INVESTIGATION OF THE LARVAL PARASITOIDS OF THE FALSE CODLING MOTH, *CRYPTOPHLEBIA LEUCOTRETA* (MEYRICK) (LEPIDOPTERA: TORTRICIDAE), ON CITRUS IN SOUTH AFRICA

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NOMAHLUBI SISHUBA

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ABSTRACT

The study examined the larval parasitoids of *Cryptophlebia leucotreta* (Meyrick) on citrus in South Africa and aimed to improve the existing rearing techniques of *C. leucotreta* with a view to mass rearing of biological control agents. The biological characteristics of *Agathis bishopi* Nixon (Hymenoptera: Braconidae) were studied, with an emphasis on parasitism rates in the field, host stage preference, developmental rate, life span and offspring sex ratios.

Two larval parasitoids, *A. bishopi* and *Apophua leucotretae* (Wilkinson) (Hymenoptera: Ichneumonidae), and an egg parasitoid, *Trichogrammatoidea cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae), were recorded on *C. leucotreta* on citrus. *A. bishopi* was the more abundant of the larval parasitoids and exhibited density dependent parasitism. The highest parasitism rates were observed in December with up to 38 % in Sundays River Valley and 34 % in Gamtoos River Valley, at a time when the highest false codling moth infestations were observed.

Agathis bishopi was recorded only in the Eastern Cape Province. The sex ratio of *A. bishopi* was biased towards females throughout the study (77% in Gamtoos River Valley and 72% in Sundays River Valley). *Agathis bishopi* is a solitary, koinobiont, larval-pupal endoparasitoid. The species showed a preference for 1st and 2nd instar hosts. Females regulate the sex of their progeny according to the size and larval stage of the host, ovipositing unfertilised eggs in younger, smaller larvae (1st instars) and fertilised eggs in

older, larger larvae (2^{nd} instars). The developmental rate of *A. bishopi* is in synchrony with that of the moth and adults emerge when adult moths that have escaped parasitism emerge.

Agathis bishopi and *T. cryptophlebiae* compliment each other because they have different niches and strategies of attack. Integrating *A. bishopi* and *T. cryptophlebiae* into the management of citrus orchards has potential to suppress false codling moth.

Larger rearing containers seemed ideal for large-scale rearing of false codling moth. A higher percentage of adults was obtained from larvae reared in larger containers than in smaller ones. The width of the sponges used as stoppers prevented escape of the larvae. Media prepared in larger containers are easier and simpler to prepare than in smaller ones, thus eliminating many precautions otherwise necessary to prevent contamination. Moth production was greatly reduced by the high concentration of Sporekill used for egg decontamination.

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1 BACKGROUND REVIEW AND PROJECT PROPOSAL: FALSE CODLING MOTH, *CRYPTOPHLEBIA LEUCOTRETA* (MEYRICK) (LEPIDOPTERA: TORTRICIDAE), ON CITRUS

1.1 Introduction

False codling moth, *Cryptophlebia leucotreta* (Meyrick), is native to South Africa and its injurious nature on citrus has been known since the early 1900s (Gunn, 1921). Fortunately, however, it is one of those insects that is not equally troublesome every year, and with oranges, at least, a season of considerable loss may be followed by one in which the damage is comparatively slight.

False codling moth derived its name because it greatly resembles the true codling moth, *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae), in the general appearance of its life stages, in its habits of feeding singly on the flesh of fruits, and in the nature of its damage (Annecke & Moran, 1982). However, although the true codling moth is essentially a pest of apples, pears and quinces, it is not known to attack citrus fruits. The false codling moth is more particularly a pest of oranges and mandarins, but does not attack apples, pears and quinces. There are, however, some fruits that are shared by both insects, such as walnuts and, to a minor extent, certain stone fruits (Gunn, 1921). In mixed gardens where both insects are equally common, no interchange of their principal host fruits has ever been observed (Annecke & Moran, 1982).

1.2 Taxonomy

Fuller (1901) was the first to describe the nature and habits of the false codling moth on citrus. He described its development, habits, appearance, nature of damage it caused in KwaZulu Natal and the methods of control. He referred to this moth as the Natal codling

moth, *Carpocapsa* sp.. Eight years later it was recorded as the Orange codling moth, *Enarmonia batrachopa*, from the Transvaal by Howard (1909). Thereafter it was generally referred to as the false codling moth and was described taxonomically by Meyrick (1912) as *Argyroploce leucotreta* (Meyrick) (Eucosmidae, Olethreutidae). Later, Clarke (1958) transferred it to *Cryptophlebia leucotreta* (Meyrick). False codling moth is also known as the tea seed borer and the red bollworm of cotton (Newton, 1998).

1.3 Distribution

The false codling moth overlaps in its distribution and host range with three members of the same genus in southern Africa, the litchi moth, *C. peltastica* (Meyrick), the macadamia nut borer, *C. batrachopa* (Meyrick) (Newton & Crause, 1990) and *C. rhizophorae* Vari (Vári, 1981). This moth is known to occur in citrus in South Africa, Zimbabwe, Mozambique and Swaziland (Hepburn, 1947; Stofberg, 1954; Pearson & Darling, 1958). The Commonwealth Institute of Entomology supplied a full account of the distribution of the false codling moth throughout sub-Saharan Africa and the nearby islands (CIE, 1976). It has been recorded from Côte d'Ivoire (Pearson & Darling, 1958), Kenya (Gunn, 1921), and Uganda (Reed, 1974) as a pest of cotton; and from Malawi (LaCroix & Tindwa, 1986) as a pest of cotton, citrus and macadamia. False codling moth is an important pest of citrus in all of the major citrus producing areas of South Africa (Catling & Ascheborn, 1974). Outside Africa, it occurs as a pest of maize, peaches and castor oil in Mauritius (Moore, pers. comm., 2002).

1.4 Host plants

False codling moth occurs as a pest of a wide range of crop plants throughout sub-Saharan Africa and nearby islands in the Atlantic and Indian Oceans (CIBC, 1984). The pest has a catholic range of wild and cultivated host plants but has become notorious for its infestations of cotton in moist equatorial areas (Pearson & Darling, 1958; Reed, 1974), citrus in Southern Africa (Catling & Ascheborn, 1974), maize in West Africa (Schulthess *et al.*, 1991) and as a serious borer of macadamia nuts in Malawi (LaCroix & Tindwa, 1986). This wide range of wild and cultivated host plants, together with mild tropical and subtropical winters and the

presence of out-of-season fruits, ensures that the pest is a year-round threat to its crop host plants in most areas of its distribution (Newton, 1990b).

This moth has been recorded from 21 cultivated and 14 indigenous wild host plants in southern Africa alone (Schwartz, 1981). Of the cultivated crops, it is particularly severe on citrus, but also attacks many other deciduous, tropical and subtropical fruits (Newton, 1998). It is also known as a pest of acorns, walnuts, olives, tea seeds, peach, oak, sorghum and almonds (Catling & Ascheborn, 1978). It attacks cotton in Zimbabwe and Central Africa, and feeds on the heads of sorghum and the cobs and stems of maize in Central Africa (Pearson & Darling, 1958). In South Africa it is also known as a pest of macadamias (LaCroix & Tindwa, 1986). In the case of oranges, the navel cultivar appears to be the most heavily attacked. Mandarins are also highly susceptible, followed by grapefruit. In lemons and limes, larval development is rarely, if ever, completed. Perhaps this is because of their greater acidity and excessive juiciness (Newton, 1990a).

There is no record of the false codling moth attacking cotton in South Africa (Newton, 1998). Pearson & Darling (1958) suggested that under South African and Zimbabwean conditions the moth prefers the ripening citrus fruits during late summer and winter when cotton bolls might be susceptible. They speculated that this might be because the cotton crops of these countries have a single, early, rather steep bolling curve, succeeded by a cool, dry season, and it is likely that there would be insufficient time for this pest to build up a noticeable infestation during the short period when suitable food is available. They suggested that it might also be because it has no diapause and must therefore breed continuously in order to survive, and in regions with long, dry seasons it is at a disadvantage unless an irrigated crop is available that provides an abundant food supply in the dry season.

1.5 Life history and habits in citrus

1.5.1 Egg

When first laid the egg is a translucent cream coloured, flat, oval-shaped disc with a granulated surface. It measures approximately 1 mm in diameter (Daiber, 1979a). In laboratory cultures, eggs are laid on any clean flat surface, whereas on oranges they are laid

inconspicuously in depressions of the rind (Newton, 1998). Oviposition on physically damaged and early ripening oranges is much greater than on healthy navel or Valencia oranges in a normal stage of development (Newton, 1989).

The eggs are laid singly and are generally placed a little distance from each other, although occasionally two eggs may be found touching one another. The majority of eggs are deposited on the rind of citrus fruit, but some are placed on leaves and exceptionally a few can be found on the twigs (Newton, 1998). Up to 65 eggs have been observed on a single fruit but such high numbers are rare (Stofberg, 1954). As population size increases in citrus, not only are more fruits infested but also there is a tendency for more eggs to be laid on each fruit (Catling & Ascheborn, 1978). Some days after being laid, the fertile egg turns reddish and shortly before hatching it turns black as the head capsule forms and becomes visible through the transparent egg-shell (Daiber, 1979a).

Hatching occurs at all times during the day (Daiber, 1979a). On citrus the incubation period is 9-12 days in winter and 6-8 days in summer (Newton, 1998). In laboratory cultures the incubation period varies considerably depending on the temperature the eggs are exposed to. If kept in a constant environment room at 25°C the incubation period takes 3-5 days (Daiber, 1979a). The eggs are susceptible to parasitism by trichogrammatid parasitoids for about half of its typical life span of 5 to 12 days. When parasitised, the egg appears quite black.

1.5.2 Larva

When emerging, the larva eats its way out of the egg-shell (Stofberg, 1954). The larva is a typical lepidopterous caterpillar and is at first creamy-white with a dark brownish-black head capsule and is about 1.4 mm in length. With age the body takes on a characteristic pinkish-red colour, which is paler ventrally. The fully-grown larva is 15 to 20 mm in length (Catling & Ascheborn, 1978). The legs and prolegs are the same colour as the abdomen, and there are inconspicuous white hairs on the body (Fuller, 1901). The colour of the head may vary from brown, through dark brown to almost black. The neck shield ranges from brown to dark brown. An attempt to find distinct larval characters for easy identification was not successful (Stofberg, 1948).

This species has five larval instars, of which the first is extremely delicate and suffers high mortality. Low humidity causes egg and first instar mortality in laboratory eultures, while low winter temperatures are lethal to these life stages in the field (Catling & Ascheborn, 1978). The young larva is often cannibalistic towards eggs and larvae and this behaviour has been associated with the fact that rarely more than one larva completes its development in a single fruit (Annecke & Moran, 1982). However, many hundreds of larvae can be reared in single containers containing artificial diet in laboratory cultures.

The first instar finds a suitable host fruit and a suitable entry point. In navel oranges, it seems to prefer the navel end (Newton, 1998). The last instar leaves the fruit through a conspicuous, frass-filled exit hole and commonly drops to the ground on a silken thread, or else emerges after the fruit has fallen (Catling & Ascheborn, 1978). Once a fruit has dropped to the orchard floor, it plays host to a wide range of fungal invaders, and vertebrate and invertebrate scavenger feeders. Mature larvae escape relatively quickly to the pupal stage in surface litter, but immature larvae in already decaying fruit are more likely to succumb to these causes of mortality (Newton, 1989).

On citrus the larval development is completed in 35-67 days in winter and 25-35 days in summer (Newton, 1998). Daiber (1979b) found that poor food quality increases larval mortality and the duration of the larval stage in laboratory cultures.

1.5.3 Pupa

The pupal stage consists of two sub-stages, the pre-pupal and the pupal stage (Stofberg, 1954). On the ground the larva spins a silky cocoon of trash and soil particles. Larvae in artificial media form cocoons in the cotton wool stopper fitted into the opening of the rearing container (Fig 4.1). Inside the fresh cocoon the pre-pupa is found, this being the fifth instar larva that has stopped feeding. The pre-pupa molts into a pupa, which is at first beige and soft skinned until the chitin hardens and becomes dark brown. Pupae from which female moths emerge are larger than those from which male moths emerge (Daiber, 1979c). The abdominal segments are flexible and each has a number of small spines. Those on the terminal segment are considerably longer and thicker than on the others (Gunn, 1921).

Daiber (1979c) found that the average duration of the pre-pupal stage was 2 days at 25°C. He also found that the duration of the pupal stage for male moths was longer than that for female moths: females taking 11 days and males taking 12 days at 25°C. In the field, the pupal stage lasts from 12 to 24 days in summer and from about 29 to 40 days in winter (Stofberg, 1954).

1.5.4 Adult

The adult stage commences when the moth emerges from the cocoon. The adult moths have a superficial likeness in size and tone of colour to the real codling moth except that the false codling moth lacks the coppery patches on the wings that are present in the true codling moth (Fuller, 1901). The adult is a rather small, inconspicuous, dark brown to grey moth. It is active at night from about dusk onwards and is therefore seldom actually seen in citrus orchards (Annecke & Moran, 1982). The forewings are mottled while the hindwings have a paler, more even colour and are fringed with hairs. The male is smaller than the female and is 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 (Catling & Ascheborn, 1978).

The sex ratio of field populations is close to unity. Females are reported to mate shortly after emergence with pre-oviposition periods of 5-6 days in the field and 1-2 days in laboratory cultures (Newton, 1998). However, Daiber (1980) found that the pre-oviposition period could vary from 1-22 days with 50% of the eggs being laid 6-23 days later at 25°C and 10°C respectively. In the field, adults live for a week or two (Annecke & Moran, 1982). In captivity, the average life span of a male can vary from 34 days at 15°C to 14 days at 25°C, and that of females from 48 days at 15°C to 16 days at 25°C (Daiber, 1980). Some authors observed that the adult feeds little or not at all, while others contend that water is essential and may extend longevity (Catling & Ascheborn, 1978).

Total development period is approximately 2.5 to 4 months in winter and 1.5 to 2 months in summer, with five to six poorly defined overlapping generations per year (Newton, 1998). Daiber (1980) observed generation times in artificial media of 32 and 114 days at average ambient temperatures of 26.3°C and 13.7°C, respectively. Stofberg (1954) recorded 37 days

for the shortest and 107 days for the longest generation time in the field. There is no diapause.

1.6 Seasonal history

Little is known about the seasonal history of the false codling moth. The insect breeds throughout the year in groves where out-of-season fruit is present. The pest can maintain itself in orange groves consisting of navel and Valencia oranges since larvae escaping just prior to the picking of navel oranges in May or June have a cocoon stage lasting about 35 days, thus emerging as moths during July to August to oviposit on Valencia oranges, which normally become infested from June onward (Stofberg, 1954). The first eggs are laid on in-season fruit between October and December, and reach large population numbers towards late summer and then gradually decline with the onset of low winter temperatures (Newton, 1998). However, recent studies by Moore & Richards (pers. comm., 2002) in the Eastern Cape Province show that false codling moth infestations generally peak in December. Where no out-of-season fruit is present populations are extremely low or even absent until the setting of the new crop the following season (Newton, 1998).

1.7 Nature and extent of injury

The false codling moth female lays most of its eggs directly on the surface of citrus, litchis and guavas. Upon emergence from the egg, the young larva bores its way into the rind and, in most instances, into the centre of the fruit. The entrance is rendered more or less conspicuous by the frass thrown out by the insect. When fully-grown, the larva bores its way out of the fruit to seek a site for pupation (Newton, 1998). Around the point of infestation the rind takes on a yellowish-brown colour as the tissue decays and collapses. In the early stages of decay the symptoms are relatively easy to recognize. Infested green fruit ripen prematurely and the wounding process leads to fruit abscission. Fruit already showing colour-break tends to be a deeper tone than usual and the area around the point of entry tends to be paler than the background colouration (Newton, 1990a).

Oviposition on physically damaged and early-ripening fruits is much greater than on healthy fruit in a normal stage of development. Owing to the physiological state of these fruits, they tend to abscise earlier than fruits which are at a normal state of development at the time of penetration by the larva, and there maybe insufficient time for complete larval development. Once a fruit has dropped to the orchard floor, it plays host to a wide range of fungal invaders, and vertebrate and invertebrate feeders. Immature larvae in already decaying fruit are more likely to succumb to these causes of mortality (Newton, 1989).

Oranges that are infested become mouldy through the development of spores that become lodged in the hole made by the larva in the rind. Since fungi can invade other lesions, the only sure procedure to determine if a fruit has been infested or not is by cutting it open to examine it for the presence of a larva or the granulate excreta produced by larvae. This becomes more difficult as decay develops (Newton, 1990a). It is not possible to form a correct estimate of the losses sustained by citrus growers through the attack of the false codling moth because the damage is highly variable from orchard to orchard and from season to season. The opinion amongst some is that the loss ranges from below 2% to as high as 90% (Newton, 1998).

1.8 Control measures

False codling moth is an extremely difficult pest to control. Eggs are laid continually during the fruiting period of citrus and upon emergence the larva immediately bores into the fruit making it hard to reach as a target (Newton, 1998).

1.8.1 Sanitation

There has been no basic change in the orchard sanitation measures suggested by Fuller in 1901. Georgala (1969) was of the opinion that orchard sanitation measures are expensive and laborious and do not guarantee satisfactory control in some seasons. The aim of orchard sanitation is to interrupt the completion of the insect's life cycle by preventing mature larvae entering the pupal stage. This involves the removal of infested fruits, from both under and on

the trees, at least once, but preferably twice, a week commencing as soon as the first infested fruit drop. The fruit must be buried or else pulped in a hammer mill and dried out in trenches.

Sanitation is facilitated by skirting the trees (Newton, 1990a). Schwartz (1974) found that the sanitation programme should be carried out from the beginning of November in all areas where the false codling moth occurs, in order to achieve satisfactory results. Sanitation programmes do not end at harvest. The programme is complete only after all fruit rejected during picking, and all out-of-season fruit left on the tree, have been removed (Newton, 1990a). Previous studies show that regular and vigorous orchard sanitation, particularly of navel oranges, reduces damage by the false codling moth, especially during seasons of high infestation rates (Ullyett, 1939; Hepburn, 1947; Stofberg, 1954). Ullyett (1939) observed that larval parasitoids of this pest are adversely affected by orchard sanitation.

1.8.2 Disposal of infested fruit

It is imperative to effectively dispose of the fruit that is removed from orchards during the sanitation process as these can serve as a source of infestation for false codling moth (Schwartz, 1974). There are several ways in which the infested fruit can be destroyed.

1. **Burying**

   This is an efficient method if carefully carried out (Stofberg, 1939). It is suitable for large plantings (Newton, 1990a). It involves removing infested fruits from the orchard and dumping them in a deep trench. The infested fruit must immediately be covered with a layer of compacted earth to a depth of 30 to 40 cm or more. In this way false codling moth larvae and pupae are prevented from escaping to the soil surface through the solid earth (Stofberg, 1939; Hepburn, 1947; Georgala, 1969; Newton, 1990a). The system of dumping infested fruit in a pit and only covering the fruit when the pit is full is a dangerous one (Stofberg, 1939). The infested fruit should be buried as far from the orchard as possible. Infested fruit can be collected and stored in fertiliser bags. However, fruit should be kept for at least four weeks before being discarded, so that complete decay can take place (Schwartz, 1974).

2. Pulping

Crushing of the whole fruit is not effective because they are not easily broken and larvae often pass through to the ground, without being killed, where they can pupate. It is advisable to cut fruit before pulping with a hammermill (Stofberg, 1939; Hepburn, 1947; Schwartz, 1974). The remains of the pulped fruit should be buried in a deep trench or spread out in the sun to dry (Schwartz, 1974). If the pulp is simply discarded on the orchard floor, it can become infected with green mould during periods of rain. This infection can increase the spore load on the crop and the danger of fruit wastage (Georgala, 1969).

3. Immersion in water

This method is especially suited to the small citrus grower (Stofberg, 1939). It involves pouring a thin layer of old engine oil into drums that are half-filled with water, and immersing the stung fruit in the drums for a week (Hepburn, 1947; Schwartz, 1974). The drums should be tightly closed with metal lids. Care should be taken not to throw the liquid near citrus trees because the products of fermentation produced in the drums may adversely affect the trees through their roots. Infested fruit should also not be thrown into river pools because larvae can escape and swim ashore (Hepburn, 1947).

1.8.3 Chemical control

Currently five products, Cypermethrin (25 ml/100 litres), Alsytin (10 or 20 ml/100 litres), Nomolt (40 ml/100 litres), Penncap-M (100 ml/100 litres) and Meothrin (30 ml/100 litres), are registered for the control of false codling moth on citrus (Moore, pers comm., 2002). However, none of these products are entirely compatible with an integrated pest management programme. They are all detrimental to natural enemies and therefore prone to causing secondary pest repercussions and the moth has developed resistance to Alsytin (Hofmeyr, 1983a; Hofmeyr, 1983b; Hofmeyr, 1984; Pringle & Hofmeyr, 1998; Moore, 2002b).

1.8.4 Biological control

1. Egg parasitoids

The focal point of biological control of this pest has been the trichogrammatid egg parasitoids (Newton, 1988a). Reference to egg parasitism was first made by Fuller (1901) who reported that 80-90% of the eggs may be parasitised at some times of the year. In South Africa, Catling & Ascheborn (1974) observed up to 80% egg parasitism of the false codling moth by *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatoidae) (later correctly identified as *T. cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatoidae) in late summer. In another orchard, they observed that the parasitoid was absent early in the season when *C. leucotreta* was active (November to January) but increased rapidly and reached 59-89% parasitism between February and April when host populations were steadily declining. They concluded that although the level of egg parasitism was irregular and tended to be absent or at very low levels in the early part of the season, the parasitoid helps to limit host populations in the latter part of the season, and speculated on the usefulness of releasing large numbers of parasitoids in early November when *C. leucotreta* populations begin to increase in the developing fruit. However, Newton (1988a) concluded that there was little evidence that rates of egg parasitism were dependent on host density, suggesting that native parasitism does not play a regulatory role at the orchard level.

Following the recommendation by Catling & Ascheborn (1974), inundative releases of *T. cryptophlebiae* led to crop savings that were regarded as economically cost-effective (Schwartz, 1980; Newton & Odendaal, 1990). Although the first experimental trials with releases of *T. cryptophlebiae* against *C. leucotreta* in citrus were successful, commercial applications produced variable and unpredictable results (Newton, 1990).

Newton (1988b) found that rates of parasitism at the tops or bottoms of trees or at the four cardinal aspects were not significantly different overall but should have been to compensate for the linear increase in numbers of host eggs with height, and the larger numbers on the warmer northern and eastern sides of the trees. Newton (1988a) found that effective parasitism (when all eggs on a fruit are parasitised) was generally lower than total percentage parasitism, and as pest population size increased, and more large

patches occurred, the difference between these two measures became greater and the degree of fruit protection decreased. Therefore, both aspects of parasitoid searching at different heights and aspects of the tree, and its functional response to patchy distributions of host eggs seemed to be responsible for this.

2. Larval parasitoids

Five larval, one egg-larval and three larval-pupal parasitoids of the false codling moth in citrus are known to occur in South Africa (Table 1.1).

Apanteles leucotretae Ullyett (Hymenoptera: Braconidae) and *Apanteles typhon* Nixon (Hymenoptera: Braconidae) are both internal larval parasitoids. They are not host specific and *A. leucotretae* has been recorded as a parasitoid of the codling moth (Prinsloo, 1984). These two species are known to occur only in the former Transvaal Province.

Apophua leucotretae (Wilkinson) (Hymenoptera: Ichneumonidae) was originally described as *Glypta leucotretae* (Wilkinson) (Hymenoptera: Ichneumonidae) from Zimbabwe, where it is a well-known parasitoid of the false codling moth on citrus and it is interesting to note that Ullyett (1939) suggested that it be introduced into South Africa. It is a primary larval parasitoid, which appears to be exclusively parasitic of the false codling moth. It is known to occur in limited areas of Mpumalanga and in Zimbabwe (Prinsloo, 1984).

Nothing is known about the biology of *Oxycoryphe edax* (Waterston) (Hymenoptera: Chalcididae) except that it is a larval parasitoid (Newton, 1998). This parasitoid has only been recorded from the former Transvaal Province (Prinsloo, 1984).

The egg-larval parasitoid *Chelonus curvimaculatus* Cameron (Hymenoptera: Braconidae) is also known to parasitise the potato tuber moth. The life cycle of this parasitoid is synchronised with that of its hosts. The adult parasitoid emerges when the adult moths, which have escaped parasitism, emerge. When the host has a diapause, the synchrony is maintained and adults of *C. curvimaculatus* may emerge after 3 years from a diapausing host. This species is wide spread and occurs in potato fields throughout the country

(Broodryk, 1969), but has only been recorded from citrus in certain parts of the former Transvaal Province.

Agathis bishopi (Nixon) (Hymenoptera: Braconidae) (Figs 2.1 & 2.2) superficially resembles *Agathis leucotretae* (Nixon) (Hymenoptera: Braconidae), known from Zimbabwe, which is also a parasitoid of the false codling moth (Prinsloo, 1984). *Agathis bishopi* was originally described as *Microdus* sp. from materials collected in the Eastern Cape Province (Ullyett, 1939). It has been recorded as a larval parasitoid of false codling moth (Prinsloo, 1984), but it was discovered in this study that it is actually a larval-pupal parasitoid (Chapter 2).

3. Predators

There are very few records of the predators of false codling moth, suggesting either an almost complete neglect of this group of natural enemies, or their ineffectiveness. An *Orius* species (Anthocoridae) and *Rhynocoris albopunctatus* (Stål) (Reduviidae) have been recorded as predators of this pest, as well as undetermined mites, ants and shrews (CIBC, 1984). Bownes (2002) showed that spiders and ants are also important predators of this pest.

4. Pathogens

Bacterial and viral infections of false codling moth larvae have been recorded in citrus (Catling & Ascheborn, 1974). Gunn (1921) observed that the pupae are killed by fungus in wet seasons. Entomopathogenic viruses have been found infecting the false codling moth in West Africa and have been studied as possible biocontrol agents. Mixed infections of a granulosis virus and a cytoplasmic polyhedrosis virus were found to cause heavy larval mortality (CIBC, 1984). Moore (2002a) found that *Cryptophlebia leucotreta* Granulovirus (CrleGV) occurs naturally in false codling moth larvae. He also recorded up to 60% reduction of false codling moth infestation of navel oranges by application of a CrleGV suspension as a spray. Begemann (1989) isolated *Beauveria bassiana* (Balsamo) from false codling moth pupae. He achieved 97% mortality of larvae in laboratory trials.

Field trials were not as successful, most probably due to low relative humidity. Moore (2002a) found *Aspergillus alliceus* Thom & Churych emend. Fennel & Warcup attacking false codling moth larvae in the field.

1.9 Laboratory rearing of the false codling moth

In many studies of the false codling moth, such as production of natural enemies for biological control, chemical and biological insecticide screening, execution of a sterile insect technique (SIT) programme and production of a pathogen as a microbial biocontrol agent, it is important to have a reliable and abundant supply of false codling moth larvae. This can be achieved by establishing a laboratory colony of the moth. A laboratory colony can be established by mass rearing field-collected larvae on an artificial medium or by obtaining insects from an already established laboratory colony.

The first method of mass rearing the false codling moth on an artificial medium was developed by Ripley *et al.* (1939) and slightly modified by Theron (1947). The insect develops satisfactorily in this medium, but the difficulty is keeping it free from contaminants that destroy the larvae. Bot (1965) developed a medium that is kept aseptic with methyl p-hydroxybenzoate. Moore & Richards (pers. comm., 2001) developed a similar medium. Both of these media are based on maize meal to which other nutrients and antibiotic agents are added. The preparation of these diets and methods for rearing false codling moth larvae are discussed in detail in Chapter 3.

1.10 Research objectives

Since chemical control of the false codling moth with currently registered insecticides has proved to be difficult and often ineffective, and since egg parasitism rates are highly variable, more research is needed on the natural enemy complex of this pest and related species from other parts of the world in order to develop integrated control programmes. The objective of this project was to examine and describe the larval parasitoids that are associated with this pest in geographically distinct citrus producing areas of the country: Citrusdal, Gamtoos River Valley, Sundays River Valley and Nelspruit. This involved establishing larval

parasitism rates in each of the four study sites and mass rearing techniques of the parasitoids that show sufficient potential for exploitation. Once the mass rearing techniques of the parasitoids had been established, experiments were conducted to examine their biology.

In order to carry out these investigations, it was very important to have reliable and large numbers of the false codling moth larvae. This was achieved by mass rearing the insect on an artificial rearing medium. Systems and techniques currently used for mass rearing this pest are labour intensive, and therefore expensive and time consuming. A final part of this project was to modify and simplify the existing rearing medium to formulate a less labour-intensive and more efficient rearing medium.

Table 1.1 Parasitoids associated with the false codling moth, *Cryptophlebia leucotreta*, on citrus in South Africa.

Parasitoid	Distribution	Reference
Egg parasitoids		
Trichogrammatoidea cryptophlebiae Nagaraja	Wide spread	Newton, 1998
Larval parasitoids		
Apanteles typhon Nixon	Former Transvaal Province	Prinsloo, 1984
Apanteles leucotretae Ullyett	Former Transvaal Province	Prinsloo, 1984
Apophua (=*Glypta*) *leucotretae* (Wilkinson)	Former Eastern Transvaal Province	Prinsloo, 1984
Oxycoryphe edax (Waterston)	Former Transvaal Province	Prinsloo, 1984
Phanerotoma curvicarinata Cameron	Former Transvaal Province	Ullyett, 1939
Egg-larval parasitoids		
Chelonus curvimaculatus Cameron	Wide spread	Broodryk, 1969
Larval-pupal parasitoids		
**Agathis bishopi* (Nixon)	Eastern Cape Province	Prinsloo, 1984
***Mintha* sp.	Unknown	Begemann, 1994
***Silbavirescens* sp.	Eastern Cape Province	Moore, pers. comm., 2002

*Previously recorded as a larval parasitoid but discovered in this study to be a larval-pupal parasitoid (see Chapter 2).

**Dipterans, whereas all the other parasitoids are Hymenopterans.

2 INVESTIGATION OF THE LARVAL PARASITOIDS OF THE FALSE CODLING MOTH, *CRYPTOPHLEBIA LEUCOTRETA* (MEYRICK), (LEPIDOPTERA: TORTRICIDAE), ON CITRUS

2.1 Introduction

False codling moth infestations lead to premature fruit drop and waste in export fruit. It is one of the most important citrus pests for which no completely satisfactory control measures exist.

While most research effort is directed at biological control and cultural methods of suppression, growers suffering from heavy losses seek an immediate solution (Newton, 1987). However, attempts to control the moth with insecticides have lead to secondary pest repercussions and development of pesticide resistance in the moth (p. 10). The trichogrammatid egg parasitoids have been the focal point of biological control of this pest (pp. 11). Larval parasitoids of the false codling moth have been discussed by Ullyett (1939), CIBC (1984) and Prinsloo (1984). They speculated that, perhaps due to the inaccessibility of the host, they do not seem to be important mortality factors. Ullyett (1939) found that many of the larval parasitoids were poorly distributed and suggested the exchange of parasitoids between the different provinces of South Africa. Table 1.1 lists all the parasitoids known to parasitise the false codling moth on citrus in South Africa.

Knowledge of the natural enemies of a pest species and the control they exert is important when considering commercial control measures. This chapter reports on surveys of the larval parasitoids of the false codling moth on citrus in four geographically distinct areas of the country.

2.2 Materials and Methods

2.2.1 Egg parasitism

The data used for analysis of egg parasitism were obtained from surveys conducted by Moore & Richards (pers. comm., 2002) on navel orchards in the Eastern Cape Province. The data were used to investigate the interaction and relationship between egg and larval parasitoids in their natural environment. Orchard 3 on Cormic Farm, Gamtoos River Valley, and Orchard 28 on Moedskepvlakte Farm, Sundays River Valley, were used as the study sites. Both orchards were also used for larval parasitoid surveys. The cultivar, rootstock, tree age and geographic co-ordinates of the farms used as the study sites are presented in Table 2.1.

Ten adjacent trees were selected and marked in the middle of each orchard. Random inspections of fruit for false codling moth eggs were made weekly. Once eggs were detected, counts of egg numbers on 10 fruits on each data tree were conducted weekly. Parasitised eggs were recorded as such. Only viable eggs were considered. Simultaneously, fruit that had dropped from the data trees was collected, inspected and dissected, and cause of drop established. This was continued until harvest.

2.2.2 Larval parasitism

To investigate larval parasitism, navel oranges were collected from Citrusdal, Nelspruit, Gamtoos River Valley and Sundays River Valley from December 2001 to May 2002. Four navel orange orchards were used as the study sites and are given in Table 2.1. The collection dates, number of fruits collected, percentage of infested fruits obtained and total number of each instar obtained are given in Table 2.2.

Navel oranges were collected once every month from each orchard. Collections from Citrusdal were made by Hendrik Hofmeyr, and from Nelspruit by Peter Stephen (both of Citrus Research International), and were then couriered to me at Citrus Research International in Port Elizabeth for analysis. Most of the fruit was collected from the ground but was also removed from trees if numbers were low on the ground. In the months with high fruit drop, only obviously infested fruit were collected, i.e. fruit with a frass filled hole and

those with a yellowish-brown colour around the point of infestation. Random collections were made in the months with low fruit drop.

An artificial rearing medium was prepared in a glass dish (diameter 22 cm). The constituents and composition of the artificial medium used to supply the nutritional needs of the larvae are given in Table 2.3. The dish was covered with foil and autoclaved at 121°C for 20 minutes and then allowed to cool. The rearing medium was transferred to glass vials (diameter 25 mm; height 75 mm) by plugging the medium from the dish using a glass vial. Approximately 40 g of the medium was transferred to each glass vial.

Larvae were collected from infested fruits by thinly slicing the rind around the point of infestation until the larva was spotted. The larva was then removed from the fruit and transferred to a glass vial containing the prepared rearing medium. A tightly fitting cotton wool plug was inserted into the opening of each glass vial. The life stage of the larva in each vial and the orchard from which it was collected were recorded on the vial. These were monitored daily for parasitoid emergence. The life stage from which the parasitoid emerged and the sex of the parasitoid were recorded. Percentage parasitism was calculated using the following formula:

$$\% \text{ Parasitism} = \frac{\text{Total number of parasitoids}}{\text{Total number of larvae}} \times \frac{100}{1}$$

2.3 Results and Discussion

2.3.1 Egg parasitism

Fig 2.3 shows that in Sundays River Valley there was no egg parasitoid activity for the first six weeks when false codling moth infestations were decreasing steadily. Egg parasitism was first noticed on January 24 and increased steadily within the next five weeks to reach a level of 90% on February 27. Egg parasitism continued at high levels for the rest of the season varying from 50 to 100 % with a mean of 69% for the February to May period. In Gamtoos River Valley (Fig 2.4), the egg parasitoid was either absent or at low levels for the first fifteen weeks of recording when false codling moth infestations were declining steadily. Egg

parasitism continued at varying levels to reach a level of 80% on April 22. No parasitoid activity was observed during May. The highest peaks of false codling moth infestation were observed in December and early January in both orchards.

2.3.2 Larval parasitism

Two larval parasitoids were reared from *C. leucotreta*. *Agathis bishopi* Nixon (Figs 2.1 & 2.2) was reared from larvae collected from Sundays River Valley and Gamtoos River Valley, Eastern Cape Province, whereas *Apophua leucotretae* (Wilkinson) (Prinsloo, 1984) was reared from larvae collected from Heksrivier, Citrusdal. No parasitoids were reared from larvae collected from Crocodile Valley, Nelspruit (Fig 2.5).

Agathis bishopi was the more abundant of the two. The sex ratio was biased towards the females in both Sundays River Valley (2.3:1) and Gamtoos River Valley (2.6:1). No parasitoids emerged from larvae that were older than the fourth instar when collected (Table 2.4), implying that only the early instars are parasitised. It is possible, though, that the parasitoid retards host development and the larvae appeared younger than they really were. *Agathis bishopi* always emerged from the pupae. Parasitoids that oviposit in host larvae but continue to develop within the larvae and emerge from the host pupae are termed larval-pupal parasitoids (Hagen, 1964). Based on this definition, *A. bishopi* is therefore a larval-pupal parasitoid and not a larval parasitoid as previously recorded by Prinsloo (1984).

The highest parasitism rates by *A. bishopi* were observed in December with up to 38% in Gamtoos River Valley and 34% in Sundays River Valley (Fig 2.5). Although the level of activity was irregular and there was a tendency for it to be at very low levels in the latter part of the season, there is little doubt that the parasitoid assisted in limiting false codling moth populations in the early part of the season in Sundays River Valley and Gamtoos River Valley. This can be concluded as host populations decreased steadily from early December to early January in Gamtoos River Valley (Fig 2.4) and from early December to late January in Sundays River Valley (Fig 2.3), at a time when the egg parasitoid, *T. cryptophlebiae*, was either absent or very low levels.

Although Figs 2.6 & 2.7 show that a low level of larval parasitism generally coincides with a high level of egg parasitism and vice versa, there is no significant correlation between the levels of parasitism of the two parasitoids; ($r = 0.028$, $p = 0.958$) in Gamtoos River Valley and ($r = -0.772$, $p = 0.072$) in Sundays River Valley. They seem to compliment each other because when one parasitoid is either absent or at low levels, the other one exhibits high parasitism rates. The lack of correlation could be due to the small sample size of larval parasitism.

The variation in percentage parasitism may be due to the variation in false codling moth populations, or to predators and environmental conditions, such as rainfall and humidity, which were not taken into account during this study. The surveys for larval parasitism were only conducted once a month and it is, therefore, possible that parasitism could have fluctuated up or down in between these times. Dead or lost larvae were not taken into account. This method of assessment may have underestimated the rate of parasitism and the number of parasitoids obtained as some larvae may have died in the host and never emerged.

Agathis bishopi seems to be limited in its distribution to the Eastern Cape Province because it was not reared from larvae collected from Citrusdal and Nelspruit. The time and manner of attack by this parasitoid is unknown.

Only one specimen of *A. leucotretae* (female) was reared from larvae collected from Citrusdal in February. It was reared from a larva that was a second instar at the time of collection but emerged from a pupa, thus making it a larval-pupal parasitoid. It appears to be a solitary parasitoid since only one individual developed per host. It is only known to parasitise the false codling moth. This is the first record of this species anywhere other than the former eastern Transvaal Province. Ullyett (1939) suggested the importation of this species from Zimbabwe. This species belongs to a small African genus of ichneumonids.

The ability of larval parasitoids to suppress larval populations may depend on factors such as whether or not citrus orchards have been sprayed with insecticides, and on the presence or absence of other hosts of the natural enemies. It is therefore very important to identify other hosts, if any, of the parasitoids described in this study. Ullyett (1939) found that orchard sanitation has adverse effects on the larval parasitoids. This is very important when conducting surveys on larval parasitoids because some parasitoids might be disposed of along

with the infested oranges. Extensive biological studies of these two parasitoids would be required to determine if their levels of parasitism could be improved sufficiently by modifications to the orchard environment. Also important to consider would be augmentation and classical biocontrol by translocation of parasitoids.

2.4 Conclusion

Two parasitoids were reared in this study from false codling moth larvae, *Agathis bishopi* and *Apophua leucotretae*. Both species are larval-pupal endoparasitoids because they attack the host larvae but continue to develop within and emerge from pupae (Hagen, 1964).

Agathis bishopi was the more abundant of the two and appears to be a valuable parasitoid of false codling moth on citrus. It seems to occur only in the Eastern Cape Province. The sex ratio of *A. bishopi* was biased towards females throughout the study. It seems to parasitise mainly the younger larval instars of its host.

Apophua leucotretae was recorded only in February (Fig 2.5). This species has previously only been recorded from the former eastern Transvaal Province but now appears more wide spread. At present, of the parasitoids that attack false codling moth larvae, only *A. bishopi* is considered worth further investigation. Other known species of larval parasitoids were absent or rare in all study areas.

Agathis bishopi and *T. cryptophlebiae* seem to compliment each other. *Agathis bishopi* exhibits high parasitism rates early in the season, at a time when *T. cryptophlebiae* is either absent or at very low levels. The egg parasitoid then increases its parasitism rates in the latter part of the season when the larval parasitoid is at low levels (Figs 2.3 & 2.4). It is interesting, therefore, to speculate on the effect of releasing large numbers of the larval parasitoid in the latter part of the season and the egg parasitoid in the early part of the season, in an integrated control programme, when wild populations of the parasitoids are often low.

The results from this chapter can only be regarded as preliminary for a more extensive survey. Extensive weekly surveys of both the larval and egg parasitoids should be conducted

in orchards so as to get a better understanding of how they interact with one another in their natural environment before the potential for any translocation can be further considered.

Table 2.1 Orchards in which the larval parasitoids of the false codling moth were surveyed.

Orchard	Farm	Area	Province	Variety	Rootstock	Trees age (yrs)	Geographic coordinates
Blikhuis	Heksrivier	Citrusdal	Western Cape	Washington Navels	Rough Lemon	25	32.6°S; 19.01°E
Cottage2	Crocodile Valley Estates	Nelspruit	Mpumalanga	Washington Navels	Empress/ Cleopatra	21	25.5°S; 30.97°E
Orchard3	Cormic	Gamtoos River Valley	Eastern Cape	Palmer Navels	Rough Lemon	18	33.7°S; 21.9°E
Orchard28	Moedskepvlakte	Sundays River Valley	Eastern Cape	McLean Navels	Rough Lemon	15	33.4°S; 25.4°E

Table 2.2 False codling moth infestation of navel oranges collected from four study sites.

				NUMBER OF EACH LARVAL INSTAR				
Farm	**Collection date**	**Fruits collected**	**% Fruit infested**	**1st instars**	**2nd instars**	**3rd instars**	**4th instars**	**5th instars**
Cormic	11/12	200	18.5	2	4	10	1	0
	15/01	126	53.17	2	3	6	6	16
	12/02	133	33.08	0	1	7	6	11
	26/03	103	22.33	3	0	5	3	2
	22/04	129	40.13	7	8	10	11	1
	20/05	81	30.86	0	5	1	4	6
Moedskep-vlakte	13/12	189	56.08	9	22	22	20	7
	24/01	123	39.02	1	1	3	5	6
	14/02	168	51.79	2	3	9	10	15
	28/03	163	36.81	0	1	7	7	12
	25/04	129	51.16	2	5	13	9	8
	22/05	96	46.88	0	10	3	8	4
Heksrivier	11/12	200	11	2	3	1	0	3
	23/01	172	51.16	1	5	12	3	12
	21/02	123	35.77	1	7	7	4	3
	28/03	110	60	0	8	5	14	8
	30/04	158	68.99	1	5	6	23	16
Crocodile Valley Estates	12/12	100	5	0	1	0	2	1
	14/01	123	26.83	0	1	2	5	8
	20/02	73	38.36	0	2	5	7	6
	25/03	77	23.38	0	1	1	3	1
	22/04	119	49.58	1	12	9	4	4
	21/05	133	38.35	0	2	3	1	3

Table 2.3 Composition of the artificial medium for rearing false codling moth (Moore & Richards, pers. comm., 2001). The quantities given below are sufficient to fill five rearing jars (500 ml) to a depth of approximately 2 cm.

Ingredients	Quantities
Maize meal	200 g
Wheat germ	20 g
Brewers yeast	10 g
Milk powder	3.65 g
Nipagin (p-Hydroxybenzoic acid methyl ester)	1.5 g
Sorbic acid	0.7 g
Distilled water	200 ml

Table 2.4 Parasitism of the different false codling moth larval instars and parasitoid sex ratios.

Farm	Collection date	% Larvae parasitised	Number of parasitised instars					% Adult parasitoids	
			1st	2nd	3rd	4th	5th	F	M
Cormic	11/12	37.04	2	4	4	0	0	70.0	30.0
	15/01	9.09	0	2	1	0	0	66.7	33.3
	12/02	8.00	0	1	0	1	0	100	0
	26/03	23.08	1	1	0	1	0	66.7	33.3
	22/04	13.51	2	1	2	0	0	60.0	40.0
	20/05	12.50	0	2	0	0	0	100	0
Moedskep-vlakte	13/12	33.75	5	6	8	8	0	70.4	29.6
	24/01	12.50	0	1	1	0	0	50.0	50.0
	14/02	33.33	2	3	8	0	0	61.5	38.5
	28/03	7.41	0	0	1	1	0	50.0	50.0
	25/04	8.11	0	3	0	0	0	100	0
	22/05	8.00	0	2	0	0	0	100	0

F = female

M = male

Fig 2.1 *Agathis bishopi* Nixon female. Note the long ovipositor.

Fig 2.2 *Agathis bishopi* Nixon male.

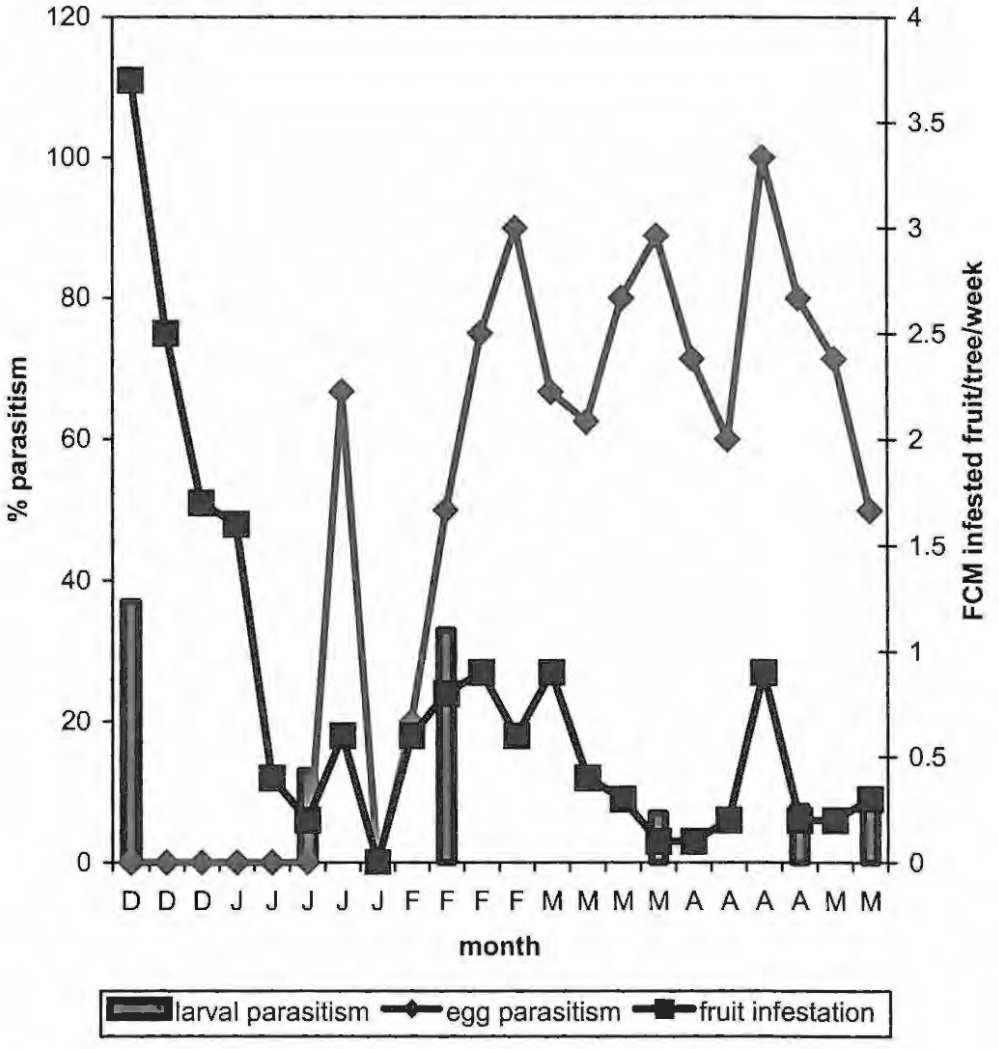

Fig 2.3 False codling moth infestation of navel oranges and parasitism of eggs and larvae at Moedskepvlakte Farm, Sundays River Valley, from December 2001 to May 2002.

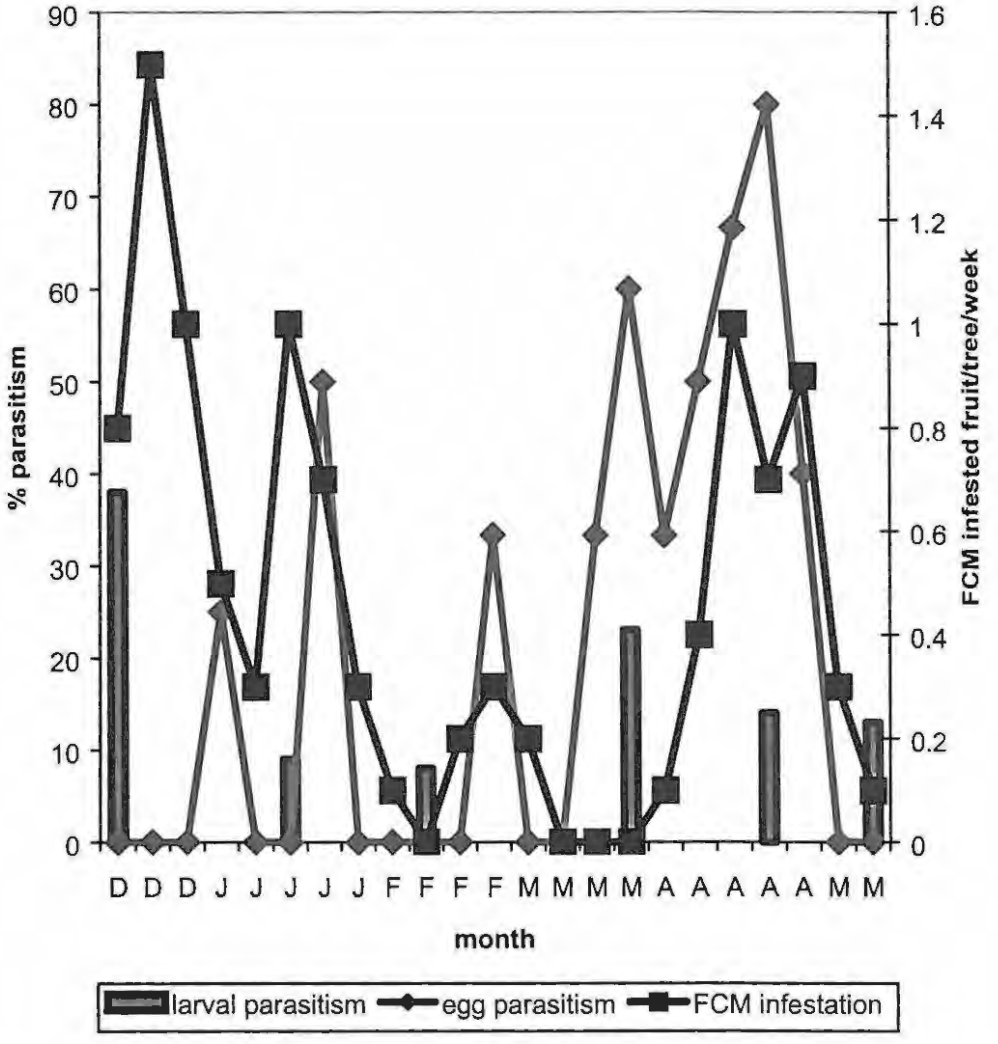

Fig 2.4 False codling moth infestation of navel oranges and parasitism of eggs and larvae at Cormic Farm, Gamtoos River Valley, from December 2001 to May 2002.

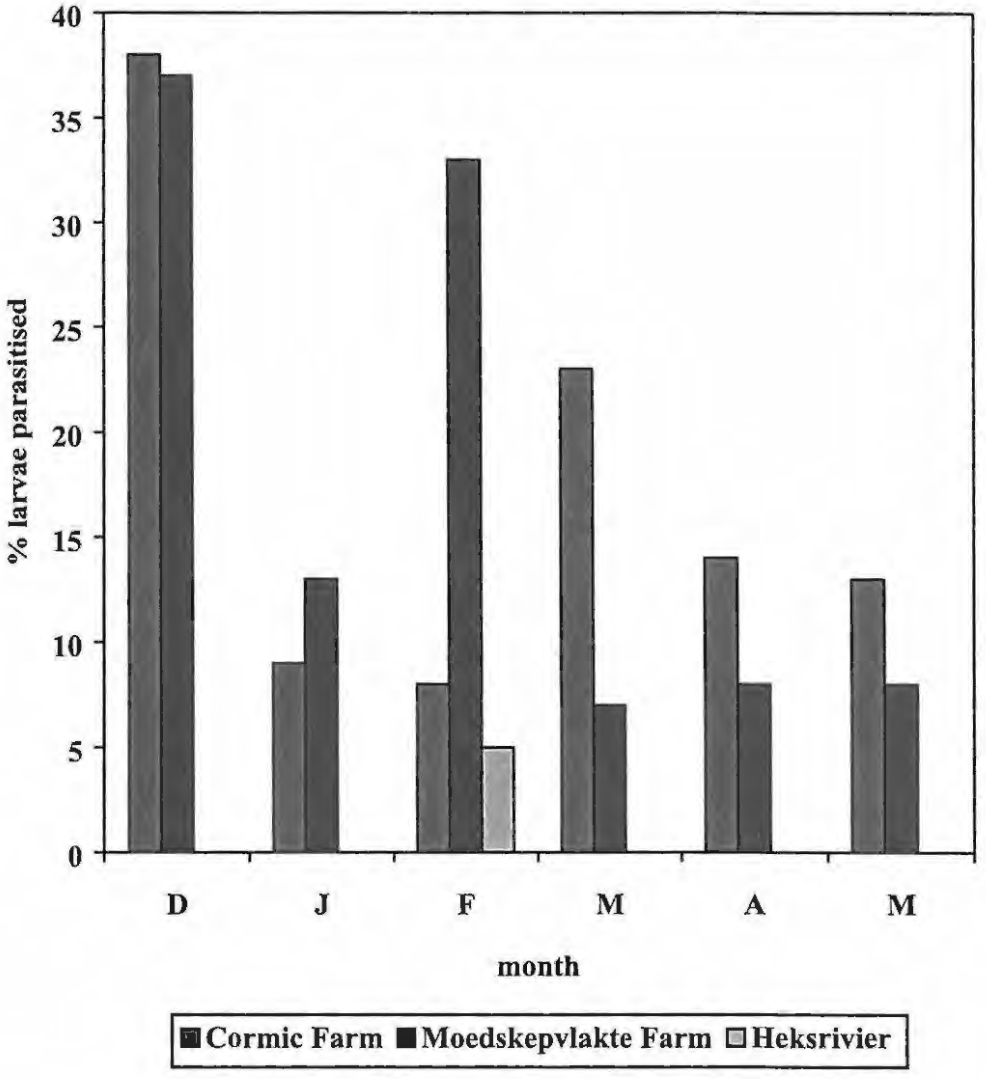

Fig 2.5 Parasitism of field collected false codling moth larvae from three navel orange orchards from December 2001 to May 2002.

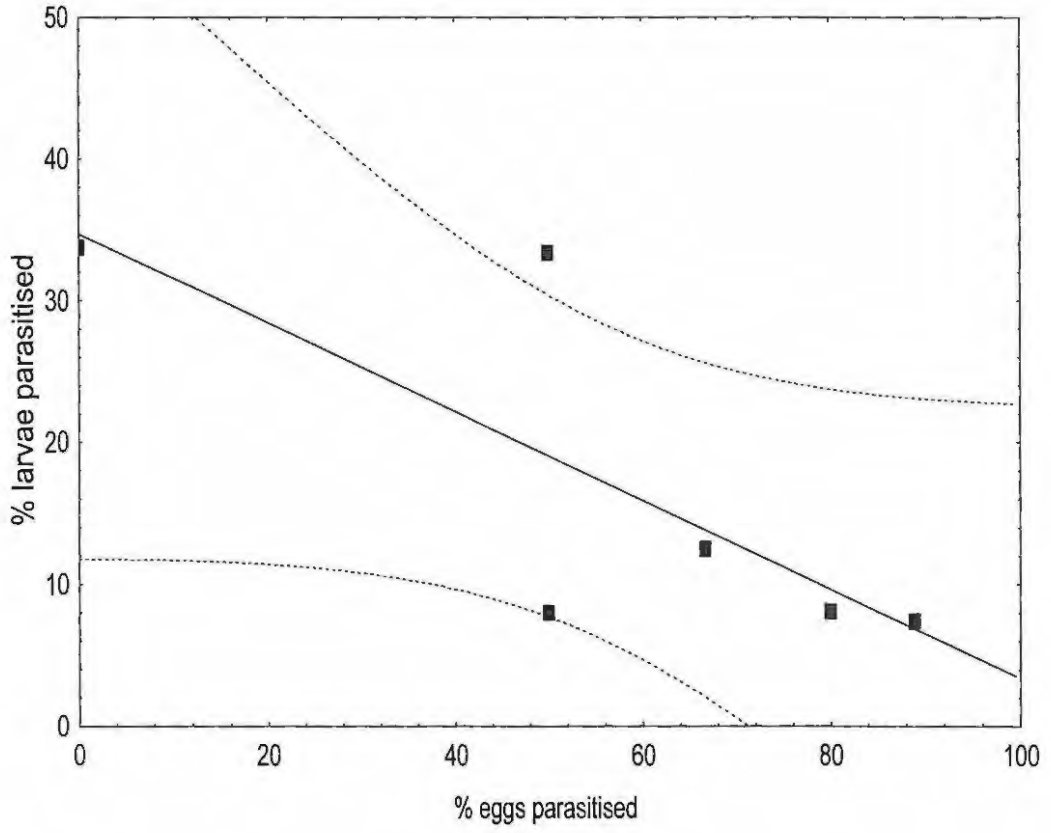

Fig 2.6 Parasitism of false codling moth larvae and eggs at Moedskepvlakte Farm, Sundays River Valley, from December 2001 to May 2002.

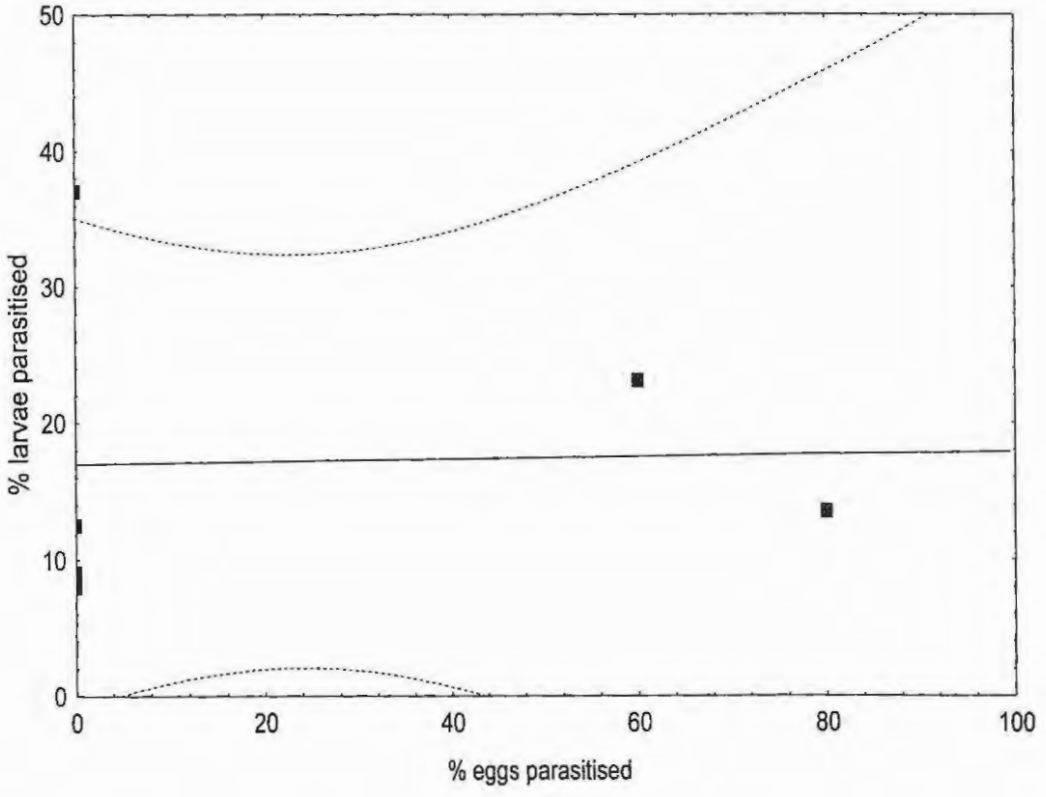

Fig 2.7 Parasitism of false codling moth larvae and eggs at Cormic Farm, Gamtoos River Valley, from December 2001 to May 2002.

3 BIOLOGICAL STUDIES ON *AGATHIS BISHOPI* NIXON, A LARVAL-PUPAL PARASITOID OF THE FALSE CODLING MOTH, *CRYPTOPHLEBIA LEUCOTRETA* (MEYRICK) (LEPIDOPTERA: TOTRICIDAE)

3.1 Introduction

It is obvious, from the studies described in Chapter 2, that *Agathis bishopi* is one of the most important natural enemies of the false codling moth in citrus. *Agathis bishopi* belongs to the family Braconidae. This family, together with the Icheumonidae, forms an extensive and important group of parasitoids of lepidopterous pests of citrus and other crops. The majority of the species in these two families are poorly known and in many cases their specific identity is still uncertain or unknown, and in urgent need of detailed study (Prinsloo, 1984).

Ullyett (1939) was of the opinion that *A. bishopi* is a valuable parasitoid in citrus but provided no data to support this. *Agathis bishopi* and *Agathis leucotretae* are similar in colour, but *A. leucotretae* is easily separated by the absence of notaulal furrows on the mesoscutum (Prinsloo, 1984). The adult *A. bishopi* is robust and strongly sclerotised. The male and female are similar in structure but differ in colour. The female is readily separated from other parasitoids of the false codling moth by its more or less uniformly brownish yellow colour and strongly protruding ovipositor (Prinsloo, 1984). It has black antennae and evenly smoked wings. The legs are the same colour as the body, except for the distal tips of the hind tibiae and the larger part of the hind tarsi, which are suffused with black. The middle and hind legs each have two tibial spurs. It has long and slender antennae with many segments and an undifferentiated club, well-developed notaulal

furrows on the mesoscutum, and tarsi with five segments. It measures about 10 mm in length, including the ovipositor, which is as long as the head and body together. The male differs from the female in that the head, thorax, abdomen and legs are usually marked in black. It measures about 5 mm in length (Prinsloo, 1984).

Knowledge of the natural enemies of a pest species, their biology and interaction in mixed populations and the control they exert is desirable when considering commercial control measures. This chapter reports on the biology of *A. bishopi*, which was studied because of the potential it has as a biological control agent of the false codling moth.

3.2 Materials and Methods

3.2.1 Acquisition of *Agathis bishopi*

To establish a culture of *A. bishopi*, wild larvae of the false codling moth were collected from navel orange orchards at Cormic Farm in Gamtoos River Valley and Moedskepvlakte Farm in Sundays River Valley in the Eastern Cape Province. The oranges were collected from the orchard floor and only obviously infested fruits were collected, i.e. fruits with a frass-filled hole and those with a yellowish-brown colour around the point of infestation. Field collections were made from April to September 2002. The tree ages, geographic co-ordinates, cultivar and rootstock of each orchard are presented in Table 2.1.

Larvae were extracted from infested fruits by cutting the rind around the point of infestation in thin slices until the larvae were spotted. The larvae were then removed from the fruits and placed individually in glass vials (diameter 25 mm; height 75mm) containing approximately 40 g of the rearing medium. The preparation of this medium and the methods for the individual rearing of larvae are exactly the same as described for larval parasitism in Chapter 2. Upon emergence, adult parasitoids of variable female:male sex ratio were placed in a plastic container (diameter 35 mm; height 55 mm) for a day to

allow them to mate. A small amount of honey was smeared on the side of each vial to serve as a food source for the adult parasitoids. Each vial was covered with a net, with a hole in the centre, held in place with an elastic rubber band. The hole in the net was blocked with a wet cotton wool plug to provide the parasitoids with water.

3.2.2 *Agathis bishopi* culture

Agathis bishopi was reared in the laboratory on false codling moth larvae. The larvae were obtained from an established laboratory culture at Citrus Research International (CRI) in Port Elizabeth, Eastern Cape Province. The larvae were placed in a clear plastic container with 50 g of the prepared rearing medium. Adult parasitoids of variable female:male ratios (2:3; 2:1; 3:1) were introduced into the container. Female parasitoids were confined with males throughout the study, unless otherwise stated. A small amount of honey was smeared on the side of the container to provide food for the adult parasitoids. The opening of the container was covered with a net, with a hole in the center, held in place with an elastic rubber band. The hole in the net was blocked with a wet cotton wool plug.

After the larvae were exposed to the parasitoids for parasitism, they were then placed individually into glass vials (diameter 25 mm; height 75 mm), containing 40 g of the prepared rearing medium, in order to complete their development without being cannibalised. The opening of the vial was closed with a cotton wool plug. When fully-grown, the moth larvae left the rearing medium and crawled to the cotton wool plug to pupate. The culture was maintained at 26.5°C and 60% relative humidity.

3.2.3 Host stage suitability, developmental rate, offspring sex ratios and life span

Both choice and no-choice tests were conducted to determine the host stage suitability of *A. bishopi*. The no-choice test was conducted after the choice test to see if the larval

instars that were not parasitised during the first test would be parasitised in the absence of the larval instars that were preferred during the choice test. In both tests, a record was made of the larval instars parasitised and the sex of emerging parasitoids.

Five larvae of 1st, 2nd, 3rd, 4th and 5th larval instars were used in the choice test. For the no-choice test, five larvae of 3rd, 4th and 5th larval instars were used. In each test, the larvae were placed in a plastic vial containing 50 g of the prepared rearing medium. Two-day–old mated adult parasitoids of variable female:male sex ratio were introduced into each vial for 24 hours to allow them to parasitise the larvae. A small amount of honey was smeared on the side of the vial. The opening of the vial was covered with a net, with a hole in the centre, held in place with an elastic rubber band. The exposed larvae were replaced with new ones every day until the female parasitoids died. The exposed larvae were reared individually in glass vial containing 40 g of the prepared rearing medium until parasitoid emergence. The duration of development from oviposition to pupation and the pupal stage were recorded to determine the duration of development of *A. bishopi*. The life span and sex of the progeny were also recorded.

Shortly after emergence, *A. bishopi* adults were placed, individually, in plastic vials and provided with honey and water every second day. False codling moth larvae were introduced into vials containing female parasitoids but not those containing males. Exposed larvae were replaced with new ones every second day. None of the parasitoids were allowed to mate, in order to determine whether unmated females would produce male offspring throughout their life span. These were monitored daily until the parasitoids died.

3.3 Results and Discussion

A thorough study of the biology of this parasitoid is necessary to confirm the results obtained in this study before biological control releases are made, since the results of this study are based on very low numbers of the parasitoid. Therefore, the statistical results

given in this chapter should be taken as a first indication of the biology of *A. bishopi* and no absolute conclusion should be drawn until further investigations are made. Moreover, because of logistical constraints, none of the tests conducted in this study were replicated, which is an important factor for demonstrating whether or not the results are of definite value.

3.3.1 *Agathis bishopi* culture

The culture of false codling moth larvae at the CRI laboratory was heavily infected with *Cryptophlebia leucotreta* Granulovirus during this study and many of the larvae that were exposed to *A. bishopi* for parasitism died before parasitoid emergence. This had adverse effects on the results obtained. The results presented in this chapter are based on results obtained from very few parasitoids and therefore more research needs to be conducted on the biology of this parasitoid. Nonetheless, the results presented here give an indication of their likely outcome.

3.3.2 Host stage suitability, developmental rate, offspring sex ratios and life span

When the females were provided with a choice between the different instars, adult parasitoids emerged only from the pupae that were parasitised as first and second larval instars (Table 3.1). No parasitoid emergence occurred in the no-choice test. This indicated that *A. bishopi* parasitises only the early larval instars (1^{st} and 2^{nd}). Female parasitoids emerged only from pupae that were parasitised as second larval instar, whereas males emerged from those parasitised as first larval instar (Table 3.2).

The parasitic Hymenoptera exhibit parthenogenesis (Quicke, 1997). In parthenogenesis, the fertilized eggs are diploid and give rise to females, whereas the azygote from the unfertilized eggs are haploid and develop into males. Mostly, females develop in larger host individuals and males in smaller hosts. Therefore, *A. bishopi* females regulate the

sex of their progeny according to the size and larval stage of the host, by placing unfertilized eggs in younger and smaller larvae and fertilized eggs in older and larger larvae. This could be used to great advantage in biological control work involving the insectary rearing of this parasitoid, where the use of large hosts would not only produce a high proportion of female parasitoids but also larger females with higher fecundity. This would result in optimum parasitoid production. It would be interesting to determine if the age of each larval instar would have any effect on the sex ratio of the progeny of this parasitoid.

Agathis bishopi appears to be well adapted to the development of the moth. The duration of development from parasitism to pupation ranged from 12 to 15 days and the pupal stage ranged from 16 to 20 days in parasitised larvae (Table 3.2). These were very similar to those observed in unparasitised larvae, which ranged from11 to 16 days for the larval stage and from 15 to 20 days for the pupal stage. It can therefore be assumed that the developmental rate of *A. bishopi* is in synchrony with that of false codling moth. The resulting *A. bishopi* adults emerge when unparasitised larvae of the same generation have completed their development and emerge as adult moths.

There was no significant difference in the duration of development from parasitism to pupation ($t = -1.93649$; $p = 0.14822$) and the pupal stage ($t = -0.568399$; $p = 0.609528$) between the first and second instar larvae. The duration of development from parasitism to pupation was 12.33 ± 0.58 and 14.00 ± 1.41 days (mean ± SD) for 1st and 2nd larval instars, respectively. The duration of the pupal stage was 17.33 ± 2.31 and 18.50 ± 2.12 days (mean ± SD) for 1st and 2nd larval instars, respectively. The non-significant p-value should be interpreted with reserve because the possibility of a Type II error is very strong with such a small sample size.

The mated female parasitoids produced both female and male offspring. The unmated females produced no offspring. There are far too many possible causes for this, and the data set is not large enough to make definite statements about the sex determination mechanisms of this parasitoid. But, based on the results obtained from field collections

(Chapter 2), it is obvious that the sex ratio is always biased towards females and because, when mated, they produce both male and female offspring, it can be speculated that they are arrhenotokous.

In this study, females of *A. bishopi* generally lived longer than males. Females lived for six to eight days and males for three to four days (Table 3.2). Previous studies have shown that it is a general trend amongst the different families of parasitoids for females to have a greater life expectancy than males. These studies also show that parasitoids are generally found to fare better when provided with moisture or, even better, dilute honey (Doutt, 1959; Ooi, 1980; Moore & Kfir, 1996). Temperature is known to play a very important role in the adult life span of Hymenoptera (Doutt, 1959; Broodryk, 1969; Moore & Kfir, 1996; Quicke, 1997). It is therefore important that the effects that temperature, humidity and nutrition have on the life span of *A. bishopi* be examined. This information may be applied advantageously in laboratory rearing of the parasitoid.

3.3.3 General

Only one adult parasitoid developed per false codling moth larva, thus making *A. bishopi* a solitary larval-pupal endoparasitoid. Based on the biological and life history traits of *A. bishopi* observed in this study and those documented by Quicke (1997) on parasitoids in general, it can be concluded that *A. bishopi* is a koinobiont, as defined in Table 3.3.

3.4 Conclusion

It was difficult to mass-rear *A. bishopi* in the laboratory due to the high viral contamination of the false codling moth larval culture at CRI laboratories. This resulted in high mortality rates of the larvae that were exposed for parasitism before parasitoids could emerge.

Only the first and second larval instars were parasitised. Pupae that were parasitised as second larval instars gave rise to females and those parasitised as first larval instars gave rise to males. The unmated females produced no offspring.

The developmental rate of *A. bishopi* appears to be in synchrony with that of false codling moth and the adult wasp emerges when the adult moths that have escaped parasitism emerge. *Agathis bishopi* can be regarded as a solitary, koinobiont, larval-pupal endoparasitoid.

Table 3.1 Parasitism by *A. bishopi* of first and second instar larvae of false codling moth in the host choice test.

Instar	**No. of exposed larvae**	**No. parasitoid obtained**
1st	20	3
2nd	20	2

Table 3.2 Duration of development of *A. bishopi* in false codling moth larvae and pupae, and sex of resulting progeny at 26.5°C and 60% R.H.

Larval instars parasitised	**Duration from parasitism to pupation**	**Duration of the pupal stage**	**Sex of progeny**	**Adult life span (days)**
1st	12	16	M	3
1st	13	16	M	3
1st	12	20	M	4
2nd	13	20	F	6
2nd	15	17	F	8

F = female
M = male

Table 3.3 Biological and life history traits of *A. bishopi* and those associated with koinobiont strategists (Quicke, 1997).

Koinobiont traits	A. bishopi traits
Endoparasitism	It is an endoparasitoid
Attack larvae, often early instars	Known to attack only 1st and 2nd instar larvae
Specialists	Only known to parasitise the false codling moth
No or temporary host paralysis	False codling moth larvae temporarily paralysed during parasitism after which they carry on with their development until the pupal stage from which the parasitoid emerges
Delayed larval development	*A. bishopi* delays its development such that it emerges when unparasitised larvae give rise to adult moths
Host-feeding uncommon	No host-feeding was observed during this study
Short adult life span	Adult parasitoids survived for a maximum of eight days
Host stage attacked often smaller than wasp	1st and 2nd instar larvae are ± 1 and 3 mm long, respectively, whereas A. bishopi females are ± 10 mm long

4 LABORATORY REARING OF THE FALSE CODLING MOTH, *CRYPTOPHLEBIA LEUCOTRETA* (MEYRICK) (LEPIDOPTERA: TORTRICIDAE)

4.1 Introduction

The false codling moth, *Cryptophlebia leucotreta*, is one of the most important pests of citrus in South Africa for which no completely satisfactory control measure exists. The development of an artificial rearing medium that can ensure reliable and large numbers of false codling moth larvae is a prerequisite for many studies of the false codling moth.

Ripley *et al.*, (1939) found mass breeding of the false codling moth in oranges to be impracticable. Although they could find conditions under which caged moths were induced to oviposit readily on the fruit, cannibalism among the larvae, particularly among the early stages, necessitated having only one or two larvae in each orange. They found that even when the eggs were laid on very green oranges, decomposition of the fruit was too rapid to allow larval growth, without stunting, to be completed within the original orange, and the larvae would not travel from a rotting orange to a fresh one placed next to it. They simply remained in the original orange and died of the toxic effects of the fermentation products. They then concluded that even though healthy larvae are highly resistant to some types of fermentation under natural conditions and often finish their development in very rotten oranges, under laboratory conditions, where fruits have to be picked before they are artificially infested, toxic fermentation products play a much more important role than in nature. Based on these shortcomings, they were convinced that the

only efficient system for mass rearing this moth was by rearing it in an artificial medium that was kept under sterile conditions to prevent microbial contamination.

The original rearing medium of false codling moth larvae developed by Ripley *et al.*, (1939) was slightly modified by Theron (1947). The larvae developed satisfactorily in both media, but the difficulty was keeping the media free of contaminants that destroy the larvae. Bot (1965) developed a medium that is kept aseptic with methyl p-hydroxybenzoate. Moore & Richards (pers. comm., 2001) developed a similar medium. Both of these media use maize meal as the basis to which other nutrients and antibiotic agents are added.

The consistency and nutritional properties of an efficient rearing medium of the false codling moth larvae are the most important factors. The consistency of a rearing medium is the determining factor in stimulating feeding in newly hatched larvae because the stimuli for feeding at this stage are mechanical rather than chemical (Ripley *et al.*, 1939). Jelly-like media are refused, but fibrous and granular ones are eaten. A marked degree of stunting results when the medium is too dry, pupae hardly one-fourth of the normal size are produced and the larval stage is greatly prolonged, requiring about 8 weeks, whereas approximately 3 weeks suffice if the medium is wet when kept at 26-30°C and ± 80 % R.H.. It is very important to use containers that are very tightly fitted with cotton wool to retain moisture throughout the larval stage. When the nutritional factors are ideal the tendency toward cannibalism is considerably reduced (Ripley *et al.*, 1939).

This chapter reports on the trials conducted to formulate a less labour-intensive and more efficient rearing method by improving the existing rearing medium and rearing containers to suit large-scale mass production of false codling moths.

4.2 Materials and methods

4.2.1.Outline of the existing rearing technique

The false codling moth larval culture was kept at approximately 27°C and 35 % relative humidity. The moths were kept at the same temperature but at 60 % R.H.

1. Preparation of medium

The ingredients and quantities of the rearing medium used to provide the nutritional needs of the larvae are given in Table 2.3. The dry ingredients were mixed into 500 ml glass jars and 40 ml of distilled water was added to each. Each jar was plugged very tightly with cotton wool. Tin foil was used to cover the cotton wool plugs and was held in place with an elastic rubber band. The medium-filled jars were then autoclaved at 121°C for 20 minutes and thereafter allowed to cool under a laminar flow hood.

2. Egg decontamination

Wax paper, on which false codling moth eggs were oviposited, was cut into small squares with an undetermined number, estimated at approximately 150, of eggs on each. These were placed in a 2-ℓ jar. They were surfaced sterilised, to prevent contamination of the rearing medium, by fumigating them with formaldehyde for 6 hours under the laminar flow cabinet. Approximately 0.055 ml of formaldehyde were dripped onto a filter paper, which was placed over the mouth of the jar, and sealed within the 2-ℓ jar with the egg squares. This method was later modified by Moore (2002b) as follows: egg squares were dipped for 15 minutes in 0.15 % Sporekill (poly-dimethyl-ammonium chloride; Hygrotech International, South Africa). After the eggs were surface-sterilised, they were placed on the prepared rearing medium using sterile forceps.

Once the eggs had been introduced into the jars, the jars were stoppered tightly with the same cotton wool plugs. The jars were removed from the laminar flow cabinet and placed on shelves in the larval culture room, where eggs hatched and larvae developed on the rearing medium. The full-grown larvae tend to leave the medium and crawl up the jars to pupate in the cotton wool plugs (Fig 4.1). As soon as most of the larvae had pupated, the cotton wool plugs were removed from the jars and placed in wooden emergence boxes.

3. Oviposition

A 2-ℓ Consol bottle was fastened over a hole on the side of the emergence box (Fig 4.2). Emerging moths were attracted towards the light and moved through a hole into the bottle. Each day moths were transferred from the Consol bottles into inverted sieves (90 mm diameter), by knocking them through a funnel inserted into a 42 mm hole in the center of each sieve. The hole in the sieve was then blocked with a wet cotton wool plug to provide the moths with moisture in order to extend their longevity (Catling & Ascheborn, 1978). Each sieve was placed on five sheets of wax paper onto which the moths oviposited (Fig 4.3). The moths were therefore retained for five days. The top wax paper, with eggs on, was removed daily from under each sieve.

Moore & Richards (pers. comm., 2001) later modified this method. They built and tested a cage incorporating moth eclosion and oviposition into one unit (Fig 4.4). The moths were confined in a cage covered with a net, with a hole at the top, which was attached to the wooden emergence box into which the pupae were placed. A roll of wax paper was fed into the cage through a thin slit at the bottom of the cage, covering the floor of the cage and exiting through a similar slit on the opposite side of the cage. The hole in the net was blocked with a wet cotton wool plug, which was wetted daily to sustain the moths. Emerging moths being attracted to light moved through a hole into the cage. The wax paper was advanced every second day so as to remove the portion covered with eggs, thus exposing a new portion for the moths to oviposit on.

4.2.2 Diet improvement

Four trials were conducted to find an efficient and less labour intensive rearing medium as an alternative to the existing medium. This could be achieved by producing a medium that does not require autoclaving and by using larger containers. Each medium was prepared in five 500 ml jars and one airtight plastic (1-ℓ Tupperware) container in all of the trials, unless otherwise stated. The ingredients and quantities used to prepare the media are given in Table 4.1 for the jars and Table 4.2 for the airtight containers. One-day-old eggs were used in all four trials. The eggs were surface sterilised by dipping egg sheets in 15 % Sporekill for 15 minutes. The eggs were placed onto the media under a laminar flow cabinet as a precautionary measure against any microbial contamination. Once the eggs were placed onto the media, they were transferred to a constant environment room that was kept at 27°C and 35 % relative humidity.

Three different media were prepared in the first trial. These were based on maize meal, chickpea flour and soya flour to which the same nutrients and antibiotic agents of the same quantities were added. The dry ingredients (47.17 g in each jar and 188.68 g in each Tupperware container) were mixed together and different volumes of boiled distilled water were added to the mixtures prepared in the jars (maize meal = 50 ml; soya flour = 60 ml; chickpea = 30 ml) and in the Tupperware containers (maize meal = 320 ml; soya flour = 360 ml; chickpea flour = 300 ml). The media were allowed to cool under a laminar flow cabinet. Approximately 120 surface sterilised eggs were placed onto the medium in each jar and approximately 480 eggs into each 1-ℓ container. The jars were then stoppered with very tight fitting cotton wool plugs and the Tupperware containers were covered with a layer of cotton wool, which was held in placed with an elastic rubber band. The duration of development of each life stage, sex ratio and moth production per jar were recorded.

In the second trial four different media were prepared in 500 ml jars only. These were based on maize meal, chickpea flour, soya flour and soya mince. The preparation of these media was exactly the same as described for the first trial, except for the maize meal

medium, which was autoclaved at 121°C for 20 minutes. Approximately 120 surface-sterilised eggs were placed into each jar. The duration of the different life stages, moth production per jar, sex ratio and average mass of the adults was recorded.

In the third trial, media based on soya flour, oatmeal, chickpea flour and a combination of oatbran and oatmeal were prepared. The media were prepared in exactly the same way as described in the first trial. Media were only prepared in 500 ml jars. Oatmeal, soya flour and chickpea flour based media of different consistencies were prepared (oatmeal = 75 or 130 ml distilled water; soya flour = 100 or 110 ml; chickpea = 60 or 65 ml; oat bran = 80 ml; oat bran + oatmeal = 95 ml). Approximately 120 surface-sterilised eggs were placed into each jar.

Media prepared in the fourth trial were based on soya flour, oatmeal and oatbran. The media were prepared in 1-ℓ Tupperware containers. Dry ingredients (94.34 g in each container) were mixed together and different quantities of boiled distilled water were added to the mixtures (oatmeal = 150 ml; oat bran = 250 ml). Decantable (with 300 ml distilled water) and porridge-like (with 200 ml distilled water) soya flour media were prepared. These were left uncovered under the laminar flow cabinet for 3 hours to allow them to cool.

Approximately 300 surface sterilised eggs were placed into each container. The containers were then covered with tight fitting sponges (160 x 130 x 40 mm). A number of grooves were made on the underside of each sponge to provide pupation sites for the larvae. The sponges were covered with tin foil and autoclaved at 121°C for 20 minutes. The containers were sterilised by soaking in a solution of sodium hypochlorite. The duration of the different life stages, moth production per container, average weight of the moths and sex ratio were recorded.

4.3 Results and Discussion

4.3.1 Diet improvement

According to the Kruskal-Wallis test, in the first trial, there was no significant difference in the mean duration of the egg stage ($p = 0.287$) on the different media in the 500 ml jars (Table 4.3). The medium based on maize meal putrefied after seven days and no further larval development was observed. The Mann-Whitney U-test indicated that there was no significant difference in the mean duration of the larval stage ($p = 0.676$) for the larvae reared on soya flour and chickpea based media. The mean duration of the pupal stage was significantly longer for the larvae reared on the chickpea based medium than those reared on the soya flour based medium ($p = 0.012$). The approximate percentage of adults obtained from the eggs placed onto the two media was significantly different ($p = 0.047$) with more adults obtained from the soya flour based medium (Table 4.3). There was no statistically significant difference in the sex ratio of the adults ($p = 0.347$).

The cotton wool covering the Tupperware containers was either not held in place tightly enough or was not thick enough because most of the larvae managed to escape. It was therefore impossible to determine the duration of larval and pupal development since most larvae developed outside the containers. The duration of the egg stage was 5 days in the soya flour based medium and 3 days in the chickpea based medium in the Tupperware containers. This experiment was not repeated because the cotton wool was considered unsuitable and was subsequently replaced with sponges.

In the second trial, the mean duration of the egg stage was significantly shorter in the maize meal and soya flour based media than that observed in the chickpea flour based medium ($p = 0.024$) (Table 4.4). The soya mince based medium putrefied after 3 days and no egg hatching was observed. After 10 days, the chickpea flour based medium also putrefied and no further larval development occurred. This might have been due to the higher water content used to prepare the media in this trial than in the previous trial.

No statistically significant differences were measured in the duration of both the larval ($p = 0.602$) and pupal ($p = 1.000$) stages in the soya flour and maize meal based media. There was no significant difference in the sex ratio of the adults obtained from each medium ($p = 0.611$). Adults reared in the soya flour based medium were normal in size when compared with those reared in the existing standard medium (maize meal based medium) (Table 4.4). Since the soya flour based medium was comparable with the maize meal based medium in all respects, it was used as the standard medium in trials hereafter, in which the media were not autoclaved.

All the media that were prepared in the third trial were affected by bacterial and fungal contamination after 2 days. It is unlikely that the cause was a lack of sanitation, as the distilled water was boiled, the media were kept under the laminar flow cabinet before being transferred to the larval culture room and the cotton wool and bottles were autoclaved before use. Presumably the high level of contamination was due to too much moisture being retained inside the jars as they were plugged immediately after pouring in the hot water; hence the media in the fourth trial were allowed to cool before being sealed.

In the fourth trial, total duration of development was shorter for larvae reared on oatbran based medium than on any other medium. Development was prolonged in the decantable soya flour based medium, lasting for about 32 days, therefore longer than on any other medium (Table 4.5). This is comparable to the observations made by Ripley *et al.*, (1939) who found that the water content plays a major role in larval development.

The approximate percentage of adults obtained from eggs was highest from the oatbran (33 %) and the porridge-like soya flour (27 %) based media (Table 4.5), even in comparison with the soya flour and maize meal (existing standard medium) media in the 500 ml rearing jars (20 % and 23 %, respectively) (Table 4.4). However, these findings need to be verified by comparing the media in one trial since these findings are based on results obtained from two different trials. This is in contrast to the observations made by Ripley *et al.*, (1939) who observed that larger containers encourage cannibalism and,

therefore, produce fewer adults than smaller ones. They also reported that the chances of deleterious mould and bacterial infections are increased in larger containers. However, none were observed in this study. This contrast could be attributed to the fact that Ripley *et al.*, (1939) were referring to a septic medium in which fungal growth was promoted, whereas aseptic media were used in this study. The adults obtained from the larvae reared on larger containers were of similar size to those reared in the 500 ml jars (Table 4.5).

The fifteen minute dip in 15 % Sporekill completely eliminated all bacterial and fungal contamination. Moore (2002b) reported success with a 100 times lower concentration of Sporekill, however, his main justification for using the lower concentration was a reduction in cost. He also found that a higher concentration significantly increased egg mortality by 12.6 %. Based on his findings, the low moth production in this study might therefore be attributed to the higher concentration of Sporekill.

4.4 Conclusion

The 1-ℓ containers seem ideal for large-scale rearing of false codling moth since more adults were obtained from the larvae reared in them than those reared in the smaller jars. Large containers have two obvious advantages; (a) more individuals can be reared per container, therefore less labour is required; (b) there is an apparently greater production ratio (i.e. adult:egg) in the larger containers. The containers are well ventilated, and no problems were encountered with moisture build-up. The width of the sponge minimizes the chances of escape of larvae. The burrowing of the larvae did not damage the plastic containers. The containers are transparent, thus making it easy to inspect the development of the larvae. They cannot be autoclaved but should be sterilized by soaking in a solution of sodium hypochlorite.

The oatbran medium proved to be the best of all the new media tested since it produced the most adults and the highest percentage of females. It is easier to prepare than the existing standard medium because unlike maize meal, it does not require autoclaving.

Table 4.1 Composition of the media for mass rearing of false codling moth in 500 ml glass jars. The quantities given below are sufficient to fill five rearing jars to a depth of approximately 2 cm.

Ingredients	**Quantities (g)**
Maize meal	200
OR Soya flour	200
OR Soya mince	200
OR Chickpea	200
OR **Oatmeal	200
OR/AND **Oat bran	200
*Wheat germ	20
*Brewer's yeast	10
*Nipagin	1.5
*Sorbic Acid	0.7
*Milk powder	3.65

*Added in all media prepared in 500 ml glass jars, in the same quantities

**100 g each used in the medium based on the combination of the two

Table 4.2 Composition of media for mass rearing of false codling moth in 1-ℓ containers. The quantities given below are sufficient to fill a 1-ℓ container to a depth of 2 cm.

Ingredients	Quantities (g)
Maize meal	80
OR Soya flour	80
OR Chickpea	80
Wheat germ	8
Brewers yeast	4
Nipagin	0.6
Sorbic acid	0.28
Milk powder	1.46

Table 4.3 Development of false codling moth on three rearing media prepared in the first trial; showing duration of development and percentage of adults obtained.

Base ingredient in medium	**Duration of egg stage (d; m ± SD)**	**Duration of larval stage (d; m ± SD)**	**Duration of pupal stage (d; m ± SD)**	**Females per 5 jars (approximate %)**	**Males per 5 jars (approximate %)**
Soya flour	3-5; 3.6±0.55	10-13; 11.8±1.1	19-21; 20.2±0.8	46.93	53.07
Maize meal	3-5; 4.2±0.83	0 0±0	0 0±0	0	0
Chickpea	3-4; 4.0±0.71	13-16; 15±1.73	16-19; 17.3±1.5	47.5	52.5

m = mean

F = females

M = males

Table 4.4 Suitability of four different types of rearing media prepared in the second trial for mass rearing of false codling moth in 500 ml rearing jars.

				Production per jar			Average weight (g)	
Base ingredient in medium	**Duration of egg stage (d; m ± SD)**	**Duration of larval stage (d; m ± SD)**	**Duration of pupal stage (d; m ± SD)**	**F**	**M**	**Adults obtained (approximate %)**	**F**	**M**
Maize meal	6; 6±0	12-13; 12.4±0.55	14-16; 14.6±0.89	62	77	23	0.011	0.006
Soya flour	6; 6±0	12-13 12.2±0.45	14-16; 14.6±0.89	53	68	20	0.010	0.006
Soya mince	0; 0±0	0; 0±0	0; 0±0	0	0	0	0	0
Chickpea	6-7; 6.6±0.55	0 0±0	0 0±0	0	0	0	0	0

m = mean
F = females
M = males

Table 4.5 Suitability of four different types of rearing media for mass rearing of false codling moth in 1-ℓ rearing containers in the fourth trial.

				Production per container			Average weight (g)	
Base ingredient in medium	**Duration of egg stage (days)**	**Duration of larval stage (days)**	Duration of pupal stage **(days)**	**F**	**M**	**Adults obtained (approximate %)**	***F***	**M**
Oatmeal	5	10	11	15	18	11	0.010	0.005
Oatbran	5	9	11	48	51	33	0.010	0.007
*Soya flour1	5	10	13	43	39	27	0.010	0.006
**Soya flour2	7	10	15	4	5	3	0.010	0.004

*Porridge-like medium
**Decantable medium
F = females
M = males

Fig 4.1 Fully-grown larvae of the false codling moth, *Cryptophlebia leucotreta,* pupating in the cotton wool plug.

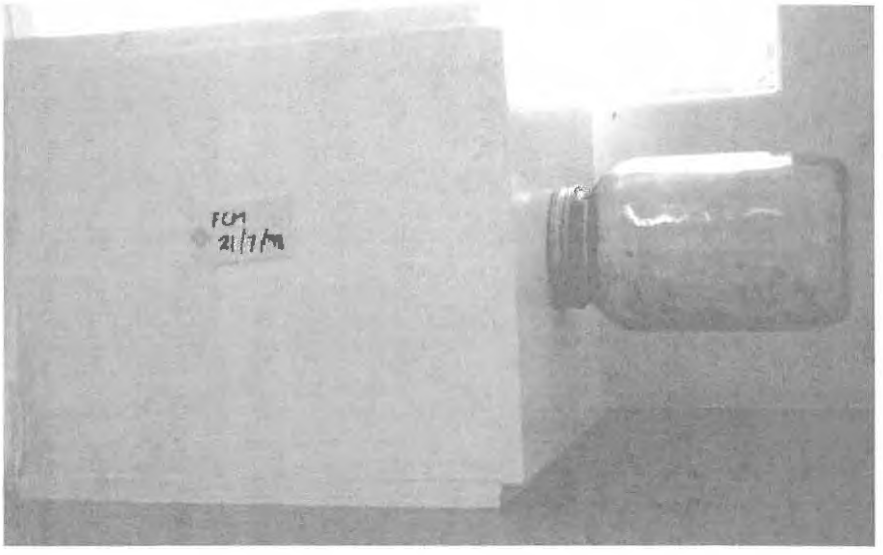

Fig 4.2 Emergence box with a 2-ℓ Consol bottle screwed on the side for collecting the emerging moths.

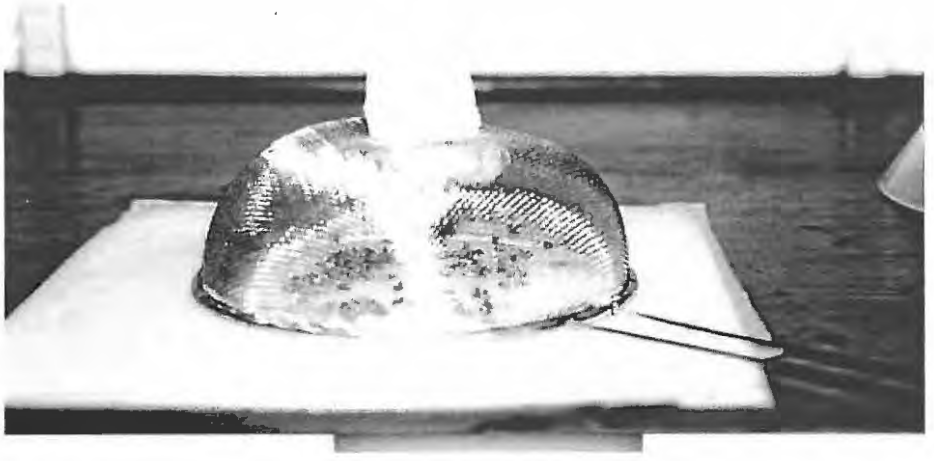

Fig 4.3 Adult false codling moths held under an inverted sieve with wax paper for egg laying.

Fig 4.4 Emergence box with cages attached incorporating both oviposition and eclosion into one unit.

5 GENERAL DISCUSSION: THE POTENTIAL FOR BIOLOGICAL CONTROL IN AN IPM PROGRAMME AGAINST *CRYPTOPHLEBIA LEUCOTRETA* ON CITRUS IN SOUTH AFRICA

5.1 Introduction

Many growers try to achieve 'zero pest tolerance' by using insecticides. The development of resistance to insecticide by the moth, resurgence of other pests and residues on the fruit within a chemical control programme is making this less of a possibility. Growers should be aware that zero pest tolerance is impractical. However, using biological agents can help in keeping false codling moth populations below the economic threshold.

Although it is unlikely that a single biological control technique could suppress false codling moth populations below economically damaging thresholds, there are several methods that are potentially valuable supplements to reduced chemical and non-toxic control programmes, such as pathogens, predators, indigenous and introduced parasitoids. Once the biological control agents are well established, outbreaks rarely occur because the agents and false codling moth populations are available in balance at low levels. If false codling moth population numbers become too low or are eradicated, biocontrol agents starve or move on and must be replaced.

Only limited data exists on the role of larval parasitoids in controlling false codling moth population numbers, and on their biology, behaviour and ecology. The purpose of this study was to examine and determine the larval parasitoids of false codling moth on citrus in South Africa as potential biocontrol agents. This involved

establishing percentage larval parasitism rates in Citrusdal, Nelspruit, Gamtoos River Valley and Sundays River Valley, mass rearing and examining the biology of the larval parasitoids that showed potential for exploitation.

5.2 Recommendations: placing the new findings in an IPM context

False codling moth infestations can cause severe yield losses; hence, it is important to control the pest to a level below the economic threshold. Control of this insect is complicated by the fact that it is a cryptic pest, in that the moth lays its eggs on the surface of oranges and, upon hatching, the larvae very quickly burrow into the orange and are then essentially in accessible. There is thus a very small window of opportunity, between hatching and burrowing, for applying chemical control measures (Hendry, pers. comm., 2002). This therefore makes chemical control of the moth difficult.

Insect resistance to pesticides is a natural phenomenon resulting from mutations in the genome of some individuals, leading to a reduced susceptibility to pesticides. It is therefore heritable from one generation to the next. The proportion of these resistant individuals in populations increases under the effect of the selection pressure exerted by the repeated application of insecticides until the treatments fail (Vaissayre *et al.*, 2002). A biologically based Integrated Pest Management (IPM) programme of the insect is therefore critical.

Ideally, a biologically based IPM programme of false codling moth on citrus should consist of augmentation of entomopathogens, egg and larval parasitoids, and routine orchard sanitation. The investigation of entomopathogens of pests on citrus world-wide and, in South Africa in particular, has received far less attention than is the case for many agricultural crops. Despite this, a review of the work that has been conducted in the southern African industry indicates that microbial control has a potential for exploitation (Moore 2002a). Entomopathogens of false codling moth on citrus in South Africa and their effectiveness are discussed in Chapter 1. An attractive feature of entomopathogens as biopesticides is their ability to mutate and change, unlike chemical agents, which are immutable thus resulting in insects becoming

resistant. Entomopathogens can change themselves thereby presenting the insect with an "altered" virus against which resistance is ineffective (Hendry, 2002). This, therefore, makes microbial control compatible within an IPM programme.

According to current information on the distribution of the natural enemies of false codling moth, the prospects for classical biological control of the moth are poor because only the egg parasitoids appear to have a significant impact (Newton, 1998). However, studies on the larval parasitoids of false codling moth (Chapter 2) show that *Agathis bishopi* plays a vital role in controlling false codling moth populations on citrus, particularly if used in IPM programmes together with the egg parasitoid, *Trichogrammatoidea cryptophlebiae.* These two species compliment each other, because they not only occupy different niches but also employ different strategies of attack. Augmentation of the egg parasitoid in the early part of the season, and that of the larval parasitoid in the latter part, when wild populations are either absent or at very low levels, would help keep false codling moth populations below the economic threshold level.

This study shows that *A. bishopi* has potential for pest control. In the field, the sex ratio of this species is biased towards females, and females regulate the sex of their progeny by placing fertilised eggs, which give rise to females, in larger and older larval instars and unfertilised eggs, which give rise to males, in smaller and younger larval instars. This not only ensures a high proportion of females, but also larger females with higher fecundity. Clearly, the wasp can locate the larvae inside the fruit, which is a useful trait against a cryptic pest. These are advantageous aspects when considering biological control.

It was difficult to mass-rear *A. bishopi* in the CRI laboratory due to the high viral contamination of the false codling moth larval cultures. This had adverse effects on the results of this study, because most of the larvae that were exposed for parasitism died before they could give rise to parasitoids. Apart from underlining the potential usefulness of pathogens in biological control, this logistical problem shows just how important it is to have a large culture that is free of contaminants when conducting biological studies. Hence, another objective of this study was to improve the existing rearing techniques of false codling moth.

Of the artificial media discussed in Chapter 4, the oatbran and soya flour media prepared in large rearing containers were the most efficient. The preparation of the media is simple and straightforward and the many precautions against contamination fall away, because they comprise only one fraction since the need for autoclaving is not required. The large containers are less labour intensive because more individuals can be reared per container.

5.3 Future research

Opportunities for classical biological control have not been fully exploited, yet there is a potential for the exchange of natural enemies between the different provinces of the country. Studies discussed in Chapter 2 indicate that integrating *A. bishopi* and *T. cryptophlebiae* has the potential of suppressing false codling moth.

Predators, viral and fungal entomopathogens have been identified in false codling moth although they have not been fully investigated as potential control methods. Therefore, further work should include studying the role of indigenous pathogens, parasitoids and predators in regulating wild false codling moth populations, to better understand how they can best be manipulated.

Finally, there is a need for more detailed studies on the biology and impact of *A. bishopi* and other potential natural enemies to determine whether strategies to enhance populations of native natural enemies could be sufficient or whether exotic parasitoids should be introduced.

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