UNIVERSITEIT VAN PRETORIA
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BIOLOGICAL STUDIES ON THE LEPIDOPTERAN EGG PARASITOID *TRICHOGRAMMATOIDEA LUTEA* GIRAULT (HYMENOPTERA: TRICHOGRAMMATIDAE) AT VARIOUS TEMPERATURES

BY

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Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae (Entomology)

in the Faculty of Natural & Agricultural Science

University of Pretoria

Pretoria

February 2011

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DECLARATION

I, Khethani Vincent Mawela, declare that the thesis which I hereby submit for the degree of Magister Scientiae at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature

Date: ……. February 2011

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ACKNOWLEDGEMENTS

The following people and organizations contributed in making this study successful

I would like to thank: Elsie Ringane, Alfred Mashala, Matthews Ngwato, Sam Mokoena, David Mtiang and Annelize Lube the staff of ARC-PPRI (Insect Ecology Division) Rietondale campus and Tanya Saayman of ARC-PPRI Roodeplaat campus for technical assistance.

I would like to express my greatest gratitude to Dr. Kerstin Krüger (my supervisor) and Dr. Rami Kfir (my mentor) for supervision, guidance and support throughout the study, many thanks to Robert Nofemela and Dr. David Simelane for constructive criticism. I am grateful to Agricultural Research Council for placing me in their mentoring program, for training and skills that I acquired as well as employment and financial support. Thanks to ARC-PPRI for allowing me to use their expertise and facilities for this study.

Finally, I would like also to extend my gratitude to my family; my mother Tshilidzi Mawela and my fiancé Tsumbedzo Mashau as well as my friends for their love, support and encouragement when situations got tough. Last but not least, I thank God for protecting, guiding and giving me strength.

SUMMARY

The African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a polyphagous pest that attacks many crops in sub-Saharan Africa. The pest is currently managed through chemical control, and by planting Bt-transgenic cotton. *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae) is an indigenous egg parasitoid of *H. armigera* in southern Africa. The study was undertaken to determine the potential of *T. lutea* as a biological control agent for augmentative releases as an alternative to chemical control, and to pave the way for the development of a mass-rearing method. The biology of *T. lutea* was examined in the laboratory on *H. armigera, Chilo partellus* (Swinhoe), and *Cadra* (formerly *Ephestia*) *cautella* (Walker) (Lepidoptera: Pyralidae). The objectives of the study were to determine (i) the exposure time of UV-irradiation required for killing the embryos of the hosts and the effect of UV-irradiated eggs on life history parameters of *T. lutea*, (ii) which host(s) and temperature(s) (18, 21, 24, 27, 30 and 35 °C) are suitable for mass-rearing of *T. lutea*, and (iii) the longevity and age-related reproductive biology of *T. lutea* on *H. armigera.*

Findings of this study showed that 13 minutes of UV-irradiation were sufficient to kill embryos of all three host species. Life history parameters were not influenced by UV-irradiation but by host species.

Parasitism, number of progeny per parasitized egg, proportion of females, and developmental time of *T. lutea* varied on different host species at temperatures ranging from 18 to 30 °C. *Trichogrammatoidea lutea* did not develop at 35 °C. Overall parasitism by *T. lutea* was higher on *H. armigera* and *Cadra cautella* compared to *Chilo partellus*. The number of progeny per parasitized egg was highest

on *H. armigera* compared to *Cadra cautella* and *Chilo partellus.* The proportion of females was highest on *Chilo partellus,* intermediate on *Cadra cautella,* and lowest on *H. armigera.* For all species and temperatures tested, parasitism and number of progeny per parasitized egg by *T. lutea* was highest on *H. armigera* at 27 °C. The lower threshold for development of *T. lutea* on all hosts was approximately 12 °C.

Female *T. lutea* parasitized eggs of *H. armigera* soon after eclosion, with the highest parasitism achieved on the day of eclosion. Though *T. lutea* parasitized eggs for up to 14 days, it may not be economically viable to keep them in cultures for more than three days since progeny became male biased three days after eclosion. The average longevity of female and male *T. lutea* was 9 and 6 days, respectively. The life table parameters of *T. lutea*, the net replacement rate (R_o), mean generation time (*T*), and instantaneous rate of population increase (r_m) were 25.5, 9.79, and 0.33, respectively.

The timing of inundative releases of *T. lutea* must be synchronised with the time that eggs of *H. armigera* are abundant in the field. The results of this study indicate that *T. lutea* is a good candidate for further testing for augmentative biological control of *H. armigera* in the field. If successful, *T. lutea* may provide opportunities for expanding tactics in the management of *H. armigera* in southern Africa.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Lepidoptera are one of the largest insect orders comprising approximately 160 000 described species (Kristensen, 1999), most of which are primarily associated with angiosperm plants (New, 2004). Although the majority are important as pollinators, some are destructive pests in many agricultural and forestry systems (New, 2004). The economic damage caused by lepidopteran pests on field crops and on stored grain exacerbates the problem of food security and malnutrition in many developing countries (Gressel *et al.,* 2004).

Different control measures are available to reduce damage caused by lepidopteran pests. However, most farmers rely on pesticides which, among other problems, may lead to environmental contamination, risk to human health, non-target effects on natural enemies, and development of resistance (Gressel *et al.,* 2004). The problem of pesticide resistance is especially severe for resource-poor farmers since lack of pest control not only leads to severe crop losses, but also loss of the scarce funds invested. Biological control of insect pests is an environmentally friendly alternative to pesticides, and can be integrated with other control strategies.

1.2 Biological control

Biological control is the regulation of pest populations using natural enemies, including predators, parasitoids, nematodes, and microbial agents (DeBach & Rosen, 1991; Van Driesche & Bellows, 1996; Holt & Hochberg, 1997; Rosenheim, 1998; Bale *et al.,* 2008). The aim of biological control is not to eradicate pests, but rather to suppress pest populations to below economic injury levels (DeBach & Rosen, 1991; Holt & Hochberg, 1997; Bale *et al.,* 2008). As opposed to chemical control, biological

control is advantageous because it poses no threat to human health; it is environmentally friendly and host specific; and the probability of the host developing resistance is low (DeBach & Rosen, 1991; Van Driesche & Bellows, 1996; Bale *et al.,* 2008). In addition, development of a biological control agent is less expensive compared to the development of an insecticide (DeBach & Rosen, 1991; Bale *et al.,* 2008).

Biological control has been successful in both augmentative (periodic releases of biological control agents), and classical (use of exotic biological control agents for exotic pests) biological control programmes (for examples see DeBach & Rosen, 1991; Van Driesche & Bellows, 1996; Elzen & King, 1999). However, the classical biological control approach has been challenged on the basis of possible negative effects (Gutierrez *et al.,* 1999). Exotic biological control agents may pose environmental risks. Such risks may be direct or indirect, and include host range expansion, changes in food web interactions, and extinction of native species due to competition (Follet & Duan, 2000; Pearson & Callaway, 2003).

Augmentation using native or introduced, established natural enemies to control a native or exotic pest comprises two strategies, inundative and inoculative releases (DeBach & Rosen, 1991). Inoculative releases are used as a preventative measure where relatively small numbers of natural enemies are released at intervals and are expected to reproduce and keep pest populations at low levels. An example is the use of the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) for the biological control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), on vegetables and ornamental plants grown in greenhouses (Hoddle *et al*., 1998). With inundative releases, large numbers of natural enemies are released for immediate reduction of a pest population as a corrective

measure. For example, *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) is used for control of different Lepidoptera pests (Clausen, 1972; Bourchier *et al.*, 1993).

Using indigenous biological control agents for augmentative biological control programmes is advantageous because the agents are generally well adapted to local conditions, pose no environmental risks, and no import and release permits are required (Hassan, 1994).

1.3 The African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

Helicoverpa armigera is a highly polyphagous pest that has been reported from 35 crop, and 25 wild host plant species in eastern and southern Africa (Greathead & Girling, 1989). It causes damage to crops such as tomatoes, potatoes, pepper, pigeon pea, peas, soybeans, okra, sorghum, maize and cotton (Kfir & Van Hamburg, 1988; CABI & EPPO, 1990; Cunningham *et al.,* 1999). *Helicoverpa armigera* lays its eggs on different parts of crops, preferably on hairy surfaces during periods of bud burst and flower production (CABI & EPPO, 1990; Cunningham *et al.,* 1999). On cotton, for example, two larvae on a plant can destroy all bolls within 15 days. On tomatoes, larvae invade fruits, prevent development and cause falling of fruits, while on maize they consume grains (CABI & EPPO, 1990).

In South Africa, *H. armigera* is a major pest on cotton where it is currently controlled by planting Bt cotton (transgenic cotton that contains δ–endotoxin genes from *Bacillus thuringiensis virescens*) and, only to a small extent, chemically. On other crops *H. armigera* is usually controlled with synthetic pesticides. Resistance of

H. armigera to different classes of pesticides such as pyrethroids, organophosphates, and carbamates (Ahmad *et al.,* 1997; Malik *et al.,* 2003; Martin *et al.,* 2003, 2005; Buès *et al.,* 2005) has been reported in Asia, Europe, and Africa (Buès *et al.,* 2005). *Bacillus thuringiensis* transgenic (Bt) cotton and several hybrids of maize, that are not susceptible to most Lepidoptera pests, are available in South Africa (Cherry *et al.,* 2003). However, development of resistance to Bt-transgenic crops by *H. armigera* has been reported under laboratory conditions (Tabashnik *et al.,* 2008).

Biological control of *H. armigera* in Africa has been reviewed recently by Cherry *et al*. (2003). In South Africa, biological control of this species has been investigated as early as the 1930s by Taylor (1932), Parson & Ullyett (1934, 1936), and Parry Jones (1937). However, attempts at classical and augmentative biological control with exotic and indigenous natural enemies have met with limited success on high value crops in South Africa (Cherry *et al*., 2003). Reasons for the failure of biological control include the perception that parasitoids are limited (e.g. by seasonal climates) and their effectiveness is unpredictable, limited knowledge of the farmers, and the use of insecticide by neighbouring farmers (Van Hamburg & Guest, 1997; Cherry *et al*., 2003). In Africa, different natural enemies have been recorded on *H. armigera* including predators, and egg and larval parasitoids of which the majority are polyphagous (Cherry *et al.,* 2003). However, their distribution and impact is limited by geographical zones, seasons, and cropping systems (Parry Jones, 1937; Van Den Berg & Cock 1993). For southern Africa, egg parasitoids were found to be the most important mortality factor (Van Hamburg & Guest, 1997), though their populations are very low in the field. The low population and parasitism contribution by egg parasitoids in the field (Parson & Ullyett 1934, 1936; Van Hamburg & Guest, 1997;

Cherry *et al.,* 2003) show that augmentative release of egg parasitoids may increase parasitism levels of *H. armigera* in southern Africa.

1.4 *Trichogramma* species as biological control agents

Parasitoids of the genus *Trichogramma* (Trichogrammatidae: Hymenoptera), and to a lesser extent the related genus *Trichogrammatoidea,* are the most widely studied and successfully used parasitoids for biological control (Greenberg *et al.,* 1996). These parasitoids attack insect eggs, mainly those of Lepidoptera (Hassan, 1994). At least nine *Trichogramma* species are produced commercially for biological control purposes worldwide (Li, 1994; Smith, 1996; Greenberg *et al.,* 1996; Khan *et al.,* 2004; Doyon & Boivin, 2005). An area of over 32 million hectares of agriculture and forestry in 30 countries was treated annually with *Trichogramma* species (Li, 1994; Smith, 1996). Examples of commercially available species that have been used successfully to suppress lepidopteran pests include *Trichogramma evanescens* Westwood, and *Trichogrammatoidea minutum* Riley (Elzen & King, 1999; Schöller & Hassan, 2001).

Of importance for augmentative biological control is the ease of rearing the agent. It is, therefore, advantageous that *Trichogramma* species not only target many economically important lepidopteran pests, but that they can be mass-reared easily on factitious hosts, which is usually less expensive than rearing the parasitoid on the target host.

Trichogrammatids are minute, ranging from 0.2 to 1.5 mm in size (Romeis *et al.,* 2005). They parasitize the eggs of more than 400 lepidopteran pest species (Hawkins & Sheehan, 1994; Khan *et al.,* 2004; Doyon & Boivin, 2005). Although they are capable of flying, their foraging is primarily through walking and short jumps

(Romeis *et al.,* 2005). Female trichogrammatids use different olfactory and visual cues to search for hosts. These include sex pheromones of the adult hosts, moth scales left close to eggs and other volatiles associated with host eggs, egg shape and colour (Godfray, 1994; Romeis *et al.,* 2005). When a female trichogrammatid encounters a host egg, it antennates (gathering information with its antenna by touching) or drums the egg, then walks across and around the egg to evaluate its size to determine the clutch size if the parasitoid is facultative gregarious (more than one parasitoid develops per host), and the sex of the progeny to be allocated to the host (Godfray, 1994; Romeis *et al.,* 2005). Larger hosts are allocated larger clutch sizes and a greater proportion of females (Godfray, 1994; Luck *et al.,* 1999). Host size evaluation is followed by host quality evaluation through probing the host with the ovipositor. This may lead to oviposition or rejection depending on host quality. In addition, the age of the female parasitoid and experience with previous hosts and host age may influence decisions on host acceptance, clutch size and sex ratio (Godfray, 1994).

1.5 Factors affecting success of trichogrammatids as biological control agents

The success of augmentative biological control programmes is influenced by various factors. These include host quality, fitness (e.g. fecundity, host finding, longevity, mobility etc.) of laboratory reared parasitoids, host-parasitoid synchrony, environmental conditions during rearing and release of the parasitoids, as well as pesticide usage (Etzel & Legner, 1999). Of these, host and environmental conditions are known to be the main factors affecting the effectiveness of parasitoids (Godfray, 1994; Etzel & Legner, 1999) because, for all immature parasitoids, the single host represents the only nutritional source (Colinet *et al.,* 2005), and temperature affects

development and survival of all insects (Pedigo, 1996; Dent, 1991; Hohmann & Luck, 2000).

Most factitious host species for mass-rearing Trichogrammatidae have relatively small eggs which produce small adults which, in turn, generally have lower fecundity and shorter longevity (Kfir, 1981; Godfray, 1994), and lower walking speed than their larger counterparts, and this may affect parasitism in the field (Etzel & Legner, 1999; Doyon & Boivin, 2005). As outlined above (section 1.4), female Trichogrammatidae evaluate the quality of the host for the developmental requirements of their progeny (Godfray, 1994). Therefore, females are expected to lay more eggs which develop into females on large and high quality hosts, because the fitness of male progeny suffers less from being small than does female progeny (Godfray, 1994; Luck *et al.,* 1999; Colinet *et al.,* 2005).

The use of non-developing hosts for rearing Trichogrammatidae is frequently practised (Etzel & Legner, 1999). Freezing and radiation are some of the techniques commonly used to halt development of the rearing host to preserve quality. However, few studies have been published on the effect of non-developing hosts on the biology of parasitoids. As a result some parasitoids fail to complete development on such hosts (Etzel & Legner, 1999).

Apart from rearing Trichogrammatidae on factitious hosts, Greenberg *et al.* (1996) reported the *in vitro* rearing of *Trichogramma* using wax-Vaseline capsules, hanging drops and plastic capsules together with an artificial diet containing 27-50 % of oak-silkworm or eri-silkworm pupal haemolymph, chicken egg yolk, malt and salt mixtures. The *in vitro* rearing technique proved suitable for the development of several *Trichogramma* species, such as *T. dendrolimi, T. chilonis,* and *T pretiosum* (Greenberg *et al.,* 1996). This *in vitro* method for *Trichogramma* species, however,

was not adopted because of low adult emergence (Zhong-Neng *et al.,* 1997), longer developmental time, and high emergence of deformed adults (Norlund *et al.,* 1997).

Development of an efficient biological control programme with Trichogrammatidae involves the selection of strains with high efficiency against target pests in a given environmental condition (Kalyebi *et al.,* 2005; Bale *et al.,* 2008). Environmental factors influence rate responses of activities such as feeding, dispersal, egg laying, and immature development (Pedigo, 1996). Amongst all environmental factors, temperature is the most important, influencing the life of insects because all insects are poikilothermic; their body temperature is influenced by ambient temperature (Pedigo, 1996; Dent, 1991; Hohmann & Luck, 2000). In addition, temperature plays an important role in tritrophic interactions among poikilotherms as it influences the level of control that natural enemies exert in biological control programmes (Kalyebi *et al.,* 2005).

Knowledge of the thermal requirements and the influence of temperature on biocontrol agents is crucial in all phases of a biological control process, i.e. ranging from the selection of natural enemies, prediction of distribution and population dynamics of pest and natural enemy, to the development of mass-rearing and field release techniques (Dent, 1991; Hohmann & Luck, 2000). The developmental rate determines the generation time, which is inversely related to growth rate of the parasitoid population (Colinet *et al.,* 2005). This influences performance among different parasitoid species attacking the same host during the same season and habitat (Colinet *et al.,* 2005; Doyon & Boivin, 2005). The parasitoid with the shortest generation time will emerge and parasitize first, while those with longer generation times will emerge later and may have difficulties of finding or locating hosts (Doyon & Boivin, 2005).

It is essential to develop and implement good quality control criteria as part of mass rearing programmes to ensure the production of high-quality parasitoids which are capable of finding the target host, parasitize and develop in it (Bourchier *et al.,* 1993). The quality of parasitoids is measured through life history parameters such as percentage parasitism, percentage emergence, developmental time, sex ratio, adult longevity and size (Khan *et al.,* 2004; Kalyebi *et al.,* 2005).

1.6 *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae)

Trichogrammatoidea lutea is indigenous to southern Africa (Parson & Ullyett, 1936; Parry Jones, 1937; Kfir, 1982). It has been recorded as an egg parasitoid of several lepidopteran pests, including *H. armigera*, the spotted stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), and the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Kfir, 1981; Kfir & Van Hamburg, 1988). *Trichogrammatoidea lutea* is yellow in colour; it has long marginal hairs on the wings, and adult length ranges from 0.30 to 0.70 mm for males and 0.45 to 0.70 mm for females (Parry Jones, 1937). It is a facultative gregarious parasitoid. Female *T. lutea* lay 2 to 4 eggs per *H. armigera* egg (Parry Jones, 1937). A study by Kfir (1982) on the reproduction of *Trichogramma brasiliensis* Ashmead and *T. lutea* on eggs of *H. armigera,* found that *T. lutea* is arrhenotokous with a haplo-diploid genetic system (unfertilized eggs develop into haploid males through a form of parthenogenesis known as arrhenotoky and fertilized eggs develop into diploid females), and that it has a larger clutch size than the deuterotokous (unfertilized eggs develop into males and females) *T. brasiliensis*.

Trichogrammatoidea lutea is well synchronized with *H. armigera* in the field in South Africa, being the most active egg parasitoid when the host is most abundant (Parry Jones, 1937; Kfir, 1982), suggesting that it has potential for biological control of *H. armigera*. However, due to relatively low population levels of *T. lutea* in the field, levels of parasitism are not sufficient to control *H. armigera*. Against this background, augmentative biological control with inundative releases of laboratory-reared *T. lutea* may prove to be an option to achieve high levels of parasitism in the field. Kfir and Van Hamburg (1988), examining interspecific competition between *Telenomuos ullyetti* and *T. lutea* on eggs of *H. armigera,* found that the population of *T. lutea* increased at high temperature (28 °C) and decreased at low temperature (22 °C) as compared to *Telenomuos ullyetti,* indicating that it is abundant from spring to the end of summer. Available information shows that *T. lutea* has potential as a biological control agent, and that it could be incorporated with other control methods as an integrated pest management (IPM) programme to control *H. armigera*. For example, Mellet and Schoeman (2004) reported that parasitism of *H. armigera* eggs by *T. lutea* did not differ among Bt cotton, non-Bt cotton and Bt-sprayed cotton.

Further studies on the biology of *T. lutea* on different host species and temperature regimes are required to lay the foundation for the development of an effective mass-rearing technique, which in turn is the key to implementing a successful biological control programme (Etzel & Legner, 1999).

1.7 Potential hosts for rearing *Trichogrammatoidea lutea*

The African bollworm *H. armigera*, the spotted stem borer *Chilo partellus,* and the almond moth *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) are some of

the most damaging lepidopteran pests in Africa. They were selected as potential hosts for *T. lutea* because host rearing techniques were already available, and all three species are presently being reared at the ARC-Plant Protection Research Institute (ARC-PPRI) in Pretoria.

Information on *H. armigera* has been provided in section 1.3. *Chilo partellus* is an exotic stemborer that was accidentally introduced, and invaded Africa in the early 20th century from Asia (Rebe, 2002). It is widely distributed as a pest in countries such as India, Pakistan, Indonesia, Sri Lanka, Thailand (Kumar, 1997) and southern and East Africa (Kfir *et al.,* 2002). *Chilo partellus* is a serious pest of maize, sorghum, rice, and sugar cane (Ashfaq & Ahmad, 2002; Rebe, 2002; Gressel *et al.,* 2004). Pre-harvest crop losses due to stemborers in general, amount to about 40 % on maize and sorghum, with *Chilo partellus* and the indigenous *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) being the most prevalent and damaging stemborers in Africa (Kumar, 1997; Gressel *et al.,* 2004). Both *B. fusca* and *Chilo partellus* are found at low and high altitudes (Gressel *et al.,* 2004). Kfir (1997) reported that *Chilo partellus* is competitively displacing indigenous stemborers in East and southern Africa. Adult females of *Chilo partellus* lay eggs close to the mid-rib on leaves. After the eggs have hatched, the first- and second-instar larvae feed on leaves making small holes. The third- to sixth-instar larvae tunnel into the stem tissue destroying growing or vegetative tissues, and causing drying of leaves and a stunted and rugged appearance (Van Hamburg, 1980; Kumar, 1997; Ashfaq & Ahmad, 2002).

Cadra cautella is a major pest of stored products such as grain, nuts and dried fruits (Bowditch & Madden, 1996; Sabbour, 2003; Ryne *et al.,* 2004). Adult females of *Cadra cautella* prefer to oviposit on substrates or food sources already contaminated by larvae (Bowditch & Madden, 1996). The damage results from

webbing in grains or other food substrates, and on the surface of bags, for example, forming large lumps that render food unsuitable for consumption. *Cadra cautella* is a pest throughout the warmer parts of the world (Bowditch & Madden, 1996). Its management is based on regular fumigation of stores with pesticides (Bowditch & Madden, 1996).

1.8 Objectives

The main objective of this study was to improve our knowledge on the biology of *T. lutea,* and to assess the possibility of mass rearing and testing this species for release in augmentative biological control programmes for *H. armigera*. The specific objectives were to determine (i) the exposure time of UV-irradiation required for killing the embryos of the hosts and the effect of UV-irradiated eggs on life history parameters of *T. lutea*, (ii) which host(s) and temperature(s) (18, 21, 24, 27, 30 and 35 °C) are suitable for mass-rearing of *T. lutea*, as well as (iii) the longevity and age-related reproductive potential of *T. lutea* on *H. armigera*.

1.9 Thesis outline

Following Chapter 1, the General Introduction, Chapter 2 provides information on rearing *T. lutea* on three lepidopteran host species, *H. armigera, Chilo partellus,* and *Cadra cautella*.

In this study, the host eggs used for the experiments had to be irradiated to halt embryo development because the larvae of *H. armigera* are cannibalistic (Kfir & Van

Hamburg, 1988). Cannibalism may have a negative effect on parasitism as a result of *H. armigera* larvae feeding on parasitized eggs.

Chapter 3 examines the effect of ultraviolet (UV) irradiation of eggs of the three hosts on the development of *T. lutea,* and the shortest duration required to kill the host embryos to limit the detrimental effects UV-irradiation might have on the quality of the hosts.

Chapter 4 deals with the effects of UV-irradiated hosts on the biology of *T. lutea* at different constant temperatures. Life history parameters, including percentage parasitism, number of progeny emerging per host (clutch size), duration of development, and sex ratio of progeny of *T. lutea* were examined to determine the most suitable host species and temperature for development.

Chapter 5 covers the longevity and reproductive biology of *T. lutea* on *H. armigera* at an intermediate temperature.

Chapter 6 provides a general discussion of this research, and evaluates the potential for *T. lutea* to be mass reared and released as a biological control agent for *H. armigera.*

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CHAPTER 2

MAINTENANCE OF INSECT CULTURES: *HELICOVERPA ARMIGERA, CHILO PARTELLUS, CADRA (EPHESTIA) CAUTELLA* AND *TRICHOGRAMMATOIDEA LUTEA*

Maintenance of insect cultures: *Helicoverpa armigera, Chilo partellus, Cadra cautella* and *Trichogrammatoidea lutea*

2.1 Lepidoptera hosts

Cultures of *H. armigera* and *Chilo partellus* were maintained in the insectary at the ARC-Plant Protection Research Institute (ARC-PPRI) Rietondale Campus (25°44'S, 18°13'E) in Pretoria, South Africa.

The culture of *H. armigera* (Fig. 2.1a) was established in 1974 with specimens collected from the Springbok Flats (29°40'S 17°53'E) and supplemented in 2006 with newly emerged larvae from eggs collected near Groblersdal (25°15'S, 29°25'E) in Mpumalanga, South Africa. The larvae of *H. armigera* were reared as described by Kfir (1994) in glass vials on an artificial diet consisting of the following ingredients: kidney beans, yeast, methyl-4-hydroxybenzoate, ascorbic and sorbic acid, formaldehyde and distilled water (Fig. 2.1b,c). The moths were kept in small Perspex buckets (17 cm high × 20 cm diameter) with the top covered by gauze held tightly with rubber bands (Fig. 2.1d). The moths were supplied with a 10 % sugar solution to feed on. White filter paper was placed on top of the gauze overnight for the *H. armigera* females to deposit eggs. The culture was kept in controlled-environment rooms and maintained at 25 ± 2 °C, 60 to 70 % RH and natural photoperiod.

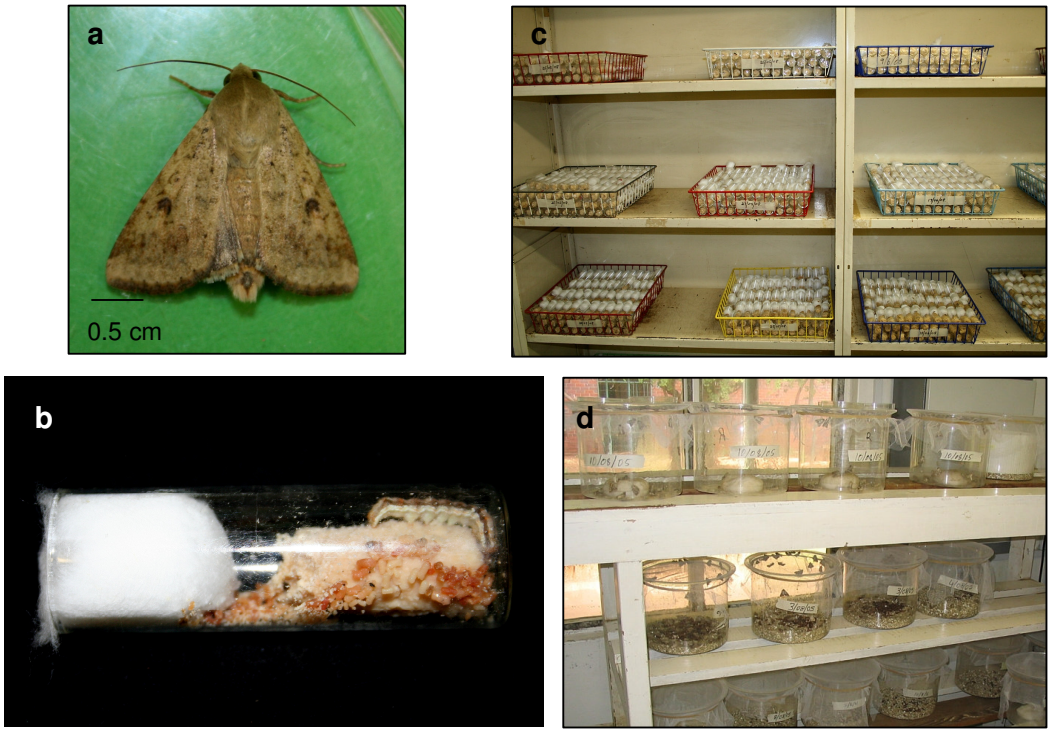

Fig. 2.1 Rearing system for *Helicoverpa armigera*; (**a**) adult *H. armigera,* (**b**) baskets with glass vials and artificial diet used to rear larvae of *H. armigera*, (**c**) close-up of glass vial used to rear larvae of *H. armigera* on artificial diet and (**d**) buckets used to maintain adult *H. armigera*.

The culture of *Chilo partellus* (Fig. 2.2a) was established with specimens collected from the Springbok Flats (29°40'S 17°53'E) in 1975 (pers. comm. Rami Kfir, ARC-PPRI). To improve genetic variability, the culture was supplemented several times with specimens collected at the Brits Research Farm (23°25'S, 27°76'E) of the ARC – Industrial Crops Institute, North West Province, South Africa. Larvae of *Chilo partellus* were reared on artificial diet in glass jars (12 cm high × 7 cm diameter) (Fig. 2.2b). The diet consisted of the following ingredients: chickpea powder, sugar, yeast, ascorbic and sorbic acid, methyl-4-hydroxybenzoate, distilled water, and ethyl alcohol (Kfir, 1992). The moths of *Chilo partellus* were maintained

in Perspex buckets as described above (Fig. 2.1d). However, corrugated paper was placed around the buckets on the inside for the adults to deposit eggs on. Moths were supplied with 10 % sugar solution to feed on. The culture was kept in a controlled-environment room and maintained at 27 ± 2 °C, approximately 70% RH and 16L: 8D photoperiod.

Fig. 2.2 Rearing system for *Chilo partellus;* (**a**) adult *Chilo partellus* and (**b**) glass jars used to rear the larvae of *Chilo partellus* on artificial diet.

The culture of *Cadra cautella* (Fig. 2.3a) was started in the late 1980s with specimens collected from stored grain in several locations in South Africa and the larvae were maintained in glass jars (22 cm high x 14 cm diameter) (Fig. 2.3b) on artificial diet in the insectary of the ARC-PPRI Roodeplaat Campus (25°39'S, 28°20'E) near Pretoria. The diet of *Cadra cautella* larvae consisted of ingredients: yeast, glycerine, honey, wheat and maize meal. Adults were maintained in containers that were divided by a sieve into halves, the adult moths being kept in the upper half such that the eggs that the females laid fell through the sieve onto the filter paper placed in the bottom of the lower half (pers. comm. Tanya Sayman, ARC-PPRI). The

eggs were subsequently collected and sent to ARC-PPRI Rietondale campus daily for the duration of experiments.

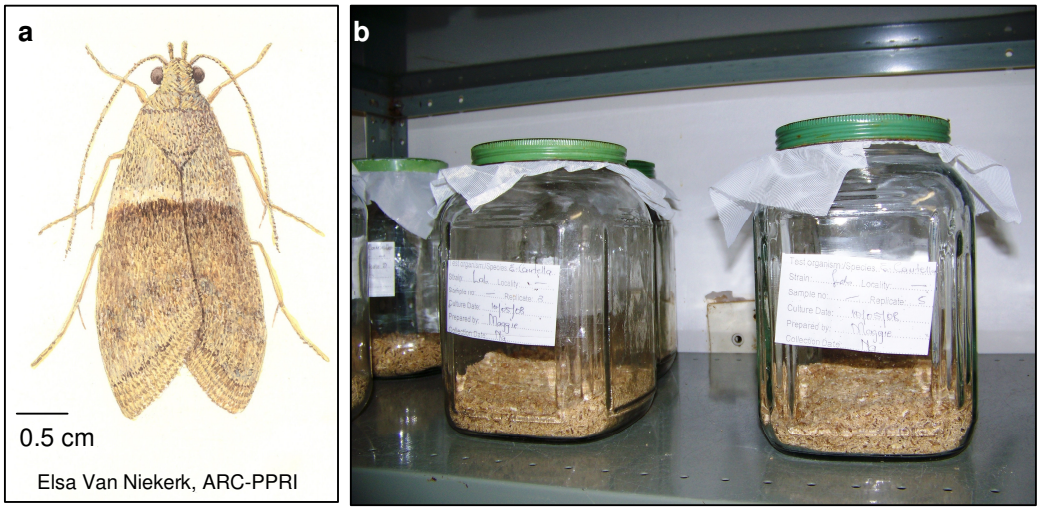

Fig. 2.3 Rearing system for *Cadra cautella;* (**a**) adult *Cadra cautella* and (**b**) glass jars used for rearing larvae of *Cadra cautella* on artificial diet.

2.2 *Trichogrammatoidea lutea*

A culture of *T. lutea* was established in 2006 from parasitized eggs of *H. armigera* collected from cotton fields in Groblersdal (25°15'S, 29°25'E) and Rust de Winter (25.1986 S, 28.6092 E), Gauteng, South Africa (pers. comm., Rami Kfir). The parasitoid was maintained on eggs of *H. armigera, Chilo partellus* and *Cadra cautella* in the insectary of the ARC-PPRI in Rietondale campus. *Trichogrammatoidea lutea* was reared in wooden cages (floor 42 x 30 cm x height 30 cm, Fig. 2.4a) in an environment-controlled room at 26 °C, approximately 60 % RH and 16L: 8D photoperiod. Adult *T. lutea* were supplied with eggs of *H. armigera* on filter paper, *Chilo partellus* on corrugated paper and *Cadra cautella* glued on filter paper with a

non toxic glue (Pritt ® Glue Stick) daily. Papers with parasitized hosts were removed daily and placed in ventilated glass jars until adults emerged (Fig. 2.4b). Water and honey were provided in the cages for *T. lutea* to feed on.

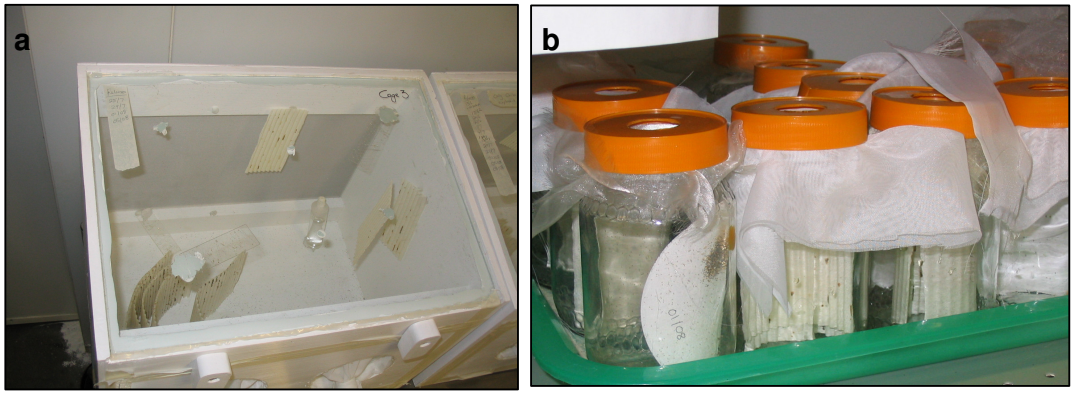

Fig. 2.4 Rearing system for *Trichogrammatoidea lutea*: (**a**) wooden cage used to expose the eggs of *Helicoverpa armigera, Chilo partellus* and *Cadra cautella* on paper strips to *T. lutea* and (**b**) glass jars used for keeping parasitized eggs of the three host species by *T. lutea*.

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CHAPTER 3

HOST SUITABILITY OF UV-IRRADIATED EGGS OF THREE LEPIDOPTERA SPECIES FOR REARING *TRICHOGRAMMATOIDEA LUTEA* GIRAULT (HYMENOPTERA: TRICHOGRAMMATIDAE)

Host suitability of UV-irradiated eggs of three Lepidoptera species for rearing *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae)[1]

Abstract

To develop a rearing programme for the egg parasitoid *Trichogrammatoidea lutea* for augmentative biological control, the effects of ultraviolet (UV) irradiated host eggs were evaluated on the number of eggs parasitized, development time, number of progeny and sex ratio of *T. lutea*, using eggs of the lepidopteran species *Helicoverpa armigera*, *Chilo partellus* and *Cadra cautella* as hosts. UV-irradiation of 13 min was sufficient to kill embryos of all three hosts. The highest mean percentage parasitism on UV-irradiated and un-irradiated eggs occurred on those of *H. armigera* (47.8 and 33.3 %) and *Cadra cautella* (33.2 and 55.5 %), followed by *Chilo partellus* (6.6 % for both). The mean number of individuals emerged per single parasitized host of UV-irradiated and un-irradiated eggs was highest for *H. armigera* (2.5 and 2.1) and lower for *Chilo partellus* (1.3 and 1.4) and *Cadra cautella* (0.9 and 0.8). The sex ratio of *T. lutea* was female-biased on UV-irradiated eggs of *H. armigera,* on UV-irradiated and un-irradiated eggs of *Cadra cautella,* and male-biased for *Chilo partellus* eggs and un-irradiated eggs of *H. armigera.* The duration of development of *T. lutea* from egg to adult male and female differed slightly between UV-irradiated and un-irradiated eggs. Overall, UV-irradiation of host eggs had negligible effects on percent parasitism, number of progeny per parasitized egg and sex ratio of *T. lutea.* Of the three lepidopteran species evaluated, *H. armigera* appears to be the most suitable

[1] **Published as**: K.V. Mawela, R. Kfir & K. Krüger (2010) Host suitability of UV-irradiated eggs of three Lepidoptera species for rearing *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae). *Journal of Applied Entomology* **134**: 737–744. Doi: 10.1111/j.1439-0418.2009.01472.x. The content of the article has been slightly modified for a more uniform presentation of chapters in the thesis.

host for mass-rearing of *T. lutea.* The results indicate that UV-irradiation of Lepidoptera eggs is suitable for mass rearing of *T. lutea.*

3.1 Introduction

Mass production of parasitoids for augmentative biological control can be simplified by rearing parasitoids on non-growing hosts (Etzel & Legner, 1999). Irradiation of host eggs is one of the methods used for this purpose (e.g. Smith, 1996; Etzel & Legner, 1999). Amongst radiation methods, ultraviolet (UV) irradiation of host eggs is frequently used for rearing egg parasitoids. It is known to improve survival of parasitoids during immature stages by preventing cannibalism (Kfir & Van Hamburg, 1988) and limiting unknown mortality factors of early immature stages (Cônsoli *et al.,* 2000). UV-irradiation also assists with preservation of hosts (Lianzhong *et al.,* 1993) and has been suggested to limit occurrence of pathogens on egg surfaces (Guerra *et al.,* 1968). An advantage of UV radiation over ionizing radiation techniques is that it is non-radioactive and therefore safer and simpler to use.

Trichogramma species and members of the related genus *Trichogrammatoidea* (Hymenoptera: Trichogrammatidae) have been successfully used as biological control agents for lepidopteran pests in agriculture and forestry in many countries throughout the world (Li, 1994; Greenberg *et al.,* 1996; Smith, 1996). Several trichogrammatid parasitoids have been reared on irradiated eggs without any detrimental effects on their life history. For example, rearing *Trichogramma brasiliensis* Ashmead on eggs laid by gamma-irradiated females of potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) had no detrimental effects (Harwalker *et al.,* 1987). However, when given a choice between non-viable eggs from ^{60}CO-irradiated

adults and un-irradiated viable eggs of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), *Trichogramma platneri* Nagarkatti parasitized significantly more viable than non-viable eggs (Zhang & Cossentine, 1995). *Trichogrammatoidea lutea* has been reported to parasitize and complete development on UV-irradiated eggs of the African bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Kfir & Van Hamburg, 1988). However, the exposure time to UV-irradiation required to kill embryos of host eggs and the effects of UV-irradiated host eggs on life history parameters, which are crucial for the development of mass rearing techniques, of *T. lutea* have not been previously examined.

Trichogrammatoidea lutea is a facultative gregarious egg parasitoid (Parry Jones, 1937; Kfir, 1982) of a number of lepidopteran pests in Africa (Kfir & Van Hamburg, 1988, Wahner *et al.,* 2008). Previous studies show that *T. lutea*, which is indigenous to South Africa, may have potential as a biological control agent for augmentative releases in this country. For example, a study on interspecific competition between *T. lutea* and *Telenomus ullyetti* Nixon (Hymenoptera: Scelionidae) parasitizing eggs of *H. armigera* reported that in South Africa *T. lutea* is dominant in summer and *T. ullyetti* in winter (Kfir & Van Hamburg, 1988). This suggests a good synchronization of *T. lutea* with pests which are active during the summer months, such as *H. armigera* (Cherry *et al.,* 2003). In addition, *T. lutea* is being evaluated as a biological control agent for the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Wahner *et al.,* 2008).

In this study, the eggs of three lepidopteran species, the natural host *H. armigera* and two factitious hosts, the spotted stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and the almond moth *Cadra* (formerly *Ephestia*) *cautella* (Walker) (Lepidoptera: Pyralidae), were chosen for rearing *T. lutea*. The three species

are some of the most important lepidopteran pests in many African countries (Kumar, 1997; Cherry *et al.,* 2003; Gressel *et al.,* 2004; Mutambuki & Harberd, 2004) and are reared in insectaries locally. Unlike for *Chilo partellus* and *Cadra cautella,* the larvae of *H. armigera* are cannibalistic (Kfir & Van Hamburg, 1988).

Exposure time to UV-irradiation required for killing the embryos of the embryos of the hosts and the effect of UV-irradiated eggs on life history parameters, i.e. parasitism development time, number of progeny per host and sex ratio, of *T. lutea* on three potential lepidopteran hosts were investigated as part of developing a mass-rearing technique.

3.2 Materials and methods

3.2.1 Insect colonies

Helicoverpa armigera eggs were obtained from a culture that was established with specimens collected from the Springbok Flats (29°40'S 17°53'E) in 1974 and supplemented in 2006 with specimens collected from cotton plants near Groblersdal (25°15'S 29°25'E) in Mpumalanga, South Africa. The culture of *Chilo partellus* was established with specimens collected from maize and grain sorghum plants at Springbok Flats in 1975 and then supplemented several times with specimens collected at the Brits Research Farm (23°25'S 27°76'E), North West Province, South Africa. The culture of *Cadra cautella* was started in the late 1980s with specimens collected from stored grain from several localities in South Africa. A culture of *T. lutea* was established in 2006 with parasitized eggs of *H. armigera* collected from cotton fields at Groblersdal and Rust de Winter (25°13'S 28°28'E), Gauteng, South

Africa. All cultures were kept in insect rearing facilities of the ARC-Plant Protection Research Institute (ARC-PPRI) in Pretoria.

Trichogrammatoidea lutea was reared in wooden cages (30 x 30 x 42 cm) consisting of a glass top, gauze back and gauze sleeves in front. The cages were kept in an environment-controlled room maintained at 26 °C, 60% RH and 16L: 8D photoperiod. Adult *T. lutea* were supplied with hosts daily and parasitized hosts were removed daily and placed in glass jars until adults emerged. Water and honey were provided in the cages for adult *T. lutea* to feed on.

3.2.2 Minimum exposure time to UV-irradiation required for killing the embryos of the host species

The sensitivity of insects to irradiation varies with order, species, strain and developmental stage (Beard, 1972; Barki *et al.,* 2005). Therefore, the exposure time required for killing all embryos of the three hosts was determined to reduce any detrimental effects on the quality of irradiated host eggs that may affect subsequent parasitism and parasitoid development. One-day-old eggs of each host species were separated into batches of 20. The eggs were evenly distributed and pasted onto a filter paper with non-toxic glue. The batches of eggs were irradiated using a new UV light tube (Philips TUV 30W/ G30T8, 254 nm, in a fitting with a reflective aluminium backing) (Fig. 3.1), placed 6 cm from the eggs and using nine different exposure times: 1, 3, 5, 7, 10, 13, 15, 20 and 25 minutes. A batch of 20 un-irradiated eggs of each host species served as control. After irradiation, the eggs were incubated in a growth chamber (Labcon™ LTGC 20) at 25 °C, 60 % RH and 16L: 8D photoperiod. The emerged Lepidoptera larvae were counted and recorded daily. The experiment was replicated five times for each host and exposure time. The shortest time required

to prevent larval emergence completely was used as the minimum exposure time to UV-irradiation required for all three moth species.

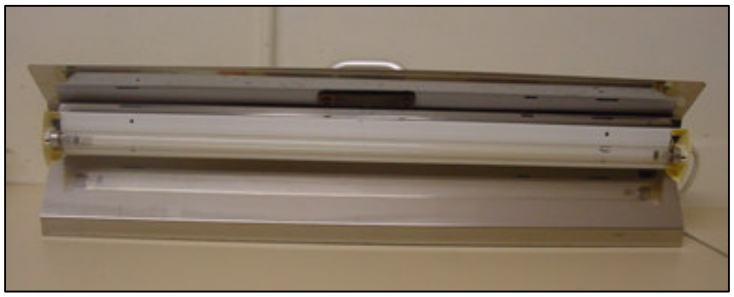

Fig. 3.1 Ultraviolet radiation light tube (Philips TUV 30W/ G30T8, 254 nm, in a fitting with a reflective aluminium backing) used to irradiate the eggs of three moth species (*Helicoverpa armigera, Chilo partellus* and *Cadra cautella*).

3.2.3 Effects of UV-irradiated host eggs on life history parameters of Trichogrammatoidea lutea

To compare the effects of irradiated and non-irradiated host eggs on parasitism, sex ratio, number of progeny per host and developmental time of *T. lutea,* one-day-old eggs of each host were separated into batches of 20. For each host, 32 replicates with 20 eggs each were irradiated for 15 minutes. Thereafter, the eggs were exposed to a one-day-old pair (1 female and 1 male) of *T. lutea* for 24 hours in glass vials (85 mm high × 10 mm diameter) in a growth chamber maintained at 25 °C, 60 % RH and 16L: 8D photoperiod. Concurrently, a control treatment consisting of 32 replicates with 20 one-day-old non-irradiated eggs each of each host were exposed to one-day-old pairs of *T. lutea* for 24 hours under the same conditions as the irradiated eggs. For both treatment and control, the glass vials were streaked with honey to provide food for the adult parasitoids. After 24 hours the wasps were removed and

eggs were incubated in a growth chamber at 25 °C, 60 % RH and 16L: 8D photoperiod. Observations were made once daily and the number of parasitized eggs, developmental time, sex ratio and number of progeny were recorded. As *T. lutea* is a facultative gregarious parasitoid, percent parasitism was determined by the number of parasitized eggs, identified by the black chorion (Kfir, 1981). In addition, the number of lepidopteran larvae hatched was recorded and larvae were removed during each observation.

3.2.4 Data analysis

Effect of UV-irradiation time on larval emergence from host eggs was analysed using non-linear regression. Life history parameters of *T. lutea* were analysed using analysis of variance (ANOVA) for unbalanced designs. Transformation of data did not result in stabilization of treatment variances and the test level was therefore set at 1 %. Where differences were significant *t*-probabilities of pairwise differences were computed to separate means at $P < 0.01$. Pearson's product-moment correlations were calculated between the number of larvae emerged from un-irradiated host eggs and the number of eggs parasitized by *T. lutea*. Analyses were carried out using GenStat® (Payne *et al.,* 2007) and Statistica (Version 7.0, StatSoft, Inc, 1984-2004).

3.3 Results

3.3.1 Minimum exposure time to UV-irradiation required for killing the embryos of the host species

The highest larval emergence occurred from un-irradiated eggs, ranging between 85 % for *H. armigera* to 68 % for *Chilo partellus* and *Cadra cautella*. Hatching of larvae from UV-irradiated eggs decreased with increasing exposure time. To prevent larval emergence completely, the exposure times required were 13 minutes for *H. armigera*, 10 minutes for *Chilo partellus,* and 5 minutes for *Cadra cautella*. The effect of UV-irradiation on larval emergence was similar for all three species (Fig. 3.2).

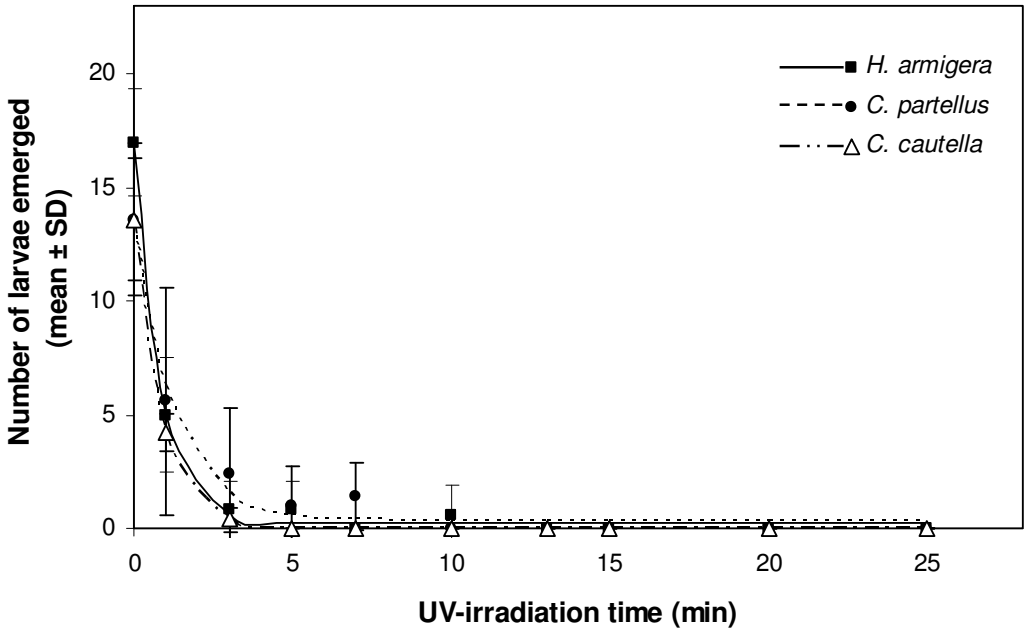

Fig. 3.2 Larval emergence from eggs of three lepidopteran host species after different durations of UV-irradiation. *Helicoverpa armigera*: $y = 0.213+16.774*e^{-1.241x}$, $R^2_a = 94.4$, $P < 0.001$; *Chilo partellus*: $y = 0.355+13.038*e^{-0.793x}$, $R^2_a = 78.3$, $P < 0.001$; *Cadra cautella*: $y = -0.005+13.606*e^{-1.174x}$, $R^2_a = 96.1$, $P < 0.001$.

3.3.2 Effects of UV-irradiated host eggs on life history parameters of Trichogrammatoidea lutea

The number of eggs of the cannibalistic *H. armigera* parasitized by *T. lutea* was significantly negatively correlated with the number of *H. armigera* larvae emerged from un-irradiated eggs ($P < 0.0001$), but not for *Chilo partellus* (Fig. 3.3). The number of larvae emerged from un-irradiated eggs of *Cadra cautella* was too small to analyse. *Helicoverpa armigera,* with 39 %, had the highest rate of larval emergence from un-irradiated control eggs exposed to *T. lutea*, followed by *Chilo partellus* with 26 % and *Cadra cautella* with 1 %. Overall percent parasitism by *T. lutea* was highest on eggs of *Cadra cautella* (44 %), and *H. armigera* (41%) and lowest on eggs of *Chilo partellus* (7 %) ($F_{2,161} = 32.22$; $P < 0.001$).

For UV-irradiated hosts percent parasitism within species was similar for *H. armigera* and *Chilo partellus* and lower for *Cadra cautella* compared to un-irradiated eggs ($F_{2,161} = 5.88$; $P = 0.003$) (Table 3.1). Percent parasitism was lowest for *Chilo partellus* for both UV-irradiated (7 %) and un-irradiated eggs (7 %) and highest on un-irradiated eggs of *Cadra cautella* (55 %) and UV-irradiated eggs of *H. armigera* (48 %).

Overall, the mean number of progeny emerged per single parasitized host was highest for *H. armigera* (2.3), followed by *Chilo partellus* (1.4) and *Cadra cautella* (0.8) ($F_{2,122} = 68.98$, $P < 0.001$). UV-irradiation did not affect the number of progeny emerged per species ($F_{2,122} = 1.81$; $P = 0.169$). The mean number of adult *T. lutea* emerged per parasitized UV-irradiated or un-irradiated egg was highest for *H. armigera* (2.5 and 2.1), followed by *Chilo partellus* (1.3 and 1.4) and was lowest for *Cadra cautella* (0.9 and 0.8) (Table 3.1).

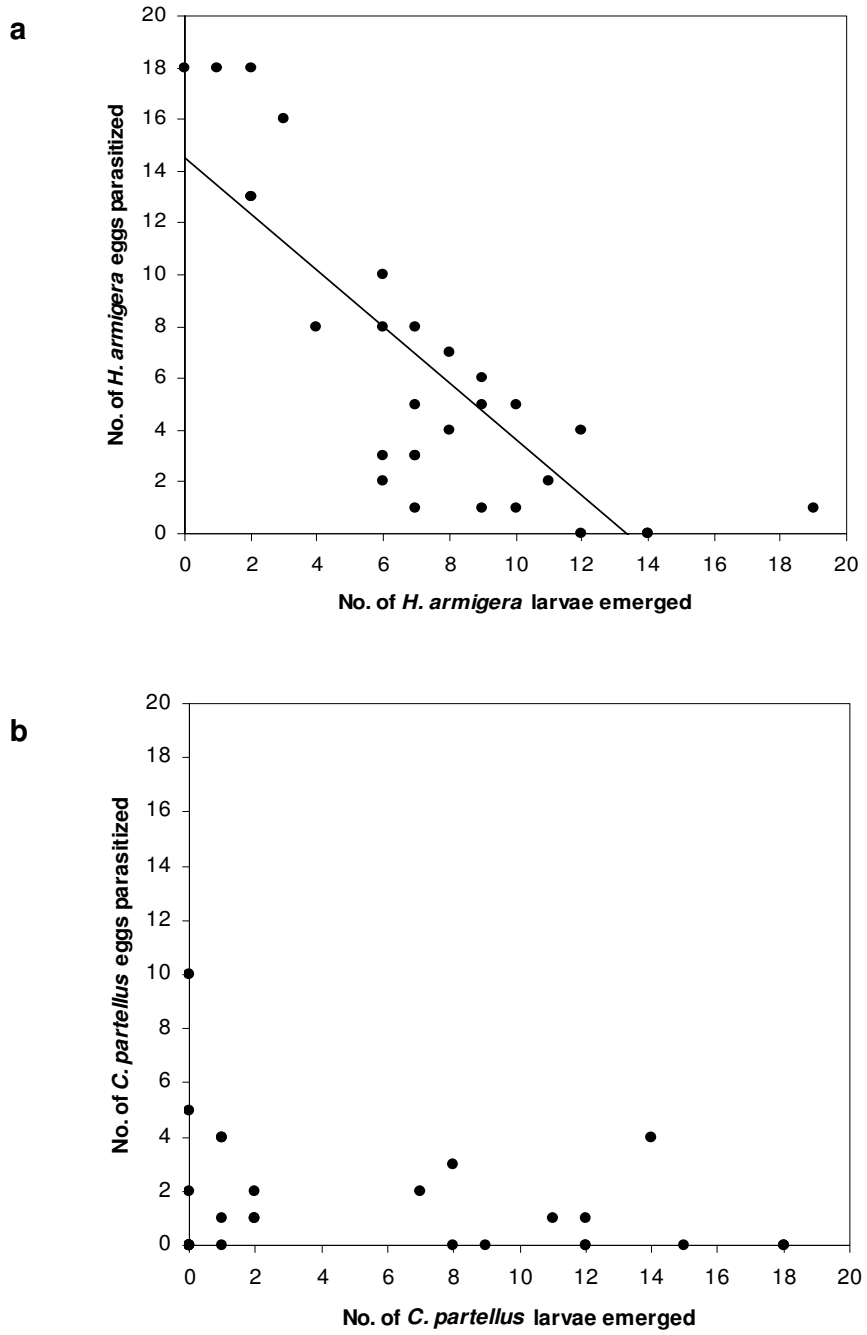

Fig. 3.3 Relationship between the number of Lepidoptera larvae emerged from batches of 20 un-irradiated eggs and the number of remaining eggs parasitized by *Trichogrammatoidea lutea*. (a) *Helicoverpa armigera*: $y = 14.483 - 1.087*x$, $r = -0.812$, $t = -7.230$, $n = 29$, $P < 0.0001$, (b) *Chilo partellus*: r = -0.192, $t = -1.037$, $n = 30$, $P = 0.3085$).

Table 3.1 Effects of UV-irradiated host eggs of three host species on parasitism, number of progeny produced and proportion of females emerged of *Trichogrammatoidea lutea* (mean ± SE).

Host	Treatment	Parasitism %	Number of progeny/host egg	Proportion of females
Helicoverpa armigera	UV-irradiated	47.83 ± 4.48 ab (n = 30)	2.54 ± 0.09 (n = 28)	0.61 ± 0.05 (n = 29)
	Un-irradiated	33.33 ± 5.48 b (n = 30)	2.11 ± 0.16 (n = 27)	0.32 ± 0.07 (n = 26)
Chilo partellus	UV-irradiated	6.55 ± 1.72 c (n = 29)	1.30 ± 0.26 (n = 13)	0.20 ± 0.09 (n = 12)
	Un-irradiated	6.56 ± 1.91 c (n = 32)	1.43 ± 0.24 (n = 15)	0.38 ± 0.13 (n = 14)
Cadra cautella	UV-irradiated	33.20 ± 8.41 b (n = 25)	0.91 ± 0.03 (n = 17)	0.72 ± 0.05 (n = 17)
	Un-irradiated	55.45 ± 8.16 a (n = 22)	0.78 ± 0.06 (n = 28)	0.58 ± 0.08 (n = 27)

Means followed by the same letter within columns are not significantly different (P < 0.01, t probabilities of pairwise differences).

The sex ratio of the progeny of *T. lutea* differed among hosts. Overall, the parasitoid had the highest mean proportion of females (0.6) on *Cadra cautella*, followed by *H. armigera* (0.5) and *Chilo partellus* (0.3) ($F_{2,119}$ = 8.14, P < 0.001). The sex ratio of *T. lutea* was female-biased for both UV-irradiated and un-irradiated eggs of *Chilo partellus* and un-irradiated eggs of *H. armigera*, whereas it was male-

biased for *Chilo partellus* and irradiated eggs of *H. armigera* (Table 3.1). However, differences were not significant at the 1 % test level ($F_{2,119}$ = 4.03, P = 0.02).

The developmental time of *T. lutea* from egg to pupa was approximately 1.5 days shorter on eggs of *H. armigera* and *Cadra cautella* compared to eggs of *Chilo partellus* ($F_{2,1279}$ = 102.48, P < 0.001). Among the three host species, irradiation did not affect the developmental time of *T. lutea* from egg to pupa on *H. armigera* and *Chilo partellus* eggs but this period was slightly shorter (5 hours) on un-irradiated eggs compared to irradiated eggs of *Cadra cautella* ($F_{2,\ 1279}$ = 6.64; P < 0.001) (Table 3.2).

The mean developmental time of *T. lutea* from egg to adult male and female was longest on *Chilo partellus* (10.3 and 10.5 days), intermediate for *Cadra cautella* (9.4 and 9.3 days) and shortest on *H. armigera* (8.6 and 8.6 days) (males: $F_{2,860}$ = 236.26, P < 0.001; females: $F_{2,1029}$ = 187.75, P < 0.001). Overall, the mean developmental time of *T. lutea* from egg to adult on un-irradiated compared to UV-irradiated eggs was slightly shorter (4 hours) for males ($F_{1,860}$ = 28.34, P < 0.001) and slightly longer (2 hours) for females ($F_{1,1029}$ = 12.00, P < 0.001). The effect of irradiation on development time was the same for all host species (male: $F_{2,860}$ = 2.11, P = 0.122; female: $F_{2,1029}$ = 0.64, P = 0.526) (Table 3.2).

Table 3.2 Mean (± SE) developmental time in days of *Trichogrammatoidea lutea* from egg to pupa, egg to adult male and female on un-irradiated and UV-irradiated eggs of three host species.

Host	*Treatment*	*Egg-pupa*	*Egg-male*	*Egg-female*
Helicoverpa armigera	UV-irradiated	3.60 ± 0.06 a (*n* = 292)	8.76 ± 0.05 (*n* = 277)	8.66 ± 0.04 (*n* = 458)
	Un-irradiated	3.68 ± 0.07 a (*n* = 105)	8.44 ± 0.05 (*n* = 271)	8.52 ± 0.06 (*n* = 128)
Chilo partellus	UV-irradiated	5.00 ± 0.16 b (*n* = 44)	10.58 ± 0.19 (*n* = 38)	10.52 ± 0.37 (*n* = 17)
	Un-irradiated	4.96 ± 0.15 b (*n* = 42)	10.12 ± 0.16 (*n* = 43)	10.53 ± 0.45 (*n* = 15)
Cadra cautella	UV-irradiated	3.56 ± 0.03 a (*n* = 316)	9.46 ± 0.00 (*n* = 83)	9.45 ± 0.01 (*n* = 204)
	Un-irradiated	3.35 ± 0.03 c (*n* = 486)	9.35 ± 0.03 (*n* = 154)	9.22 ± 0.04 (*n* = 213)

Means followed by the same letter within columns are not significantly different ($P < 0.01$, *t* probabilities of pairwise differences).

3.4 Discussion

Irradiation of host eggs for rearing egg parasitoids for augmentative biological control is common (e.g. Zhang & Cossentine 1995; Romeis *et al.,* 1997; Tunçbilek *et al.,* 2009). The minimum exposure to UV-irradiation for killing the embryos of hosts have been examined by several authors (e.g. Guerra *et al.,* 1968; Calderon & Navarro

1971; Romeis *et al.,* 1997; Faruki *et al.,* 2007). In this study, larval emergence of *H. armigera*, *Chilo partellus* and *Cadra cautella* decreased with an increase in the exposure time to UV-irradiation. Emergence of larvae from eggs of all three species ceased after irradiation of 13 minutes. Similarly, larval emergence decreased with increasing exposure time from UV-irradiated (wavelength 254 nm) eggs of *Cadra cautella* (Calderon & Navarro, 1971; Faruki *et al.,* 2007), *H. armigera* (Romeis *et al.,* 1997), and *H. virescens* (F.) and *H. zea* (Boddie) (Guerra *et al.,* 1968). In accordance with the current study, UV-irradiation was estimated to be lethal at 5 min for *Cadra cautella* by Calderon & Navarro (1971), whereas egg hatch continued for eggs irradiated for 24 min in the study by Faruki *et al.* (2007). Likewise, percentage egg hatch decreased to 6 and 3 % after 10 minutes of UV-irradiation (254 nm) for *H. virescens* and *H. zea,* respectively (Guerra *et al.,* 1968), which is similar to *H. armigera* where egg hatch was 3 % after 10 min of UV-irradiation. However, Romeis *et al.* (1997) recorded ca. 25 % egg hatch of *H. armigera* after 10 minutes of exposure to UV-irradiation (254 nm). The difference between this study and those of Romeis *et al.* (1997) and Faruki *et al.* (2007) could be due to the distance between the irradiation source and the eggs and the age of the irradiation source. For example, after long usage of a UV light tube, eggs had to be irradiated for longer periods to prevent emergence of *H. armigera* larvae (RK, pers. obs).

Romeis *et al.* (1997) observed that *T. chilonis* Ishii did not differentiate between UV-irradiated and un-radiated eggs of *H. armigera*, although parasitoid survival was lower in irradiated eggs. Carpenter *et al.* (2004) reported that a species closely related to *T. lutea*, *Trichogrammatoidea cryptophlebiae* Nagaraja, accepted and successfully developed and emerged from gamma-irradiated false codling moth (FCM) *Thaumatotibia* (formerly *Cryptophlebia*) *leucotreta* (Meyrick) (Lepidoptera:

Tortricidae) eggs, but the parasitoid preferred un-irradiated eggs. Likewise, Saour (2004) found that three *Trichogramma* species, *T. cacoeciae* Marchal, *T. evanescens* Westwood, and *T. principium* Sugonyaev and Sorokina, preferred to parasitize un-irradiated compared to gamma-irradiated eggs of the potato tuber moth (PTM) *Phthorimaea operculella* (Zeller). The results of the current study show that percent parasitism was influenced by the different host species, rather than by UV-irradiation of the host eggs.

Similarly, the sex ratio of *T. lutea* and the number of progeny emerged per host were not influenced by irradiation but by the different hosts. This is in agreement with El-Mandarawy & Rizk (2002), who found no differences for parasitism and fecundity of *T. evanescens* and *T. bacterae* Nagaraja from gamma-irradiated and un-irradiated eggs of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae). The findings on the sex ratio of *T. lutea* in this study are in agreement with Romeis *et al.* (1997), who recorded no differences in the sex ratio of *T. chilonis* reared on UV-irradiated and un-irradiated eggs of *H. armigera*.

Gregarious parasitoids adjust clutch size and sex allocation according to host quality and size, with more females being allocated to better quality hosts. Therefore, the male-biased sex ratio and lower number of progeny of *T. lutea* on *Chilo partellus* in the present study indicates lower preference by the female parasitoids. The eggs of *Chilo partellus* are flattened, ovoid (up to 1.01 mm in length) whereas those of *H. armigera* and *Cadra cautella* are spherical (approximately 0.57 and 0.40 mm in diameter, respectively). Compared to *H. armigera* and *Cadra cautella* which lay eggs singly, *Chilo partellus* oviposits in batches. As *Chilo partellus* eggs are tightly clumped together within a batch, *T. lutea* was unable to measure the sizes of

individual *Chilo partellus* eggs. The higher percentage parasitism of *Cadra cautella* eggs was due to the small-sized eggs of this species, in which only one parasitoid develops compared to *H. armigera* in which two or more can develop (Parry Jones, 1937; Kfir, 1981). Accordingly, the number of progeny developing per host egg was higher in *H. armigera* than in the other two species. Although *H. armigera* is a natural host of *T. lutea,* the number of parasitized eggs was the same as on *Cadra cautella,* a factitious host. The relatively low rate of parasitism and lower proportion of females on un-irradiated eggs of *H. armigera* compared to irradiated eggs observed in this study is likely to be due to the high number of *H. armigera* larvae emerging from un-irradiated eggs and subsequent cannibalism; the higher the number of larvae emerged, the lower the percentage parasitism of remaining eggs.

Duration of development of *T. lutea* from egg to adult males and females in un-irradiated eggs compared to irradiated eggs was slightly shorter for males, Similarly, developmental time of *T. platneri* was shorter on un-irradiated compared to eggs laid by ^{60}CO-irradiated codling moth *C. pomonella* (Zhang & Cossentine, 1995). El-Mandarawy & Rizk (2002), however, reported that gamma irradiation of host eggs had no significant effect on developmental time of *T. evanescens* and *T. bacterae* on *C. maculatus* and *C. cephalonica*.

Results of this study suggest that UV-irradiation of host eggs had little effect on percent parasitism, number of progeny, sex ratio and development time of *T. lutea.* The parameters measured differed among species and showed that *Chilo partellus* is a less suitable host for *T. lutea* than *H. armigera* or *Cadra cautella*. The findings of this study show that UV-irradiation of host eggs to kill host embryos is a suitable and simple method for mass rearing of *T. lutea.*

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CHAPTER 4

TEMPERATURE AND HOST EFFECTS ON PARASITISM, DEVELOPMENT TIME AND SEX RATIO OF THE EGG PARASITOID *TRICHOGRAMMATOIDEA LUTEA* GIRAULT (HYMENOPTERA: TRICHOGRAMMATIDAE)

Temperature and host effects on parasitism, development time and sex ratio of the egg parasitoid *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae)

Abstract

Knowledge of the thermal biology and host association are the key to development of mass rearing systems for biological control programmes. Temperature and host effects on *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae) were studied on eggs of three hosts (*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Cadra cautella* (Walker) (Lepidoptera: Pyralidae)) at six constant temperatures (18, 21, 24, 27, 30 and 35 °C). *Trichogrammatoidea lutea* did not develop at 35 °C on any of the three host species. Host species had a greater influence than temperature on parasitism, number of progeny emerged per parasitized host and sex ratio of *T. lutea.* Overall parasitism was higher on *H. armigera* (26 %) and *Cadra cautella* (22 %) than on *Chilo partellus* (12 %) at temperatures ranging from 18 to 30 °C. Overall parasitism increased with temperature between 21 and 27 °C, and then decreased at 30 °C. For *H. armigera* parasitism was highest at 27 °C (58 %). For *Cadra cautella,* parasitism was highest at 27 and 30 °C (31 and 28 %) while it was highest on *Chilo partellus* between 24 and 30 °C (13 to 17 %). The number of progeny of *T. lutea* per host egg was highest on *H. armigera* (2.9), followed by *Chilo partellus* (1.3) and lowest on *Cadra cautella* (0.9). The overall sex ratio by female proportion was highest on *Chilo partellus* (0.7), followed by *Cadra cautella* (0.6) and lowest on *H. armigera* (0.5). The rate of development from egg to pupa and egg to adult was fastest on *H. armigera* and slowest on *Chilo partellus.* Lower threshold for

development and degree days (DD) of *T. lutea* from egg to adult were 12.8 °C and 105.4 DD on *H. armigera,* 11.3 °C and 141.6 DD on *Chilo partellus* and 12.9 °C and 118.2 DD *Cadra cautella*, respectively. Based on these results, *T. lutea* can be mass reared on *H. armigera* and *Cadra cautella* at 27 °C.

4.1 Introduction

Trichogrammatoidea lutea Girault (Hymenoptera: Trichogrammatidae) is a facultative gregarious polyphagous egg parasitoid (Kfir, 1982) that is indigenous to southern Africa (Parson & Ullyett, 1936). It is known to parasitize several lepidopteran pests such as the African bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Parson & Ullyett, 1936; Parry Jones, 1937), the spotted stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Nagarkatti & Nagaraja, 1977), the spiny bollworm *Earias biplaga* Walker (Lepidoptera: Noctuidae) (Nagarkatti & Nagaraja, 1977) and the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Wahner, 2008). Since the 1930s, several studies have been undertaken to assess the suitability of *T. lutea* as a biological control agent against lepidopteran pests in southern Africa (Parson & Ullyett, 1936; Parry Jones, 1937; Kfir, 1982; Kfir & Van Hamburg, 1988; Wahner, 2008).

Temperature and host have the greatest effect on population dynamics of parasitoids as these often influence their metabolic rate, flight activity, reproduction, development and survival (Godfray, 1994; Bourchier & Smith, 1996; Thomas & Blanford, 2003; Kalyebi *et al.,* 2006). Except for Parry Jones (1937) and Wahner (2008) who examined temperature-related development of *T. lutea,* no detailed studies have been undertaken to determine its thermal biology and host association, which

form a key component of identifying biological control agents, developing mass-rearing techniques and biological control programmes (Elzen & King, 1999).

The majority of Trichogrammatidae have been mass reared successfully on eggs of different factitious hosts (Greenberg *et al.,* 1996). Factitious hosts do not necessarily share a natural habitat with the parasitoid but can support mass production of the parasitoid without compromising its quality. For example, egg parasitoids such as *Trichogramma minutum* Riley and *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) have been successfully reared on eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) as factitious hosts (Greenberg *et al.,* 1996). However, different host species and host age vary in their suitability and quality and these influence the life history parameters of parasitoids (Godfray, 1994). Host species and quality influence growth and survival of developing parasitoids, as well as their sex ratio, fecundity, longevity and vigour of adults (Vinson & Iwantsch, 1980; Godfray, 1994). In solitary idiobionts (parasitoids that paralyze or kill their host after initial parasitism (Vinson, 1997)), host size has the greatest effect on the fitness of progeny, as larger parasitoids tend to develop from larger hosts and larger females have a higher fecundity compared to smaller females (Godfray, 1994; Colinet *et al.,* 2005). In addition, host size influences the sex ratio of the progeny as female offspring of solitary parasitoids is usually allocated to larger hosts (Godfray, 1994). In contrast, host size influences the number of developing siblings (clutch size) in gregarious parasitoids (where more than one parasitoid develops per host), which in turn influences the size of resulting adults (Godfray, 1994). Because *T. lutea* is polyphagous, its life history parameters on different host species are likely to vary.

Knowledge on the thermal biology of *T. lutea,* as well as determining suitable hosts and temperatures for mass rearing, will provide insight into the potential of the parasitoid for augmentative biological control of *H. armigera*. The objectives of the current study were to assess parasitism, number of progeny per host, sex ratio and development time of *T. lutea* on the eggs of three Lepidoptera host species (*H. armigera*, *Chilo partellus* and *Cadra cautella* (Walker) (Lepidoptera: Pyralidae)) at six constant temperatures (18, 21, 24, 27, 30 and 35 °C).

4.2 Materials and methods

4.2.1 Insect colonies

The insect cultures were maintained as described in Chapter 2. The cultures of *H. armigera* and *Chilo partellus* were maintained in the insectary at the ARC-Plant Protection Research Institute (ARC-PPRI), Rietondale Campus (25°44'S, 18°13'E) in Pretoria, South Africa, where all the experiments were carried out. *Cadra cautella* was obtained from a culture kept at ARC-PPRI, Roodeplaat campus (25°39'S, 28°20'E) near Pretoria.

4.2.2 Development time of Trichogrammatoidea lutea *on three host species at different constant temperatures*

The eggs of the three host species (*H. armigera, Chilo partellus* and *Cadra cautella*) were irradiated with a UV light (TUV 30W/ G30T8, Philips, Holland, 254 nm, in a fitting with a reflective aluminium backing, at a distance of 6 cm from the eggs) for 15 minutes to sterilize the eggs in order to avoid cannibalism by the emerging larvae (Kfir & Van Hamburg, 1988) and improve survival of immature

parasitoids (Cônsoli *et al.,* 2000). Batches of 50 eggs (less than 24 hours old) of each host species were exposed to 16 mated naive *T. lutea* females for 4 hours in individual glass jars (12 cm high × 6.5 cm diameter) (Fig. 4.1) at six constant temperatures (18, 21, 24, 27, 30 and 35 °C). The jars were streaked with honey for *T. lutea* to feed on. After exposure of 4 hours, the eggs were removed and placed in empty jars, and incubated (LabconTM LTGC 20 incubators; Laboratory Marketing Services cc, Roodepoort, South Africa) at the same temperature as used for parasitism. The experiment was replicated 30 times. All incubators were maintained at 16L: 8D photoperiod and approximately 60 % RH. Observations were made twice a day, in the morning between 08:00 and 09:00 and in the afternoon between 15:00 and 16:00. The data for development, parasitism, number of progeny per host and sex ratio were recorded at each observation. The time when the chorion of the egg turned black was taken to estimate the development time from egg to pupa. The date, time of pupal formation and adult emergence, and the number of male and female adult parasitoids emerged were recorded during each observation. The time of emergence was determined as the midpoint of two consecutive observations during which emergence occurred (Kirsten & Kfir, 1991).

Fig. 4.1 Glass jars used to expose eggs of *Helicoverpa armigera, Chilo partellus* and *Cadra cautella* to *Trichogrammatoidea lutea* females for parasitism in an incubator.

4.2.3 Data analysis

Percent parasitism, developmental times, number of progeny per host, and sex ratio of *T. lutea* at different temperatures on different hosts were compared using analysis of variance (ANOVA) for unbalanced design using GenStat® (Payne *et al.,* 2007). Data at 18 °C for parasitism, number of progeny per host, sex ratio, and development from egg to adults by *T. lutea* were excluded from the analysis because of low parasitism and adult emergence (1 adult) on *Chilo partellus* at this temperature. Data that were not normally distributed were transformed before ANOVA, using square root transformation for percentages and arcsine transformation for proportions. The significance level was set at $P < 0.05$, except for development time from egg to pupa where transformation of data did not result in stabilization of treatment variances and the test level was set at $P < 0.01$.

The relationship between temperature and development of insects within limits follows a hyperbola. However, when the results of developmental time are expressed as rate of development the relationship between the two variables is linear (Pfadt, 1985). The linear regression model $R(T) = a + bT$, where $R(T)$ is the rate of development or the inverse of developmental time ($1/d$), d is development time in days, a is the intercept, b is the slope of the regression and T is the ambient temperature (°C), was used to determine the developmental threshold temperature for *T. lutea* on different hosts (Davidson, 1944; Pfadt, 1985; Dent, 1991; Pedigo, 1996; Gullan & Cranston, 2000; Wang *et al.,* 2004) using Statistica (Version 6.1, StatSoft, Inc, 1984-2004). The lower developmental threshold (T_{min}) was taken as the point where the regression line intercepts the x-axis and was estimated by using the formula ($T_{min} = -a/b$). To estimate the degree-days (DD) for development of *T. lutea* from egg to pupa and egg to adult, the formula $ThC = 1/b$ was used (where ThC is the thermal constant). Degree-days are a unit used to measure the amount of heat units required by an organism to complete its development, and this determines the physiological time.

4.3 Results

4.3.1 Parasitism

No parasitoid developed on any host species at 35 °C. Overall percent parasitism was significantly higher on *H. armigera* (26 %) and *Cadra cautella* (22 %) compared to *Chilo partellus* (12 %) ($F_{2, 326} = 10.90$, $P < 0.001$) at temperatures ranging from 18 to 30 °C. In general, percent parasitism increased significantly from 9 to 30 % with an increase in temperature between 21 and 27 °C and then decreased

to 19 % at 30 °C ($F_{3, 326}$ = 15.55, P < 0.001). However, temperature did not affect all hosts equally ($F_{6, 326}$ = 9.71, P < 0.001) (Table 4.1). For *H. armigera* eggs, parasitism increased from 10 to 59 % between 21 and 27 °C and declined to 12 % at 30 °C. Parasitism was similar at 21 and 24 °C (14 and 15 %) but was higher at 27 and 30 °C (31 and 28 %), respectively, for *Cadra cautella* eggs. For *Chilo partellus,* parasitism was low at 21 °C (4 %) and similar between 24 and 30 °C (13 to 17 %). Parasitism at 18 °C varied among the three host species, being lower (1 %) for *Chilo partellus* compared to 10 % and 23 % on *Cadra cautella* and *H. armigera*, respectively.

4.3.2 Number of progeny per host

The mean number of progeny of *T. lutea* per parasitized host egg (clutch size) varied among the hosts, being highest on *H. armigera* (2.9), intermediate on *Chilo partellus* (1.3) and lowest on *Cadra cautella* (0.9) ($F_{2, 234}$ = 168.95, P < 0.001). Temperature had a lesser effect than host species on the number of progeny per host ($F_{3, 234}$ = 3.53, P = 0.016). The mean number of progeny per host was similar at different temperatures except for 21 and 24 °C where it was lower (1.5 for both) compared to 30 °C (1.8). However, the number of progeny varied between 21 and 30 °C on different hosts ($F_{6, 234}$ = 2.71, P = 0.015) (Table 4.1). For *H. armigera*, the mean number of progeny per host egg was lower at 21 and 24 °C (2.6 and 2.7), respectively, compared to 27 °C (3.4). Temperature had no effect on the number of progeny of *T. lutea* per parasitized egg of *Cadra cautella,* with progeny ranging from 0.73 to 0.96 between 18 and 30 °C. For *Chilo partellus*, the mean number of progeny per host egg was higher at 30 °C (1.8) compared to 21 to 27 °C (1.1 and 1.4), respectively. The mean number of progeny per parasitized host egg at 18 °C was

higher for *H. armigera* (3.2) and lower for *Cadra cautella* (0.73) and *Chilo partellus* (0.1) compared to those at 21 to 30 °C.

4.3.3 Sex ratio

Overall, *T. lutea* produced the highest mean proportion of females on *Chilo partellus* (0.7) and *Cadra cautella* (0.6) and the lowest on *H. armigera* (0.5) ($F_{2, 252}$ = 12.57; P = 0.001). Temperature had little influence on the sex ratio of *T. lutea* developed on *H. armigera* eggs where the proportion of females ranged between 0.4 and 0.5. Similarly for *Chilo partellus* eggs, the sex ratio of *T. lutea* was female-biased, ranging between 0.6 and 0.7 at different temperatures. However, on *Cadra cautella* eggs, the proportion of *T. lutea* females decreased with decreasing temperature from 0.8 and 0.7 at 30 and 27 °C to 0.6 and 0.5 at 24 and 21 °C, respectively ($F_{6, 252}$ = 2.34; P = 0.032) (Table 4.1). The mean proportion of females at 18 °C was similar compared to temperatures ranging between 21 and 30 °C for *H. armigera* (0.5). For *Cadra cautella*, the proportion of *T. lutea* females was higher (0.7) at 18 °C compared to that at 21 and 24 °C where it averaged 0.5 and 0.6, respectively. For *Chilo partellus*, only one adult female emerged at 18 °C.

Table 4.1 Percent parasitism, number of progeny per parasitized egg and proportion of females of *Trichogrammatoidea lutea* on three host species (*Helicoverpa armigera, Chilo partellus* and *Cadra cautella*) at five constant temperatures (mean ± SEM).

Species	Temperature (°C)[1]	Parasitism (%)	Number of progeny per parasitized egg	Proportion of females
H. armigera	18	22.6 ± 4.13 (30)	3.18 ± 0.14 (25)	0.52 ± 0.05 (26)
	21	9.87 ± 2.26 (32) cd	2.64 ± 0.14 (21) b	0.43 ± 0.09 (22) de
	24	30.67 ± 4.57 (21) b	2.74 ± 0.14 (17) b	0.51 ± 0.04 (26) cde
	27	58.80 ± 7.96 (10) a	3.35 ± 0.16 (9) a	0.52 ± 0.05 (30) cde
	30	11.52 ± 2.45 (29) cd	2.97 ± 0.17 (16) ab	0.38 ± 0.06 (18) e
Chilo	18	0.93 ± 0.33 (30)	0.08 ± 0.08 (6)	1 (1)
partellus	21	4.06 ± 1.24 (31) d	1.43 ± 0.31 (14) cd	0.72 ± 0.11 (12) ab
	24	13.86 ± 2.67 (29) c	1.11 ± 0.16 (25) def	0.68 ± 0.06 (20) abc
	27	13.29 ± 2.81 (31) c	1.19 ± 0.22 (24) de	0.64 ± 0.07 (18) abc
	30	17.35 ± 3.43 (31) c	1.85 ± 0.17 (24) c	0.67 ± 0.05 (24) abc
Cadra	18	10.07 ± 1.81 (29)	0.74 ± 0.08 (21)	0.66 ± 0.09 (20)
cautella	21	13.94 ± 3.51 (31) c	0.88 ± 0.09 (18) ef	0.46 ± 0.08 (17) de
	24	15.00 ± 2.23 (30) c	0.92 ± 0.06 (26) ef	0.57 ± 0.08 (26) bcd
	27	30.84 ± 4.85 (31) b	0.79 ± 0.06 (26) f	0.73 ± 0.05 (25) a
	30	28.06 ± 3.83 (32) b	0.96 ± 0.03 (26) ef	0.76 ± 0.02 (26) a

The number in parentheses gives the number of replicates. Means within columns followed by the same lower case letter are not significantly different ($P < 0.05$, t probabilities of pairwise differences). [1]Data for 18 °C were not included in the analyses because of low egg parasitism and adult emergence on *Chilo partellus* at this temperature.

Table 4.2 Developmental time (in days) of *Trichogrammatoidea lutea* from egg to pupa, egg to adult male and female in on three host species (*Helicoverpa armigera, Chilo partellus* and *Cadra cautella*) at five constant temperatures (mean ± SEM).

Host	Temperature (°C)	Egg-pupa	Egg-male[1]	Egg-female[1]
H. armigera	18	7.12 ± 0.04 (348) c	20.64 ± 0.03 (513)	20.69 ± 0.03 (587)
	21	4.39 ± 0.03 (157) f	12.66 ± 0.04 (272)b	12.38 ± 0.05 (144)b
	24	3.53 ± 0.02 (665) h	9.39 ± 0.02 (835)d	9.35 ± 0.02 (942)e
	27	2.76 ± 0.01 (938) j	7.43 ±0.02 (1035)g	7.50 ± 0.01 (1258)g
	30	2.35 ± 0.04 (168) m	6.32 ± 0.03 (295)j	6.39 ± 0.03 (229)i
Chilo	18	9.84 ± 0.39 (14) a	-	24.12 (1)
partellus	21	5.61 ± 0.11 (64) d	14.55 ± 0.27 (16)a	14.67 ± 0.0 (65)a
	24	4.43 ± 0.05 (201) f	11.38 ± 0.10 (101)c	11.26 ± 0.08 (190)c
	27	3.67 ± 0.04 (207) h	9.28 ± 0.09 (113)e	9.03 ± 0.07 (186)e
	30	2.67 ± 0.04 (269) k	7.53 ± 0.04 (222)h	7.65 ± 0.03 (338)g
Cadra	18	8.04 ± 0.11 (146) b	23.18 ± 0.1 (36)	23.16 ± 0.08 (87)
cautella	21	4.87 ± 0.04 (214) e	14.64 ±0.1 (117)a	14.40 ± 0.09 (88)a
	24	4.23 ± 0.04 (226) g	10.88 ± 0.07 (138)c	10.87 ± 0.08 (83)d
	27	2.94 ± 0.02 (478) i	8.59 ± 0.07 (97)f	8.28 ± 0.05 (277)f
	30	2.50 ± 0.01 (451) l	7.01 ± 0.04 (108)i	6.88 ± 0.03 (325)h

The number in parentheses gives the individual number of pupae, males and females per 30 replicates. Means within columns followed by the same lower case letter are not significantly different ($P < 0.01$, *t* probabilities of pairwise differences). [1]Data for 18 °C were not included in the analysis because only one adult emerged at this temperature on *Chilo partellus.*

4.3.4 Development time

The development time of *T. lutea* varied among host species and constant temperatures. *Trichogrammatoidea lutea* did not develop at 35 °C. Although *T. lutea* completed development on the three host species at 18 °C, only one individual emerged from *Chilo partellus,* though several eggs turned black, an indication that *T. lutea* developed until the pupal stage on this host at this temperature. Therefore, data at 18 °C were only analysed for development from egg to pupa. Developmental time from egg to pupa was significantly shortest on *H. armigera* (4 days), intermediate on *Cadra cautella* (4.5 days) and longest on *Chilo partellus* (5.2 days) ($F_{2, 4531}$ = 11.85, P < 0.001) (Table 4.2). Overall, development time of *T. lutea* from egg to pupa decreased significantly with an increase in temperature from 8.3 days at 18 °C to 2.5 days at 30 °C ($F_{4, 4531}$ = 6268.96, P < 0.001). The development time of *T. lutea* from egg to pupa differed significantly among host species and temperatures, and was shortest on *H. armigera* (7 to 2.3 days), intermediate on *Cadra cautella* (8 to 2.5 days) and longest on *Chilo partellus* (10 to 2.6 days) at temperatures ranging from 18 °C and 30 °C, respectively ($F_{8, 4531}$ = 24.05, P < 0.001) (Table 4.2). The lower developmental threshold of *T. lutea* for the development from egg to pupa was 12.2 °C on *H. armigera,* 13.4 °C on *Chilo partellus* and 12.3 C° on *Cadra cautella* (Fig. 4.2). The degree-days required by *T. lutea* to develop from egg to pupa were 40.4 DD on *H. armigera,* 44.1 DD on *Chilo partellus* and 43.8 DD on *Cadra cautella*, respectively.

Development time of *T. lutea* from egg to adult male ($F_{2, 3337}$ = 1616.26, P< 0.001) and female ($F_{2, 4113}$ = 874.61, P < 0.001) differed significantly among the three host species (Table 4.2). Mean development time from egg to adult was significantly shorter on *H. armigera* than on other host species, with *H. armigera* male and female

offspring taking 8.5 days and 8.2 days to develop, respectively, versus 9.8 and 9.2 days for *Cadra cautella* and 10.3 and 9.8 days for *Chilo partellus*. Development time from egg to adult male ($F_{3,\ 3337}$ = 10026.6, P < 0.001) and female ($F_{3,\ 4113}$ = 10423.63, P < 0.001) decreased significantly with an increase in temperature (Table 4.2). Development time of *T. lutea* from egg to adult male ($F_{6,\ 3337}$ = 36.90, P < 0.001) and female ($F_{6,\ 4113}$ = 56.29, P < 0.001) differed significantly among temperatures and hosts, with development time decreasing from 12 to 6 days on *H. armigera*, 14 to 7 days on *Chilo partellus* and *Cadra cautella* between 21 °C and 30 °C (Table 4.2). The lower developmental threshold of *T. lutea* to complete development from egg to adult was 12.8 °C on *H. armigera*, 11.3 °C on *Chilo partellus* and 12.9 °C on *Cadra cautella* (Fig. 4.3). The degree-days required by *T. lutea* to complete development were 105.4 DD on *H. armigera,* 141.6 DD on *Chilo partellus* and 118.2 DD on *Cadra cautella*.

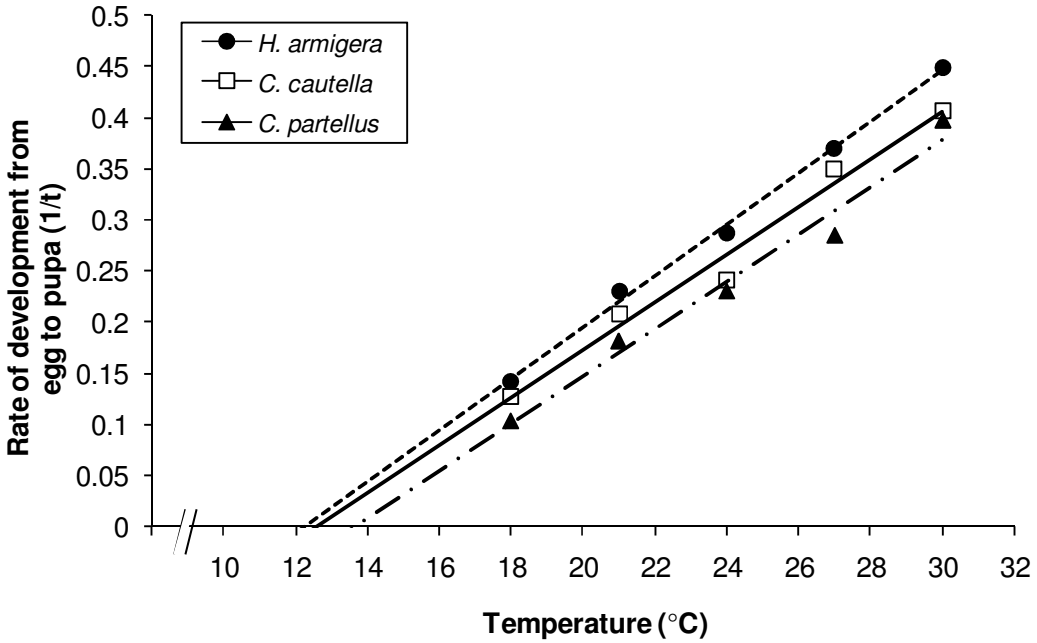

Fig. 4.2 Effect of temperature on the rate of development of *Trichogrammatoidea lutea* from egg to pupa on three host species (*Helicoverpa armigera*: y = -0.3005 + 0.02473x, $R^2_a = 0.992$, $P < 0.001$; *Cadra cautella*: y = -0.2817 + 0.02283x, $R^2_a = 0.974$, $P < 0.001$; *Chilo partellus*: y = -0.3041 + 0.02270x, $R^2_a = 0.983$, $P < 0.001$).

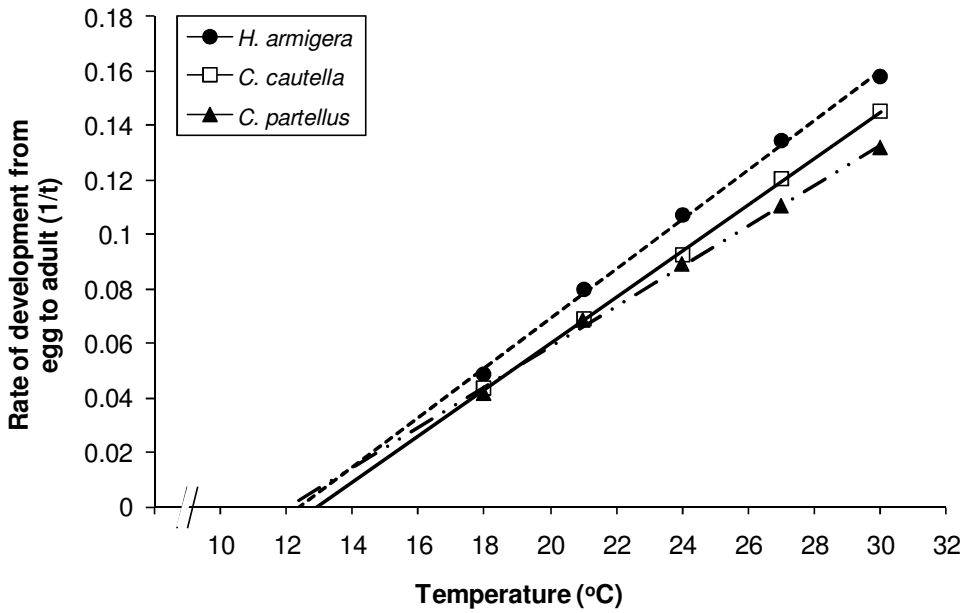

Fig. 4.3 Effect of temperature on the rate of development (1/t) of *Trichogrammatoidea lutea* from egg to adult on three host species (*Helicoverpa armigera*: y = -0.12158 + 0.009485x, R^2_a =0.997, $P < 0.001$; *Cadra cautella*: y = -0.10895 + 0.008459x, R^2_a = 0.999, $P < 0.001$; *Chilo partellus*: y = -0.079742 + 0.00706x, R^2_a = 0.999, $P < 0.001$).

4.4 Discussion

Parasitism, number of progeny emerged per parasitized egg (clutch size), sex ratio and developmental time of *T. lutea* were affected differently by host species and temperature. On all three host species, parasitism was low at 18 and 21 °C. This may be due to low activity of insects at lower temperatures. Kalyebi *et al.* (2005) also reported low parasitism of eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) by *Trichogrammatoidea* sp. nr. *lutea* collected from different altitudes at temperatures lower than 25 °C. Host species influenced parasitism in the current

study. Parasitism was significantly higher on *H. armigera* (26 %) and on *Cadra cautella* (22 %) than on *Chilo partellus* (12 %), suggesting that *T. lutea* prefers *H. armigera* and *Cadra cautella* to *Chilo partellus.* Likewise, Muli *et al.* (2010), who compared parasitism by *T.* sp. nr *lutea* on six differed host species, observed that parasitism was lowest on *Chilo partellus*. However, further studies with choice experiments may be required conclusively to determine host preference of *T. lutea*.

The number of progeny of *T. lutea* per host egg (clutch size) was more strongly influenced by host species than by temperature. The lowest number of parasitoids per host emerged from *Cadra cautella* and the highest from *H. armigera.* Though mortality of immature *T. lutea* occurred on all host species, the mean adult emergence of less than 1 parasitoid per parasitized egg from *Cadra cautella* was a result of mortality on this host. The results of clutch size of *T. lutea* on *H. armigera* in this study are similar to those reported by Kfir (1982) on *H. armigera*. Muli *et al.* (2010) also reported a clutch size of two *T.* sp. nr *lutea* adults per egg of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). The clutch size of *T. lutea* on *Chilo partellus* in the present study is similar to that of *T.* sp. nr *lutea* on *Chilo partellus* reported by Muli *et al.* (2010). In trichogrammatids, the size of the host egg is considered the most important attribute leading to host acceptance and determination of clutch size (Godfray, 1994; Oliveira *et al.,* 2003). *Trichogrammatoidea lutea* is facultatively gregarious, and therefore the larger clutch size on *H. armigera* may be a reflection of its egg features, which could in turn influence parasitism. In keeping with the optimal oviposition theory (Jaenike, 1978), the relatively larger eggs of *H. armigera* (spherical eggs, ca. 0.57 mm in diameter) were probably preferred for oviposition by *T. lutea* to those of *Cadra cautella* (spherical eggs, ca. 0.40 mm in diameter). Compared to *H. armigera* eggs, which are laid singly, *Chilo partellus* eggs (flattened,

ovoid, up to 1.01 mm in length) are laid in clumps or overlapping each other, making it difficult for parasitoids to assess their size. Trichogrammatids are known to reduce their clutch size when attacking clumped eggs because the host eggs may have less exposed surface area, and this could render the female parasitoid unable to evaluate the host egg quality (Godfray, 1994).

The sex ratio of *T. lutea* on *H. armigera* was close to 1: 1 at most temperatures except at 18 and 30 °C where males dominated. It was female-biased on *Cadra cautella* at all temperature except at 21 °C where it was male-biased. It was female-biased on *Chilo partellus* at all temperatures. Studies by Kalyebi *et al.* (2005) and Wahner (2008) found no relationship between sex ratio and temperature of *T.* sp. nr *lutea* and *T. lutea,* respectively. Different factors, including parasitoid density, host size, duration of exposure to hosts and age of parasitoids, influence sex ratio allocation in parasitoids (Godfray, 1994). For example, the influence of parent parasitoid density on the sex ratio of *T. lutea* offspring has already been shown by Kfir (1982) where the female proportion of *T. lutea* on *H. armigera* decreased from 79 % to 51 % due to increased parent parasitoid density from 1 to 8 females per 150 host eggs. In the present study, sex ratio appears to have been influenced by host species, egg shape and size where the female proportion was 50 % or less on *H. armigera* eggs and higher than 50 % on *Cadra cautella* and *Chilo partellus* eggs. In addition, facultative gregarious parasitoids such as *T. lutea* are known to adjust the sex ratio with clutch size, the sex ratio becoming male-biased as clutch size increases (Godfray 1994). Therefore, the female-biased sex ratio of *T. lutea* on *Chilo partellus* and *Cadra cautella* might be due to *T. lutea* generally allocating less than two eggs per host egg while allocating more than two eggs per *H. armigera* egg.

Trichogrammatoidea lutea failed to develop on all three hosts at 35 °C. At all temperatures tested (18 to 30 °C), the development of *T. lutea* was fastest on *H. armigera*, followed by *Cadra cautella* and slowest on *Chilo partellus.* Parry Jones (1937) and Wahner (2008) also reported that developmental time of *T. lutea* decreases with increasing temperature. The developmental time from egg to adult *T. lutea* of 7.5 days at 31 °C on *H. armigera* reported by Parry Jones (1937) is similar to 7.5 days on *Chilo partellus* but slightly longer than 7 days on *Cadra cautella* and longer than 6.3 days on *H. armigera* in the present study at 30 °C. Similarly, Wahner (2008) reported developmental times of *T. lutea* from egg to adult on *Cydia pomonella* of 14.5 days at 20 and 7.15 days at 30 °C, which is similar to the development of *T. lutea* on *Chilo partellus* at 21 and 30 °C, but higher than on *H. armigera* at 21 and 30 °C in the present study. The degree-days required by *T. lutea* to complete development were highest on *Chilo partellus*, which would explain the longer development time on this host compared to *H. armigera* and *Cadra cautella.* At temperatures lower than 12 °C, development of *T. lutea* might be favoured on *Chilo partellus* compared to the other two hosts. Because the threshold for development of *T. lutea* was estimated at between 11.3 and 12.8 °C, *T. lutea* is unlikely to develop where average winter temperature is below 11 °C but could do so at average temperatures above 11 °C provided the host species supports development at that temperature. Lower developmental threshold and thermal constant degree-day for *T. lutea* on three hosts in the current study differed from those of *T.* sp. nr. *lutea* from different elevations, reported on *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (lower developmental threshold: 7.5 to 8.9 °C; degree days: 158.0 to 169.5 DD) (Kalyebi *et al.,* 2006). Wahner (2008) reported a lower developmental threshold at 11.3 °C on *Cydia pomonella* which is similar to that of *T. lutea* on *Chilo partellus* in this study,

though the thermal constant of 123.6 DD was lower than that of 141.6 DD of *T. lutea* on *Chilo partellus*. Haile *et al.* (2002) also reported a lower threshold for development and higher degree days for *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) (8.83 °C and 188 DD) and *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) on *S. cerealella* (9.23 °C and 192 DD). The variation between these estimates might be due to a combination of factors such as different experimental protocols (host-parasitoid ratio (Kfir, 1982)), genetic variability within and amongst species (Hohmann & Luck, 2000) and different hosts used.

The results of this study show that *T. lutea* developed on all three hosts at all temperatures tested, except at 35 °C. However, *H. armigera* appears to be the most suitable host for mass rearing *T. lutea* because of relatively high parasitism, short development time, greater clutch size and a balanced sex ratio recorded at 27 °C on this host species. *Cadra cautella* may also be used as rearing host for *T. lutea,* though longer exposure times might be required because parasitism was low. Based on the results of the current study *Trichogrammatoidea lutea* should be reared at 27 °C because optimum parasitism and sex ratios were recorded at this temperature for *H. armigera* and *Cadra cautella*. The thresholds for development of *T. lutea* show that it may be abundant in summer (Parry Jones 1937), which may also be the best season for mass releases. The results from this study do not necessarily reflect what would happen in the field because field conditions are characterized by fluctuating temperatures which may lead to higher or lower rates of development compared to those recorded at constant temperatures in the laboratory.

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CHAPTER 5

LONGEVITY AND AGE-RELATED REPRODUCTIVE BIOLOGY OF *TRICHOGRAMMATOIDEA LUTEA* GIRAULT (HYMENOPTERA: TRICHOGRAMMATIDAE) ON EGGS OF *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE)

Longevity and age-related reproductive biology of *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae) on eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

Abstract

Trichogrammatoidea lutea Girault (Hymenoptera: Trichogrammatidae) is an egg parasitoid of the African bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in southern Africa. As part of determining the potential of *T. lutea* as a biological control agent of *H. armigera*, longevity, daily parasitism, fecundity, number of progeny per egg, and sex ratio with regard to longevity and maternal age were examined. The mean longevity of adult male and female *T. lutea* was 5.6 and 8.6 days, respectively. No pre-oviposition period was observed. The reproductive period of *T. lutea* lasted for up to 14 days. The mean realized fecundity of *T. lutea* was 51.9 offspring per female. Daily fecundity and sex allocation depended on maternal age. Daily percentage parasitism was highest on the day of eclosion, reaching 34 % and was lowest with no parasitism on day 15. Similarly, daily fecundity was also highest on the day of eclosion at 14.4 offspring per female, and then decreased with female age. The sex ratio was female-biased during the first three days, and thereafter became male-biased until day 14. The number of progeny per host egg was also highest at 2.17 on the day of eclosion, then decreased to 0.89 on day 8 and thereafter increased to 2.0 on day 13. Overall, sex ratio of *T. lutea* was approximately 1:1. The net replacement rate (R_o), mean generation time (*T*), and instantaneous rate of population increase (r_m) of *T. lutea* were 25.5, 9.79 and 0.33, respectively. Findings from this study show great potential for using *T. lutea* as a biological control agent of *H. armigera*.

5.1 Introduction

An ideal candidate for biological control is expected to have certain biological attributes to regulate pest populations to a level that is economically acceptable (Smith, 1996). These attributes include high life time fecundity and a female-biased sex ratio in order to maintain sufficiently high numbers of the biological control agent in the field (DeBach & Rosen, 1991; Smith, 1996; Hawkins & Cornell, 1999; Mills, 2005). Fecundity is one of the fundamental attributes that influence ecology and population dynamics of insects (Price, 1997), and is referred to as lifetime reproductive capacity of an organism in terms of total number of eggs produced (Abrahamson, 1989; Godfray, 1994; Mills & Kuhlmann, 2000). The number of eggs or ovarioles in a female wasp represents the maximum lifetime fecundity in pro-ovigenic species, while it represents the current egg load or potential fecundity in synovigenic species (Godfray, 1994). Pro-ovigenic species mature their lifetime complement of eggs prior to emergence, lay most of their egg complement just after emergence, and usually have a short oviposition period (Gordh *et al.*, 1999; Mills & Kuhlmann, 2000). In contrast, synovigenic species synchronise oogenesis with oviposition rate, meaning that they emerge with no or few mature eggs (weakly synovigenic) and have to mature them with nutrients gained from subsequent host-feeding, thus being egg-limited (Gordh *et al.*, 1999; Hawkins & Cornell, 1999; Mills & Kuhlmann, 2000; Jervis *et al.,* 2001). In addition, synovigenic wasps have a pre-oviposition period, which varies in duration depending on species, and host-feed in order to reproduce (maturation of eggs), while pro-ovigenic species host-feed mainly to improve their longevity (Jervis *et al.,* 2001). Longevity refers to the total time that an adult organism can live, and this is highly dependent on the availability of food

resources (e.g. haemolymph of hosts) for parasitoids (Godfray, 1994; Hawkins & Cornell, 1999). Every organism has a limited amount of resources available for primary activities such as growth, maintenance, and reproduction, and the amount of resources that it expends on a particular activity affects the amount of resources available for other activities (Abrahamson, 1989). It is not always practical to determine the precise fecundity of a female organism. Hence, realised fecundity (total number of lifetime viable offspring produced) is often used to estimate fecundity (Godfray, 1994; Hawkins & Cornell, 1999).

Sex ratio change in prolonged parasitoid cultures has been noted and may affect the mass-rearing of parasitoids and, eventually, success of augmentative biological control programmes (Etzel & Legner, 1999). The majority of parasitic Hymenoptera have a haplo-diploid genetic system where males develop from unfertilised eggs through a form of parthenogenesis known as arrhenotoky, while females develop from fertilized eggs (Godfray, 1994; Gordh *et al.*, 1999; Hawkins & Cornell, 1999). Haplo-diploid female parents are able to determine the sex of the offspring through the regulation of sperm access to eggs which, after copulation, is stored in the spermatheca (Godfray, 1994; Gordh *et al.*, 1999; Hawkins & Cornell, 1999). However, factors such as female age, sperm depletion, temperature, host size, host age, and species are known to influence the decision of female parasitoids on sex allocation (Godfray, 1994).

This study aimed at determining longevity of adults, age-specific fecundity (total number of viable offspring produced by a female parasitoid per day) and realized fecundity (total number of viable offspring produced during the lifetime of a female), daily parasitism, number of progeny per egg and sex ratio of the progeny of *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae). In addition, a

life table of *T. lutea* was also constructed as a tool to estimate population dynamics. The information on the reproductive biology should contribute towards the development of a rearing protocol for *T. lutea,* and to provide additional information on the potential of this parasitoid for use in augmentative biological programmes against the African bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae).

5.2 Material and methods

5.2.1 Insect colonies

Insects from cultures maintained at the insectary of the ARC-Plant Protection Research Institute (ARC-PPRI) at the Rietondale campus were used (see Chapter 2). *Trichogrammatoidea lutea* was reared on eggs of *H. armigera*.

5.2.2 Longevity and reproductive biology of Trichogrammatoidea lutea

To determine age specific reproductive biology of *T. lutea*, newly emerged adults (less than 24 hours old) were paired (1 male and 1 female) and transferred to small plastic vials (85 mm high × 10 mm diameter), with one-pair per vial (Fig. 5.1). Thin streaks of honey were provided in the vials for adult *T. lutea* to feed on. Each *T. lutea* pair was supplied daily with a batch of 20 UV-irradiated eggs (less than 24 hours old) of *H. armigera* until all parasitoids (male and female) died. This was replicated 40 times. The eggs of *H. armigera* were UV-irradiated for 15 mins with a UV light tube (TUV 30W/G30T8, Philips, Holland; 254 nm; in a fitting with a reflective aluminium backing) (see Chapter 2). The experiment was carried out in an incubator (Labcon™ LTGC 20, Laboratory Marketing Services CC, Roodepoort,

South Africa) maintained at 25 ± 1 °C, 60 ± 2 % RH and 16L: 8D photoperiod. The time and date of death of each parasitoid was recorded daily. Because *T. lutea* is facultative gregarious, percent parasitism was determined by the number of eggs that turned black (Kfir, 1981), while fecundity was taken as the total number of viable offspring produced per female in a vial. Progeny production, i.e. age specific fecundity, and the sex ratio in each replicate were determined daily. Average realized fecundity was estimated as the mean of the lifetime progeny of individual females.

Fig. 5.1 Vials used to expose eggs of *Helicoverpa armigera* on filter paper to *Trichogrammatoidea lutea* females for parasitism.

5.2.3 Life table of Trichogrammatoidea lutea *on* Helicoverpa armigera

A cohort life table was constructed using the data from reproductive potential of *T. lutea* on *H. armigera* at 25 °C, 60 ± 2 % RH and 16L: 8D photoperiod. The life table of *T. lutea* was constructed as explained in Price (1997); the following columns were used: x which represents age, l_x represent age specific survivorship of females and m_x for the number of female offspring produced per female per age. Net replacement rate

was calculated using formulae Ro = $\sum(l_x \times m_x)$, the mean generation time was calculated using $T = \sum(x \times l_x \times m_x)/\sum(l_x \times m_x)$ and the instantaneous rate of population increase as $r_m = \log_e R_o/T$ (Price, 1997).

5.2.4 Data analysis

The data on male and female longevity of *T. lutea* were analyzed using analysis of variance (ANOVA) for unbalanced design. Where differences were significant t-probabilities of pair-wise differences were computed to separate means at $P < 0.05$. Relationships between percent parasitism, fecundity, number of progeny per egg and sex ratio of progeny to age of *T. lutea* were determined using regression analyses weighted for number of females per age. Data for the females older than 11 days were excluded from the analysis in order to stabilize the variance. The significance level was set at $P < 0.05$. The data were analyzed using GenStat ® (Payne *et al.,* 2007).

5.3 Results

Female *T. lutea* lived significantly longer than males ($F_{1,78}$ =12.92, $P < 0.01$), with a mean longevity of 8.6 and 5.6 days, respectively (Fig. 5.2a). The longest longevity for females was 16 days (n = 1) while the shortest was 1 day (n = 2), and for males 14 days (n = 1) and 1 day (n = 3), respectively. *Trichogrammatoidea lutea* parasitized the eggs of *H. armigera* from the day of eclosion. Percent parasitism was highest on day 1 at 34 % and, thereafter, decreased as the females aged. Parasitism declined from 34 % to a range from 28 to 22 % between day 2 and 4, from 19 to 16 % between day 5 and 8, and then from 7 to 1.6 % from day 9 to 14 (Fig. 5.2b). No eggs

were parasitized by *T. lutea* after 14 days. The highest mean daily fecundity per female was 14 on the first day, and decreased significantly to less than 2 with an increase in age of the females (Fig. 5.3a). The average realized fecundity of *T. lutea* was 52 offspring per female, with a minimum of 8 and maximum of 93 offspring per female. The net replacement rate (Ro) was estimated at 25.5; generation time (*T*) at 9.79 days, and instantaneous rate of increase (r_m) was at 0.33. The number of progeny per egg with 2.17 was highest on the day of eclosion, then decreased to 0.89 on day 8 and, thereafter, increased up to 2.0 on the 13th day ($F_{13,247} = 18.24$, $P < 0.001$) (Fig. 5.3.b) (Fig. 5.3.b).

The daily sex ratio of *T. lutea* was significantly female biased from day 1 to day 3 and, thereafter, male biased and reached 100 % males from day 9 to day 14 (Fig. 5.3c). However, the overall percentage of male and female progeny of *T. lutea* on eggs of *H. armigera* was not significantly different, with 51 % males: 49 % females.

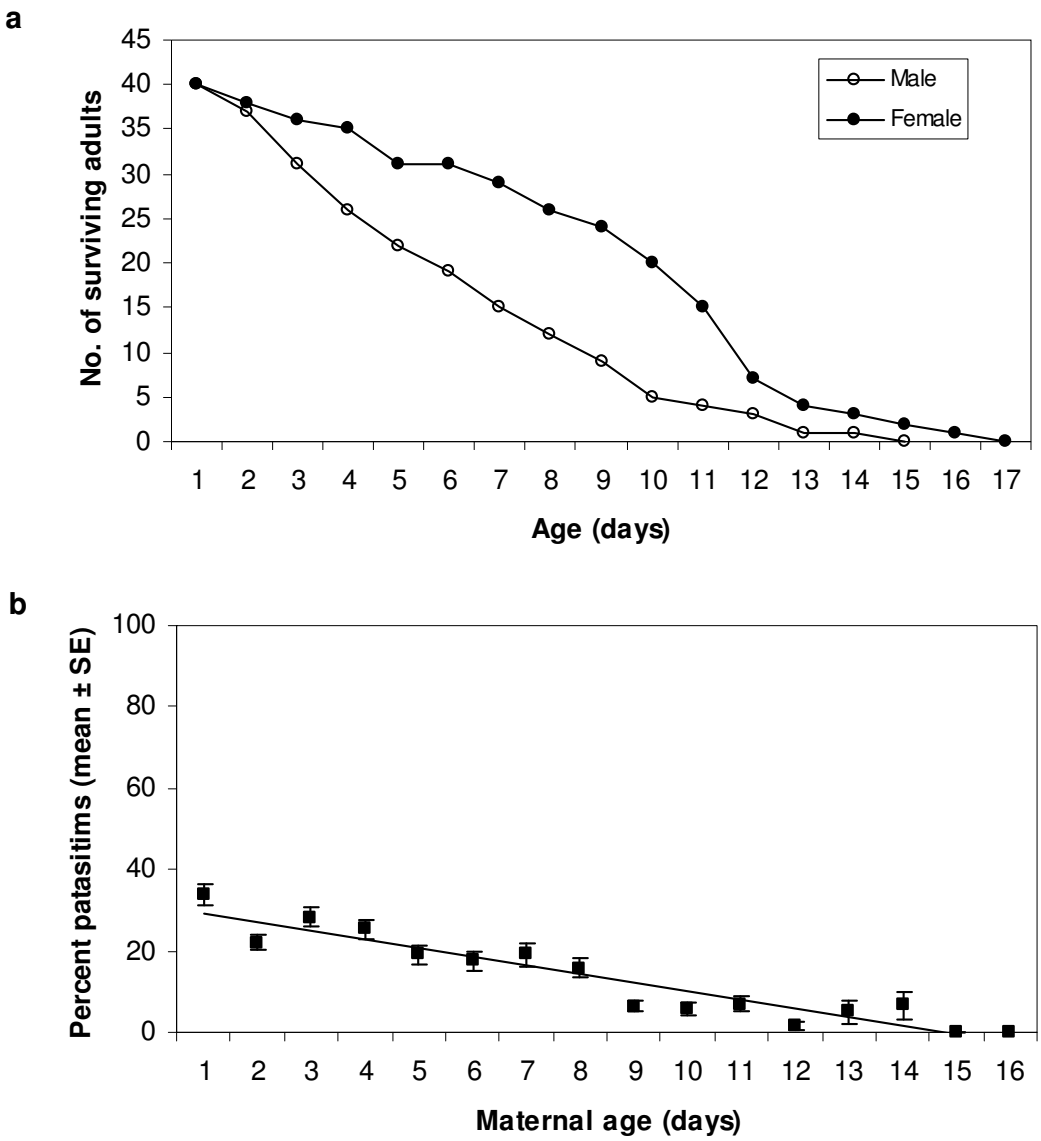

Fig. 5.2 (a) Survival of male and female *Trichogrammatoidea lutea.* (b) Relationship between percentage parasitism and maternal age of *T. lutea* parasitizing eggs of *Helicoverpa armigera* (y = 33.18 - 2.46x, R^2_a =0.85, $P < 0.001$).

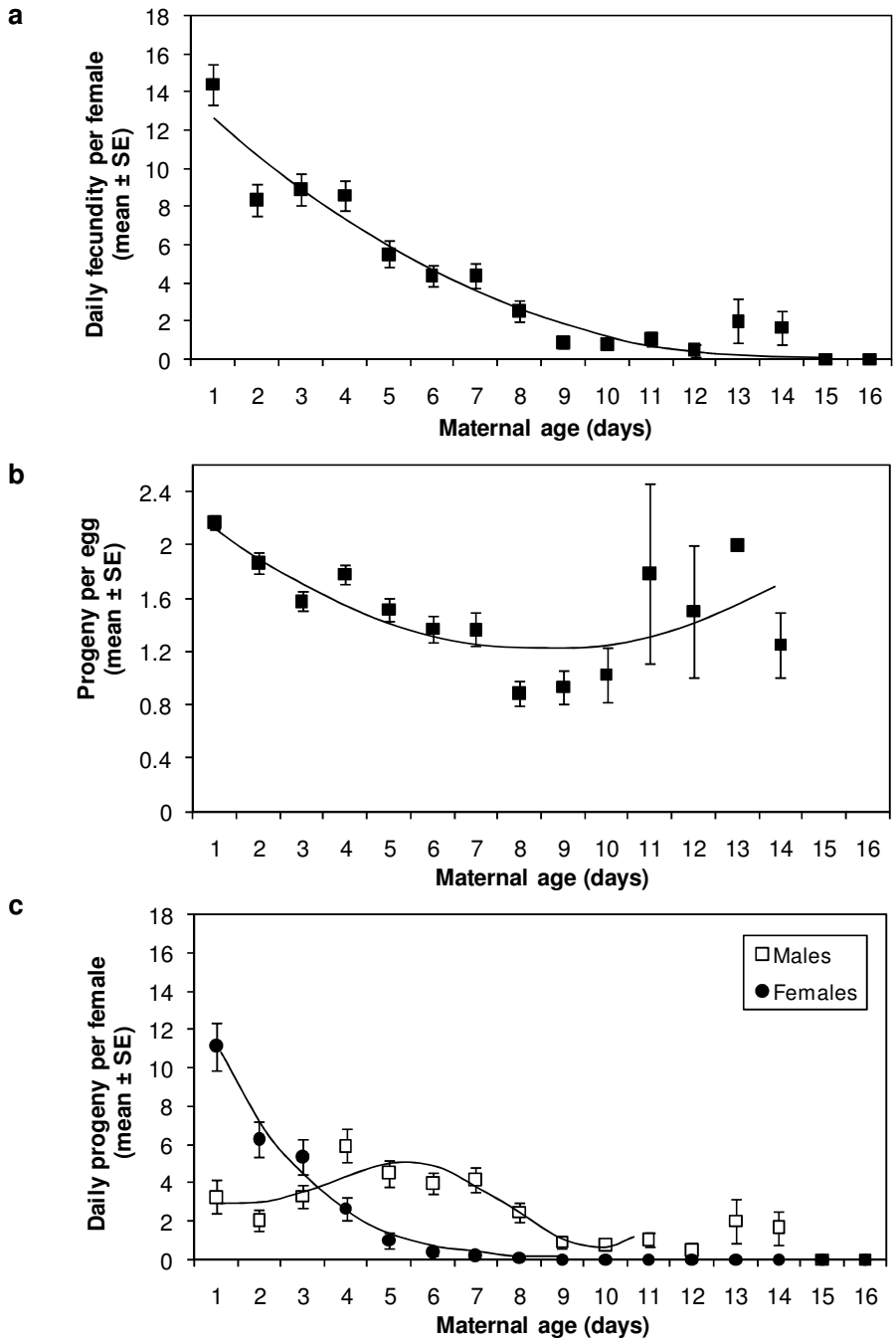

Figure 5.3 Relationships between maternal age of *Trichogrammatoidea lutea* and (a) daily fecundity per female ($y = -1.37 + 17.59 * e^{-0.183x}$, $R^2_a = 0.92$, $P < 0.001$), (b) number of progeny per egg of *Helicoverpa armigera* ($y = 2.410 - 0.2717*x + 0.01525*x^2$, $R^2_a = 0.72.6$, $P < 0.001$) and (c) male and female progeny (male: $y = 5.17 -3.62 * x + 1.685 * x^2 - 0.242*x^3 + 0.01076 * x^4$, $R^2_a = 0.69$, $P = 0.021$; female: $y= -0.462 + 17.86 * e^{-0.4431x}$, $R^2_a = 0.98$, $P < 0.001$).

5.4 Discussion

Knowledge on the reproductive biology of *T. lutea* is important for development of mass rearing systems, and development of biological control programmes (Gordh *et al.*, 1999). In this study, longevity of males and females *T. lutea,* as well as daily parasitism, fecundity, number of progeny per egg, and sex ratio with regard to age of female parents were determined. Female *T. lutea* lived longer than males, and this is common in insects as males are mainly necessary for mating. The longevity of female *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) of 8.5 days at 28 °C reported by Navarrese and Rodolfo (1997) was similar to that of female *T. lutea* in the present study. Wahner (2008) also reported similar results with a lifespan of *T. lutea* of about 9 days at 25 °C. Parry Jones (1937) reported longevity of female *T. lutea* of 7.6 and 8 days at 22 and 15 to 18.5 °C, respectively, which are slightly similar to the results in the present study. *Trichogrammatoidea lutea* did not have a pre-oviposition period since females parasitized eggs of *H. armigera* from the day of eclosion. The reproductive period of *T. lutea* lasted for 14 days with no eggs parasitized from day 15 onwards, suggesting that *T. lutea* may have a post oviposition period. Thus a 15-day-old female *T. lutea* may be unable to parasitize the hosts as a result of egg limitation which is permanent for pro-ovigenic species (Heimpel & Rosenheim, 1998). The majority of *Trichogramma* species are known to be pro-ovigenic (Hawkins & Cornell, 1999). Jervis *et al.* (2001) reported that the mean lifespan of synovigenic species (26 days) is greater than that of pro-ovigenic (9 days), which is similar to the 8.6 days for female *T. lutea* in this study. In this study, *T. lutea* was supplied with 20 eggs daily and 100 % parasitism was not achieved even on the day of eclosion. This suggests that *T. lutea* did not emerge with a full egg

complement, but rather continued to mature eggs with age. Such parasitoids are referred to as weakly synovigenic (Jervis *et al.,* 2001).

Daily parasitism and fecundity are not the same in the present study because *T. lutea* is facultative gregarious (Kfir, 1981). Nonetheless, daily parasitism and fecundity followed the same pattern. These were highest on the day of eclosion, then decreased progressively as the females aged. The levels of parasitism in this study are similar to those reported by Garcia *et al.* (2001) on *T. cordubensis* Vargas & Cabello where the total number of parasitized eggs decreased with age of the female parasitoids. Steidle *et al.* (2001) also reported similar results on *T. brassicae* (Bezdenko), *T. pretiosum,* and *T. carverae* Oatman & Pinto where the number of eggs laid daily decreased as the females aged. However, the lifetime fecundity of the latter three *Trichogramma* species as reported by Steidle *et al.* (2001) (36.4; 22.8 and 9.6; respectively), were lower than the *T. lutea* (52) in this study. Wahner (2008) found a net replacement rate (R_o) and instantaneous rate of population increase (r_m) of *T. lutea* at 11.92 and 0.26, respectively, on *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) at 25 °C and both were lower compared to those reported in the present study at the same temperature. However, the mean generation time (*T*) (9.4) reported by Wahner (2008) was similar to that in this study. The net replacement rate of *Trichogrammatoidea* near. *lutea* from low, medium, and high altitudes reported by Kalyebi *et al.* (2006) on *Coryra cephalonica* Stainton (Lepidoptera: Pyralidae) at 25 °C are also lower than that of *T. lutea* in this study. Amongst other factors, difference in estimates of life table parameters of *T. lutea* reported by Wahner (2008) and the present study could be a result of different host species used (Pratissoli & Para, 2000).

Female *T. lutea* produced both male and female offspring, showing that the female parents were mated and successfully inseminated (Mackauer & Völkl, 2002).

However, the sex ratio of the offspring changed from female biased in the first three days to male biased between day 4 and 8, and then males only from day nine. Earlier studies have shown that if female parasitoids are supplied with an unlimited number of hosts in the laboratory, the sex ratio changes and becomes increasingly male biased due to sperm depletion (Godfray, 1994; Pérez-Lachaud & Hardy, 1998). In the current study males had a shorter lifespan than females, and were not replaced after death. Therefore, the decrease in the proportion of female progeny until males only were produced could have been due to sperm depletion. Thus, female *T. lutea* may require multiple-copulation to continue producing females. The decrease in the daily number of offspring produced towards the end of the experiment could be due to egg depletion (Gordh *et al.*, 1999). In general, the two most important limiting factors for reproduction in parasitoids are likely to be the number of mature eggs for synovigenic, and the time available for pro-ovigenic species (Godfray, 1994). *Trichogrammatoidea lutea* allocated more female offspring on the first three days, and this was presumably because fitness gain of the mother is increased by producing a diploid female rather than haploid male offspring (Godfray, 1994). Furthermore, it was advantageous for female *T. lutea* to allocate fertilized eggs at an early age. The number of progeny emerging per parasitized egg decreased from day 1 to day 8 and, thereafter, increased up to day 14.

In conclusion, *T. lutea* did not have a pre-oviposition period, emerged with most of its eggs mature, had a short lifespan, and short reproductive period. Though *T. lutea* did not have a pre-oviposition period, 100% parasitism was not achieved on the day of eclosion showing that only a proportion of the eggs was matured. Therefore, *T. lutea* was presumed to be weakly synovigenic. The results indicate a strong influence of male and female longevity together with maternal age on daily parasitism,

fecundity, and sex ratio. The high net replacement rate of *T. lutea* in the present study shows good potential for using this parasitoid as a biological control agent. For efficient mass rearing of *T. lutea,* parasitoids should not be kept more than three days in cultures because the progeny became male-biased from the fourth day of the experiment and, at the same time, only 20 % parasitism was achieved. However, from the fourth day onwards, males may also be supplemented in cultures to avoid male biased sex ratio. Results from this study show great potential of mass rearing *T. lutea* for augmentative biological control programmes.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

General discussion and conclusions

The development of insecticide resistance by insect pests is a major impediment to their management throughout the world. The African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), a polyphagous pest that attacks many crops, and which is considered to be one of the most economically important pests in Africa (Greathead & Girling, 1989), has developed resistance to different classes of insecticides (Ahmad *et al.,* 1997; Malik *et al.,* 2003; Martin *et al.,* 2003; 2005; Buès *et al.,* 2005).

Biological control, using parasitoids or predatory insects, is a successful and economically viable alternative to the use of insecticides to regulate populations of insect pests in many parts of the world (Godfray, 1994; Van Driesche & Bellows, 1996; Elzen & King, 1999; Thomson *et al.,* 2003). The process of identifying effective natural enemies as biological control agents, and the development of efficient mass rearing systems are the key elements for augmentative biological control programmes (periodic release of natural enemies) (Smith, 1996). Host suitability, thermal tolerance, longevity, and fecundity are some of the main biological parameters used to assess the potential of candidates for biological control programmes (Hassan, 1994). In addition, augmentative biological control by using indigenous biological control agents is most preferred since they are established and well acclimatized to local environmental conditions and cropping systems (Hassan, 1994). Efforts to explore for exotic biological control agents (importation) should only be undertaken if indigenous parasitoids have been proved to be ineffective (Hassan, 1994).

Trichogrammatoidea lutea Girault (Hymenoptera: Trichogrammatidae) is one of several indigenous egg parasitoids that have been recorded on *H. armigera* in southern Africa (Parson & Ullyett, 1936; Parry Jones, 1937; Kfir, 1982). This parasitoid is a suitable candidate for biological control of *H. armigera* because it attacks the egg stage. Furthermore, the use of egg parasitoids for biological control is more desirable because they kill the pest before it reaches the damaging larval stage (Hassan, 1994). To pave the way for the development of a mass-rearing method, and to determine the potential of *T. lutea* as a biological control agent for augmentative releases, the biology of *T. lutea* was studied in the laboratory on three Lepidoptera hosts (*Helicoverpa armigera, Chilo partellus* and *Cadra cautella*). The study examined (i) whether UV-irradiation of host eggs to arrest development of embryos affects development of the progeny of *T. lutea*, (ii) which host(s) and temperature(s) are suitable for mass-rearing *T. lutea*, and (iii) the longevity and reproductive biology of *T. lutea* on *H. armigera.*

Ultraviolet (UV) irradiation treatment is used as host preservation method Lianzhong *et al.* (1993), to limit host defence, infection by pathogens (Cônsoli *et al.,* 2000), and possible cannibalism by emerging larvae from viable host eggs (Kfir & Van Hamburg, 1988). Romeis *et al.* (1997) reported lower survival of *T. chilonis* Ishii on UV-irradiated eggs compared to non-irradiated eggs of *H. armigera.* Mellet and Schoeman (2004) presented the possibilities of combining *T. lutea* with sterile insect technique in an IPM for *H. armigera* on cotton fields. Results from the present study show that UV-irradiation method can be employed to ease mass rearing of *T. lutea* because *T. lutea* developed without detrimental effects on UV-irradiated eggs. However, it should be taken into consideration that the effect of UV-irradiated hosts varies among species.

The main factors that influence efficacy of parasitoids in biological control programmes include: host preference (recognition, acceptance and suitability), and tolerance to environmental conditions (Hassan, 1994). In this study, the influence of temperature and the host species were determined, and it was concluded that both affected the life history of *T. lutea* in different ways. As insects are known to be poikilothermic (Dent, 1991), the rate of development of *T. lutea* increased with temperature on all three hosts. However, the rate of development was faster on *H. armigera* at all temperatures and slower on *Chilo partellus* demonstrating that the host species influenced parasitoid development. The developmental threshold of *T. lutea* on the three host species was estimated at approximately 12 °C, which is slightly higher than 11 °C estimated by Wahner (2008) for *T. lutea* developing in eggs of *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae). Kalyebi *et al.* (2006) reported developmental thresholds around 8 °C for *Trichogrammatoidea* sp. near *lutea* from different altitudes on *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in Kenya. The difference between the life history parameters in the present study compared to earlier studies could be due to the different experimental method and host species used.

Results presented in the present study and in earlier studies (Kfir & Van Hamburg, 1988; Wahner, 2008), shows that *T. lutea* has a wide thermal tolerance, and is active during the summer season (Parson & Ullyett, 1936) when *H. armigera* is also abundant (Parry Jones, 1937). The survival of *T. lutea* was poor at low temperatures (18 and 21 °C). Hence, Wahner (2008) suggested that survival of overwintering *T. lutea* is poor. The average minimum field temperatures during winter in some parts of South Africa can be lower than the developmental threshold of *T. lutea* reported in the

current and earlier studies. However, *T. lutea* may still survive when average maximum temperatures are higher than its developmental threshold.

Due to controlled environmental conditions and supply of food sources in the laboratory, the longevity of parasitoids tends to be longer than in the field. *Trichogrammatoidea lutea* lived for up to 16 days, with an average of 8.6 days for females. This is slightly longer than the 7.2 days reported by Parry Jones (1937). Therefore, regular releases of newly emerged *T. lutea* may be required to maintain sufficient numbers of parasitoids, and to achieve high parasitism levels since longevity may be shorter in the field. The realized fecundity of 51, and the reproductive period of 14 days shows that *T. lutea* may remain active for a longer period and parasitize more eggs, provided the environmental conditions are favourable and food resource are available. The life table parameters of *T. lutea* determined in the current study; net replacement rate (Ro), mean generation time (*T*), and instantaneous rate of population increase (r_m) may be used to estimate population dynamics of this parasitoid for biological control purposes. For mass rearing purposes, it may not be productive to keep more than three-day old *T. lutea* in cultures because progeny became male biased after three days.

Results from this study show good prospects for using *T. lutea* in an augmentative biological control programme against *H. armigera,* and this may provide opportunities for expanding tactics in integrated pest management (IPM) of *H. armigera* in southern Africa. However, open field studies are required to further evaluate the efficacy of *T. lutea* under field conditions.

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