control is the use of natural enemies (Rechcigl & Rechcigl 2000). It started with farmers in China and Yemen moving ant colonies between fields to control pests in tree crops, *Citrus* spp. and dates (*Phoenix dactylifera* Linnaeus).

In China spiders were also used for pest control (Huffaker & Messenger 1976; Van Driesche & Bellows 1996; Rechcigl & Rechcigl 2000; van Lenteren 2005). In 1762 the first introduction from one country to another of a natural enemy took place. This involved the mynah bird being introduced into Mauritius from India to control the red locust, *Nomadacris septemfasciata*. In the 1880s cottony-cushion scale, *Icerya purchasi* Maskell, was destroying the citrus industry in southern California. The cottony-cushion scale was found to be native to Australia, so scientists went to Australia to discover its natural enemies. Two control agents were taken back, the parasitic fly, *Cryptochetum iceryae* Williston (Diptera: Cryptochetidae), and a predacious beetle, *Rodolia cardinalis* (Coleoptera: Coccinellidae). The beetle was found to be the more effective control agent, and was then distributed to a few farmers and released into citrus orchards. This led to successful control of cottony-cushion scale (Greathead 1986; Van Driesche & Bellows 1996; Rechcigl & Rechcigl 2000; Mills 2005). In 1897 *R. cardinalis* was introduced into South Africa for the control of cottony-cushion scale on citrus. Subsequently, red scale became a major pest and it was brought under control by the introduction of its natural enemy, *Rhizobius lophanthae* Blaise (Coleoptera: Coccinellidae) (du Toit 1996). Due to such successful projects, biological control has grown more popular.

5.1.2. Targets of biological control

Biological control was initially used to control insects, mites and weeds. It now includes invertebrates, plant pathogens and some vertebrates. Biological control has mainly been used against insect pests. In the world, over 543 species have been targeted by 1200 biological control programmes. The main orders of insects targeted have been Hemiptera, Diptera, Hymenoptera, Coleoptera and Lepidoptera. A few mite families have been targeted, mainly with the introduction of predatory mites (Van Driesche & Bellows 1996). Biological control has also been used for snails, either against the herbivorous species that damage crops or the ones that are intermediate hosts for pathogens. A number of weeds have been targets for biological control in various types of habitats. For example, rats, pigs and rabbits can damage grazing lands. Biological control against these vertebrates can only be undertaken in areas where there is no conflict between the need for control and the need to protect domestic populations. For vertebrate control, native

predators or genetic methods have been adopted for their control (Van Driesche & Bellows 1996).

5.1.3. Types of biological control

There are three types of biological control: conservation of natural enemies, classical biological control and augmentation (Rehcigl & Rehcigl 2000).

Conservation: This involves preserving and enhancing the natural enemies that are already present in the environment by reducing human influences that negatively affect the environment (Van Driesche & Bellows 1996). It involves the use of pest control tactics, habitat management and the use of selective pesticides (Waage & Mills 1982; Viggiani 2000). The use of insecticides, which is often the first method used in pest management, is often the reason why conservation has not been effective, and its involvement in the case of *A. bishopi* needs investigation. Selective insecticides such as genetically engineered crops, insect pathogens and insect growth regulators are more likely to work with conservation. Pest-specific insecticides should target the pest only and not the natural enemy populations (Rehcigl & Rehcigl 2000).

Refuge crops or cover crops help to provide a more suitable habitat for the predators and parasitoid populations (Van Driesche & Bellows 1996; Rehcigl & Rehcigl 2000). Cover crops would provide food sources for adults of *A. bishopi*, which is important in extending the life spans of females in particular (Chapter 4). The *Agathis bishopi* population could be enhanced by the reduction in orchard sanitation (Chapter 2).

Classical biological control: This involves the introduction of natural enemies into new areas (Greathead 1986). Pests and other exotic or non-indigenous insects can be introduced into a new area accidentally. When this occurs the pest does not have a suite of natural enemies to control its population growth, therefore its population increases rapidly and becomes a problem in the area. When this occurs, natural enemies from the pest's region of origin are surveyed and the ones most likely to establish in the new habitat are imported into the new area and released (Van Den Bosch 1971; Van Driesche

& Bellows 1996; Rechcigl & Rechcigl 2000). This method requires a great deal of research on the newly-imported natural enemy or enemies to ensure that there are no further ecological ramifications. The agent being imported and released has to fill the following criteria: 1) it must have a narrow host range; 2) it should originate from a region with a similar climate to the one where it will be released; 3) it should be easy to capture in its native region and easy to rear in the laboratory; 4) most importantly, it must be established whether or not this agent can become a pest in the introduced area (Van Driesche & Bellows 1996; Rechcigl & Rechcigl 2000). Success using this method has been achieved in citrus. For example, research is being conducted on the control of the citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), using an egg parasitoid from the genus *Megaphragma* Timberlake (Hymenoptera: Trichogrammatidae) (Viggiani 2000). In Spain, classical biological control of the citrus leaf miner has resulted in the establishment of the parasitoid *Citrostichus phyllocnistoides* (Narayanan) (Hymenoptera: Eulophidae) that has led to the successful control of the citrus leaf miner (Garcia-Mari *et al.* 2004). To date *Agathis bishopi* is known to only occur in the Sundays River Valley. If this parasitoid is able to be mass-reared it could be released into other citrus producing areas of South Africa i.e. Citrusdal and Nelspruit, but environmental conditions could be problematic as it is different in these regions compared to the Eastern Cape.

Augmentation: This involves mass-rearing of an existing natural enemy in the laboratory, and releasing it into the field (Rechcigl & Rechcigl 2000; Viggiani 2000; Collier & Van Steenwyk 2004). This requires release of the natural enemy at various intervals to maintain the high population levels in the field. This differs from classical biological control in that these natural enemies will have their own natural enemies; therefore it is necessary to keep releasing them into the field (Van Driesche & Bellows 1996; Rechcigl & Rechcigl 2000). The augmentation of the egg parasitoid *Trichogramma* spp. has been studied the most. In Germany the use of the parasitoids *Trichogramma dendrolimi* (Matsumura) and *T. cacoeciae* (Marchal) are used for the control of *Cydia pomonella* (Linnaeus) and *Adoxophies orana* (Fisher von Rosslerstamn), respectively. The parasitoids are released at two-week intervals over the oviposition period of the pest

(Viggiani 2000). The augmentation of the egg parasitoid *Trichogrammatoidea cryptophlebia* Nagaraja, and its release initially resulted in the reduction of the population of false codling moth, but since then results have been variable (Wysoki *et al.* 2002).

Agathis bishopi is indigenous to the Sundays River Valley area; therefore probably possessing its own suite of natural enemies known as hyperparasitoids. No hyperparasitoids were reared from field collected parasitised larvae. From the data in chapter 2, the rate of parasitism of the parasitoid increases half-way through the season (around March). This means that the parasitoid population is building up its numbers later in the season. Augmentation is useful for control agents that build population numbers up only later in the season as it allows the agent to be released early in the season in high numbers, therefore allowing earlier control of the pest (Collier & Steenwyk 2004). It is for this reason that the parasitoid needs to be mass-reared in large numbers and released into the field to achieve successful control of false codling moth.

5.2. Evaluation

This involves determining the influence of the biological agent on the environment and its success in the field (Strand & Obrycki 1996; Wajnberg *et al.* 2001; Hoelmer & Kirk 2005). There are two reasons for the importance of evaluation. Firstly, evaluation is important to distinguish effective biological control agents from less effective ones. Secondly, economic evaluations are important for funds available; data are needed to show the effect of the agent on the pest population in the field to acquire more funding, from the investors of the project, to sustain the project. Evaluations are important to determine the relationship between the natural enemy and the pest in the new environment (Hoelmer & Kirk 2005); progress of the biological control programme, possibly using other natural enemies in conjunction with the present one for further control of the pest; and the effect of the pest's population on the population of the natural enemy (Van Driesche & Bellows 1996).

In the case of parasitoids, the most widely used method of evaluation for the success of the control agent is based upon percentage parasitism. Another method which

can be used is based on physical and chemical barriers (Viggiani 2000) and a third method is the analysis of life tables (Bautista *et al.* 2004). For *Agathis bishopi*, percentage parasitism was used as a measure of the success of the parasitoid. Compared to the other two methods, percentage parasitism gives a more rapid evaluation of the extent to which the parasitoid is controlling the pest, as the other methods are time consuming and complex (Viggiani 2000).

5.3. Integrated pest management

In most cases the pest is part of a complex agro-ecological system, and more than one method is used to control it (Slater 1996). In such a case the use of a biological control agent may be affected by another control method. It is important for all of the control methods used to be integrated into a programme to enhance overall crop production. Many crops have a key pest that must be kept under control i.e. false codling moth on citrus. The various control methods need to be incorporated into an integrated crop management (ICM) programme (Van Driesche & Bellows 1996). An integrated pest management (IPM) programme relies mainly on biological control, but this is often influenced by other management activities (Smith & Pena 2002). For example, the role of parasitoids in citrus orchards can be negatively influenced by the use of general insecticides (Viggiani 2000). It is possible to combine biological and chemical control in an ICM programme by using chemicals with sub-lethal activity; targeting the pest but with no or little effect, except to deprive them of hosts, on biocontrol agents (Wright & Verkerk 1995). Cultural practices and conservation of the field for enhancement of natural enemies are also important in an integrated pest management programme (Smith & Pena 2002). Most systems are pesticide-based, but this can be reduced by pest monitoring and using damage thresholds.

A major problem in the use of pesticides is the evolution of pesticide resistance by the pest. A programme which incorporates the use of all of the control methods can be successful in pest control (Van Driesche & Bellows 1996; Viggiani 2000). For an IPM programme, insect growth regulators can be successful, but more studies are required to

determine if they have any effect on parasitoids (Viggiani 2000). The chemical parathion was replaced with an insect growth regulator, pyriproxyfen (Nemesis) for the control of red scale. This was because parathion was also detrimental to the natural enemies of both red scale and mealybug, therefore not allowing effective biological control to take place (du Toit 1996).

It is important for IPM programmes to become based on biological control. Natural enemies can be incorporated into pest management systems by using the following guidelines: 1) monitor the natural enemy and develop thresholds to aid in determining the abundance of the natural enemy necessary for effective pest control; 2) if further control is required, use pesticides compatible with the biological control programme; 3) cultural practices can also affect the natural enemy population, therefore the type of cultural practice being used must also be compatible with the biocontrol programme; and 4) management of the natural enemy is important, because numbers of may be increased early in the season by inundative or augmentative releases to flood the system and get early control (Van Driesche & Bellows 1996).

The Eastern Cape citrus industry adopted an integrated pest management strategy for the control of red scale, *Aonidiella aurantii* Maskel (du Toit 1996). This was relatively easy compared to other citrus-producing regions in the country as there fewer pests and diseases which require the use of pesticides. Organophosphate and parathion pesticides had a major impact on natural enemies of the pests. Pyriproxyfen had no effect on the parasitoids in the orchard but it had a negative affect on the coccinellid beetles which controlled mealybug and cottony-cushion scale. This led to parathion being used again for the control of these pests, but only in winter or spring. An integrated pest management programme was designed that incorporated the mass-rearing of red scale parasitoids and releasing them when red scale was most vulnerable to parasitism. The pest population was monitored by using a trapping system. This programme was successful as red scale is currently under complete biological control (du Toit 1996). *Agathis bishopi* can be used successfully in an integrated pest management programme. The egg parasitoid, *Trichogrammatoidea* spp. and *A. bishopi* would compliment each

other in such a programme. The egg parasitoid and *A. bishopi* can be released together at the beginning of the season to minimize the number of eggs hatching and to kill larvae that escaped the initial parasitisation. These two parasitoids compliment each other as they attack different life stages of the false codling moth life cycle therefore minimize its population. However, the two compete with each other as they are using the same resource i.e. the host. The higher the egg parasitism the lower the number of available larvae for *A. bishopi* and the higher the larval parasitism the lower the number of eggs available in the next generation for the *Trichogrammatoidea*. For an IPM programme this is not bad as the aim is to reduce the hosts population. In a study on the parasitisation of melon fruit fly (Diptera: Tephritidae) by two parasitoids, *Fopius arisanus* and *Psytalia fletcheri*, it was found that by using two parasitoids there was greater control of the pest (Bautista *et al.* 2004). The false codling moth larvae fall to the soil when they are ready to pupate, therefore the majority of the parasitoids emergence is going to occur from the soil under the trees. Therefore it may be advisable not to practice too much orchard sanitation as one could be removing parasitoids from the system (Chapter 2). As *A. bishopi* is indigenous to the area, it would be necessary to release parasitoids continuously into the field to augment the wild population, which is currently not particularly effective (Chapter 2). With the lifespan of the female being approximately 18 days at 27°C and 60% humidity (Chapter 4), one should be releasing this parasitoid every two weeks to ensure that there is an overlap between generations and that there are always female parasitoids present in the field. It may be advisable to release the parasitoids into the field during the evening. A reason for this is that parasitoids are attracted to areas where the adult moths are present as this is most likely where the eggs or larvae of the host occur. Many of the parasitoids are drawn to the area by pheromones of the adult moths (Van Alphen & Vet 1986). Moths are most active at night and their peak egg laying occurs then (Wysoki *et al.* 2002), therefore releasing the parasitoids prior to this can promote control of the moth. The *Cryptophlebia leucotreta* granulovirus (CrleGV) can also be used as it is specific to false codling moth and therefore will not have a non-target effect on the parasitoids. One thing to take into consideration is the fact that *A. bishopi* has only been found in orchards in the Sundays River Valley area. This may have an influence on the success of this parasitoid as a biological control agent in

other citrus-producing areas in South Africa. Investigation is required on the reason for the absence of *A. bishopi* in these areas.

5.4. Conclusion

Due to the expense of pesticides, problems such as insects developing pesticide resistance (Hogsette 1999), chemical residue on the skin of the fruit and the negative impact on the environment, it has become necessary to find alternative methods for pest control. Integrated pest management offers a suitable solution. The example of the control of red scale is proof that an integrated pest management programme can be successful (du Toit 1996). The use of parasitoids in an integrated pest management system is important, especially in citrus, when the economic threshold of the pest is high. Biological and cultural controls serve to enhance the activity of parasitoids in the citrus orchards (Viggiani 2000).

Agathis bishopi has potential to be used successfully in an integrated pest management programme once the hurdle of mass-rearing has been overcome as thousands of parasitoids will be required per hectare in-order to reduce the population of false codling moth.

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