Biological control ecology of *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae) on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

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Abstract

The solitary and koinobiont endoparasitoid, *Aphidius colemani* Viereck, is produced commercially for biological control of green peach aphid *Myzus persicae* (Sulzer) and cotton aphid *Aphis gossypii* Glover around the world. However, its production cost is still high and biological control efficiency is still uncertain, probably due to the lack of knowledge on its biological control ecology. To fill the knowledge gap, I investigated the biological control ecology of the *A. colemani*-*M. persicae* system. My results show that most emergence and reproductive activities of *A. colemani* occur during the photophase. After emergence, both sexes need about 2 hours for sex maturation, but once sexually mature, age of neither sex has any significant effect on mating success. Food supply to adult females is essential to mating success. The mating behavioural sequence is similar to that of many other braconid parasitoids. My findings suggest that *A. colemani* is an effective biological control agent of *M. persicae* because reproductive outputs of the parasitoid are twice as high as the aphid, the parasitoid reaches the maximum lifetime reproductive potential about a week earlier than the aphid, and parasitised aphids contribute little to their population growth and make limited damage to plants. The parasitoid prefers to attack larger hosts but such preference is counterbalanced by greater defensive ability of larger hosts, resulting in similar parasitism rate on hosts of all ages. As a result, parasitising mid-aged hosts allows *A. colemani* females to gain maximum fitness in developmental period, body size and parasitism of their progeny. Finally, my study confirms that *A. colemani* has a Type II functional response. However, it can still successfully control *M. persicae* regardless of pest density probably because parasitoid density has significantly more effect than host density on parasitoid reproductive fitness and the low mutual interference among the searching parasitoids encourages aggregation of the parasitoids on host patches of high density. The present study provides basic knowledge on the biology of *A. colemani* for development of effective measures for laboratory handling, rearing, and field release, and brings insight into the success of aphid biological control programmes using the parasitoid augmentation approach.

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Table of Contents

on life history strategies of both parasitoid and its host: evidence from *Aphidius colemani–Myzus persicae* system. Journal of Economic Entomology 110: 400-406.

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List of Tables

List of Figures

Chapter One

General Introduction

1.1 Introduction

1.1.1 Aphids and their economic importance

Aphids are among the most economically important insect pests of agriculture and forestry (van Emden & Harrington 2007). Due to their ability of dispersal and nature of parthenogenetic reproduction, they frequently become invasive pests throughout the world (Teulon & Stufkens 2002; Messing et al. 2007). Aphids cause damage by feeding on phloem sap and transmitting a number of plant virus diseases through their piercing and sucking mouthparts (Vilcinskas 2016). About 50% of insect borne plant viruses are transmitted by aphids (Ng & Perry 2004). For example, 28 aphid species potentially transmit seven virus diseases of potato crops in New Zealand (Stufkens & Teulon 2001). Furthermore, aphids excrete honeydew on plants (Chomnunti et al. 2014), promoting sooty mould growth and reducing photosynthesis (Nelson 2008).

Among about 120 aphid species in New Zealand 90% are exotic (Teulon & Stufkens 2002; Teulon et al. 2008). Many species have significant economic impacts on arable, vegetable and fruit and ornamental crops, and forest trees (Stufkens & Teulon 2003; Sopow et al. 2017). For example, green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is one of the most important pests of agricultural and horticultural crops worldwide. It attacks plants from about 40 families, and transmits more than 100 plant virus diseases (van Emden & Harrington 2007). In New Zealand, green peach aphid is the most abundant aphid species found on potato crops (Stufkens & Teulon 2001). It can overwinter as eggs on its primary woody hosts, mainly peach, or reproduce parthenogenetically year-round on a large number of secondary hosts including potatoes, tomatoes, brassicas, beets, cereals, pasture clovers, peas, and so on (Cameron & Fletcher 2005). Some common virus diseases transmitted by green peach aphid in New Zealand include alfalfa mosaic, potato leaf roll, tomato yellow top, beet

western yellows, cucumber mosaic, lettuce mosaic, potato virus Y, watermelon mosaic virus Type 2, and zucchini yellow mosaic (Cameron & Fletcher 2005).

1.1.2 Aphid management

In New Zealand, chemical insecticides are routinely applied to control insect pests including plant virus transmitting aphids such as green peach aphid (Fellowes & Fergusson 1974; Martin 2005; van Toor & Teulon 2006; van Toor et al. 2008). For example, potato seeds are often treated with a neonicotinoid (imidacloprid), followed by up to 11 applications of insecticides from organophosphates, carbamates, pyrethroids and other chemical groups, to control aphids and aphid borne plant virus diseases (van Toor & Teulon 2006). Multiple and repeated use of insecticides, however, results in the development of insecticide resistance (Martin 2005). For example, green peach aphid has already developed resistance to about 70 types of synthetic chemical pesticides worldwide (Silva et al. 2012; Earlham Institute 2017) including Australia (Umina et al. 2014; de Little et al. 2017) and New Zealand (Fellowes & Fergusson 1974; Baker 1978; Cameron & Walker 1988). As a result, the control of green peach aphid using insecticides has recently become more difficult (Foster et al. 2003) although curative control of outbreaks using insecticides is still practiced in glasshouse crops.

Therefore, there is an urgent need for the further development and application of aphid management strategies which provide alternatives to the routine applications of insecticides (Cameron & Fletcher 2005), for example, Integrated Pest Management (IPM). IPM is an approach which maximises the use of biological control agents and cultural practices in pest and disease management with the conscious use of selective pesticides if necessary (Horne & Page 2008). Significant progress has been made for the IPM approach in New Zealand (Cameron et al. 2009; Walker et al. 2009; Horrocks et al. 2010; Walker et al. 2017).

1.1.3 Biological control of aphid pests

Biological control using natural enemies plays an important role in pest management (Waage et al. 1988; Naranjo et al. 2015). It can provide the most economical and long-lasting pest management (DiTomaso et al. 2017) and reduce the

negative impacts of chemical insecticides (van Lenteren et al. 2017). Numerous predators, parasitoids and entomopathogens attack aphids (Hajek & Leger 1994; Völkl et al. 2007). Among these natural enemies, parasitoids are more effective in managing aphid populations, for example, the success rate for aphid control by parasitoids is 21.8% (n = 193), considerably greater than that by predators (4.1%, n = 221) (Hirose 2006). The most commonly used aphid parasitoids for biological control programmes in the field and greenhouses are from the genera *Aphidius*, *Praon*, *Diaeretiella*, *Trioxys* and *Ephedrus* (Wei et al. 2005) in the hymenopteran Braconidae and Aphelinidae (Boivin et al. 2012).

Aphidius species (Braconidae: Aphidiinae) are important parasitoids in controlling green peach aphid (Mani & Krishnamoorthy 1994; Kos et al. 2008; Acheampong et al. 2012). One important parasitoid in this group is *A. colemani* (Viereck), which has been present since 1982 after unassisted introduction (Teulon et al. 2008). It is a solitary, koinobiont, oligophagous parasitoid, capable of parasitising various economically important aphid species in the field (Starý 2002). It is also used as a biological control agent in glasshouses in New Zealand (Cameron & Fletcher 2005). This species is commercially produced for aphid control in Europe, North America (Fernandez & Nentwig 1997), and New Zealand (Teulon et al. 2008; Bioforce 2017) due to its great host searching and parasitising ability (van Steenis 1995). Biological control of green peach aphid in the field can be complex because of interactions of many factors such as multiple species of biological control agents, and other pests and their control tactics. However, the practice in greenhouse is relatively simpler.

1.2 Relevance of the research

Although advancements in pest biological control programmes are giving hope for future prospects, biological control is currently practised in a relatively small scale (van Lenteren 2012). Today, growers are still less confident in biological control as compared to the application of insecticides. The main drawbacks include the efficiency of natural enemies, ease of their availability and storage, and cost (van Driesche & Heinz 2004). Furthermore, knowledge on biological control ecology of various enemy-pest systems is still far from adequate, which warrants extensive basic studies to

understand biology and ecology of both pests and their natural enemies (Bale et al. 2008).

Mass releases of *A. colemani* in glasshouses have been practised for aphid control (Goh et al. 2001). However, its mass production is still costly (Vásquez et al. 2006) and basic knowledge on its ecology is still largely lacking (Harizanova & Ekbom 1997; Goh et al. 2001; Zamani et al. 2007). To reduce its production cost, facilitate evaluation of its biological control efficiency and improve its cost-effective application in aphid pest management, we need to better understand the parasitoid's circadian activity patterns and host size preference, as well as life history strategies of both parasitoid and its hosts, and interactions between parasitoid and host densities.

Insect parasitoids usually have inherited patterns in emergence, feeding, mating and oviposition, with light providing cues for such activities (Pompanon et al. 1995; He 2008; Kant & Sandanayaka 2009; Khatri 2011). Therefore, knowledge on circadian rhythms can help determine the timing for parasitoid harvest, storage, transportation and release. Prior to the present study, almost nothing was known about the circadian activity patterns of *A. colemani*.

Parasitoids usually select hosts of certain size for better reproductive fitness (Bernal et al. 1999) but findings on *A. colemani*, for some unknown reason, are contradictory (Goh et al. 2001; Martinou & Wright 2007). Larger hosts usually contain more resources than smaller ones (Liu 1985; Mackauer 1986). Consequently, females that parasitise larger hosts produce progeny of larger body size (Liu 1985; He et al. 2005a; Henry et al. 2009), more female-biased sex ratio (Henry et al. 2005; He et al. 2005a), higher egg load at emergence (He et al. 2005a), and shorter developmental time with lower premature mortality (Jenner & Kuhlmann 2006). However, unlike idiobiont parasitoids that attack hosts with fixed resources, hosts of koinobiont species continue to grow and develop after being parasitised and thus their properties continue to change during the course of parasitoid progeny development (Mackauer 1986; Brough et al. 1990; Lin & Ives 2003). Furthermore, larger aphids may be able to defend themselves more effectively than smaller ones against parasitoid attack, costing more to parasitoids in terms of time and energy for handling (Henry et al. 2006; Wyckhuys et al. 2008; He et

al. 2011; Khatri et al. 2016). Therefore, aphidiine females may have developed strategies to accurately assess the suitability of hosts for their progeny before oviposition (Colinet et al. 2005; Henry et al. 2005) and to balance their fitness return and oviposition cost (Chau & Mackauer 2001; Henry et al. 2009). So far, none of these studies has quantified host defence behaviours, making it difficult to evaluate host-parasitoid interactions in greenhouse crops.

Essential information for evaluation of biological control potential of a parasitoid can be obtained from direct measurement of life history parameters for both parasitoid and its host (Bellows et al. 1992; van Driesche & Bellows 1998; Bellows & van Driesche 1999; van Lenteren 2009; Lins et al. 2011; Khatri et al. 2017) and subsequent construction and comparison of their life tables (Bellows et al. 1992; Bellows & van Driesche 1999; van Lenteren 2009; Lins et al. 2011; Khatri et al. 2017). However, in evaluation of the efficiency of parasitoids in suppressing aphid populations, most previous studies have not given complete pictures of life histories for both parasitoid and host species. For example, van Steenis (1993) and Torres et al. (2007) investigated the life table of *A. colemani* but did not compare it with that of *Aphis gossypii* Glover. In contrast, van Steenis and El-Khawass (1995a) and Vásquez et al. (2006) estimated the population growth of *A. gossypii* without considering that of *A. colemani*. Chi and Su (2006) analysed the life table parameters of both *Aphidius gifuensis* (Ashmead) and *M. persicae* but they did not take parasitised aphids of different ages into consideration. Prior to my PhD studies, little was known about life history strategies and life tables of the *A. colemani*-*M. persicae* system.

The success of biological control of insect pests using parasitoids usually depends on parasitoids' ability to suppress the pest populations and to build up their own populations, which is largely determined by their responses to host density and influenced by the mutual interference between the searching parasitoids (Kidd & Jervis 2005; He & Wang 2014). The functional response describes the per capita response of a parasitoid to the variation in host density (Solomon 1949), whereas demographic response reflects the change in parasitoid's reproductive output as a function of change in host and parasitoid density (Kidd & Jervis 2005; He & Wang 2014).

Several previous studies have made effort to understand the functional response of *A. colemani* (van Steenis & El-Khawass 1995b; Jones et al. 2003; Byeon et al. 2011). However, their conclusions are highly subjective in determining the type of functional response. For example, based on the shape of response cure, (Jones et al. 2003; Zamani et al. 2007; Byeon et al. 2011) suggest a type II functional response for *A. colemani* at low density of *M. persicae* but a type III functional response at medium to high aphid density. Jones et al. (2003) propose that *A. colemani* on *Schizaphis graminum* Rondani usually has a type III functional response due to the slightly higher coefficient (R^2) for that response model. In the *A. colemani-A. gossypii* system, van Steenis and El-Khawass (1995b) simply fit their data with a type III functional response model because of the limited space and time for their experiments (Pandey et al. 1982; Pandey et al. 1984). Therefore, the above studies have neither tested nor confirmed the type of functional response for their study systems. Moreover, those authors hold the parasitoid density constant (n = 1) during their studies while in nature more than one foraging parasitoid usually visit a host patch simultaneously (Chong & Oetting 2006; He & Wang 2014). In the above-mentioned systems, it is still unknown whether parasitoid reproduction is affected by the demographic response and mutual interference between the searching parasitoids.

1.3 Aim and objectives

In order to provide new and useful information for the development of effective mass rearing measures and biological control strategies, the present study aims to study aspects of biology and behavioural ecology of *A. colemani* on *M. persicae*, with four objectives:

(1) to investigate the circadian rhythm and mating behaviour of *A. colemani*;

(2) to study life history strategies of both *A. colemani* and *M. persicae*;

(3) to evaluate host size selection behaviour of *A. colemani* and its consequences in reproductive fitness;

(4) to examine the response of parasitoids of different density and age to *M. persicae* density.

Chapter Two

Literature Review

2.1 Introduction

This chapter reviews the current knowledge on *A. colemani* with some discussion on other Aphidiinae species that are relevant to my PhD studies.

2.2 Origin

It is suggested that the parasitoid originates from northern India or Pakistan, and subsequently spreads to central Asia, the Mediterranean region, Africa, and perhaps secondarily to Australasia and South America (Starý 1975).

2.3 Taxonomy and identification

2.3.1 Taxonomy

This species was first described by Viereck in 1912 and its taxonomic position is:

Order: Hymenoptera

Family: Braconidae

Subfamily: Aphidiinae

Genus: *Aphidius*

Species: *colemani*

2.3.2 Identification

Aphidius Nees is one of the most speciose genera in the subfamily Aphidiinae. The genus can be diagnosed by the following characters: transverse head; filiform antennae (with 12-24 segments); propodeum areolated with narrow, small central areola, and two upper and lateral areolae; tentorial index (tentoriocular line/intertentorial line) ≤ 0.6; triangular pterostigma in the forewing, metacarpus longer than width of pterostigma; abdomen lanceolate in female and rounded at apex in male; tergite 1 at least twice as long as wide at spiracles; ovipositor sheaths comparatively short, slightly curved upwards with sparse hairs (Starý 1973; Kavallieratos et al. 2001;

Ullah et al. 2015). Table 2.1 lists *Aphidius* species commonly found in New Zealand and their key diagnostic characters.

Table 2.1 Common *Aphidius* species present in New Zealand and their diagnostic characters

Species	Diagnoses	References
A. ervi	• Anterolateral area of petiole rugose • Antennae with 18 to 19 (sometimes 20) segments • Stigma 3.4 to 3.9 times as long as wide • Ovipositor sheath slightly concave	Tomanović et al. (2003), Tomanović et al. (2012), Rakhshani et al. (2008)
A. eadyi	• Antennae with 19 to 21 segments • Pterostigma 1.8 to 2.1 times as long as metacarpus • Number of costulae on anterolateral area of petiole ranges from 6 to 9 • Ratio of stigma length and length of Rs vein 1.6 to 2.0	Pungerl (1986), Tomanović et al. (2003), Rakhshani et al.(2008)
A.rhopalosiphi	• Antennae with 16 to 17 segments • Pterostigma slightly longer than metacarpus • Stigma 4.0 to 4.5 times as long as wide • Petiole 3.5 to 4.0 times as long as wide • 1-3 longitudinal placodes on flagellomere 2	Tomanović et al. (2003), Rakhshani et al.(2008)

A. smith	• Antennae with 19 to 20 segments • Forewing distal abscissa of R1 0.77 to 0.83 times length of stigma • Pterostigma 1.3–1.5 times as long as metacarpus.	Kavallieratos et al. (2001), Rakhshani et al. (2008)
A. sonchi	• Antennae with 15 to 19 segments • Flagellomere 1 3.0 to 3.5 times as long as median width • Petiolus 2.5 to 3.0 times as long as wide at spiracle level • Stigma 3.0 to 3.5 times as long as wide	Starý (1973), Tomanović et al. (2003)
A. colemani	• Antennae with 15 or 16 segments • Metacarpus as long as or slightly shorter than pterostigma length • Labial palpus with two palpomeres • Anterolateral area of petiole costate • Forewing stigma triangular. • Flagellomere 1 brown to light brown, metasoma brown and metasomal tergum 1 light brown to yellow	Rakhshani et al. (2008), Tomanović et al. (2012), Kavallieratos et al. (2013), Rakhshani et al. (2015)
A. salicis	• Antennae with 12 to 13 segments • Maxillary palps 4-segmented • Labial palps 3-segmented • Petiolus about 2.4 to 2.6 times as long as wide at spiracle level • Tentorial index 0.39 to 0.49	Kavallieratos et al. (2001), Tomanović et al. (2003)

Aphidius colemani is a 2-3 mm long, slender, wasp with a black thorax and head and yellowish-brown legs and abdomen (Figure 2.1). A female has pointed abdomen with an ovipositor (Figure 2.1).

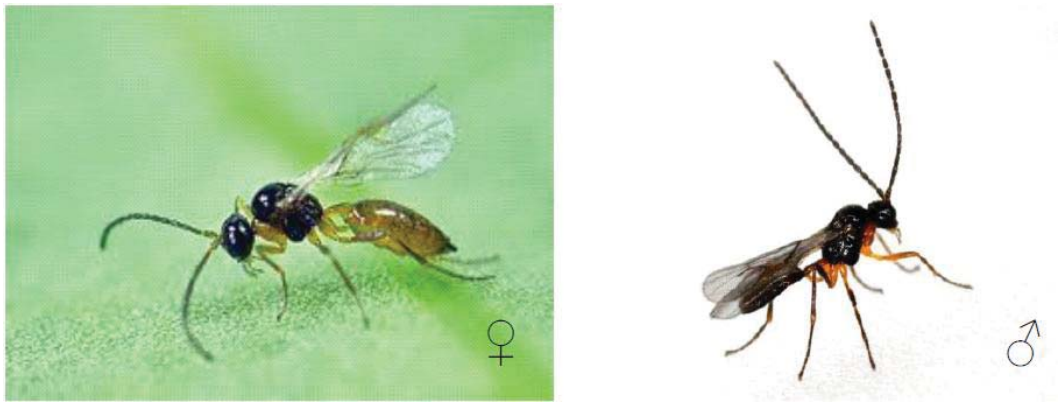

Figure 2.1 Male and female adults of *A. colemani*.

It is often difficult to differentiate between *A. colemani* and closely related species using morphological characters (Zamora Mejías et al. 2010). For example, according to Kavallieratos & Lykouressis (1999) *A. colemani* females may be distinguished from those of *A. transcaspicus* because the antennae of the former have 15 and the latter 16 – 17 segments (rarely 15). However, Garantonakis et al. (2009) state that both males and females of *A. colemani* have 15 antennal segments (rarely 16 for males and 14 for females) and 2-segmented labial palps while *A. transcaspicus* Telenga males have 17 – 19 antennal segments and females 16 (rarely 15) and both sexes have 3-segmented labial palps. In Mescheloff & Rosen's (1990) study male antennae of *A. colemani* have 15-16 segments but those of females 13-14 segments. Rabasse et al. (1985) indicate that the antennae of *A. colemani* females from southern France have 16 – 17 segments (rarely 15) and those from Brazil 15 – 16 segments (rarely 14). In Rakhshani et al. (2008), *A. colemani* adults have 14- to 15-segmented antennae and 2 labial palmomeres. Starý (1975) considers *A. colemani* as a synonym of *A. platensis* Breth and *A. transcaspicus*. Garantonakis et al. (2009) indicate that *A. colemani* and *A. transcaspicus* can interbreed and produce fertile offspring. Hence, cross mating, often used for complex species separation, may fail to reach taxonomic separation with other closely

related species. Molecular methods are increasingly used for the correct taxonomic identification of closely related species (Garantonakis et al. 2009).

The *A. colemani* population used in this study is reared on *M. persicae*. Males have 17 antennal segments and females 15 antennal segments. I sent the parasitoid specimens to Dr. Darren Ward, Curator of Hymenoptera, New Zealand Arthropod Collections (NZAC), Landcare Research, Auckland for formal identification. Based on morphological characters and DNA sequencing, the species was confirmed to be *A. colemani*.

2.4 General biology

Aphidius colemani is a solitary endoparasitoid. Females normally deposit a single egg in an aphid although superparasitism may occur when hosts are scarce. Once within the aphid, the egg expands several times its original size. The larva then hatches and begins feeding osmotically. The parasitoid passes through three larval instars inside the host (O'Donnell 1987; Muratori et al. 2004). It stops feeding at the end of the third larval stage (Muratori et al. 2004) and spins its cocoon inside the empty cuticle of the aphid, and pupates (Hågvar & Hofsvang 1991). At this stage the parasitised aphid turns into a swollen, yellowish brown mummy. The adult parasitoid cuts a lid in the upper-distal part of the mummy (between the cornicles) through which it emerges. It is a pro-ovigenic species (Byeon et al. 2011), with the potential fecundity of newly emerged females at emergence being 226.1 ± 9.0 eggs (Sampaio et al. 2008). Lifetime fecundity could be > 400 eggs on some occasions and < 100 eggs on others (Harizanova & Ekbom 1997; Goh et al. 2001).

Temperature has a significant influence on the life history characteristics of *A. colemani* such as developmental time, fecundity, fertility, parasitism rate, sex ratio and survival (Goh et al. 2001; Sampaio et al. 2007; Zamani et al. 2007; Saleh et al. 2014). In the laboratory, *A. colemani* can develop within a temperature range of 10 ~ 30^{o}C (Zamani et al. 2007; Saleh et al. 2014) but adults' longevity decreases with increasing temperature (Saleh et al. 2014). The parasitoid cannot survive when temperature is lower than 5^{o}C or higher than 35^{o}C (Zamani et al. 2007). The developmental time for male and female *A. colemani* reared on *M. persicae* at 22 ± 1^{o}C, 70 ± 10% RH, and 12

hour photophase is 12-14 and 12-13 days, respectively (Sampaio et al. 2008). Under this condition, males and females live for 14.30 ± 0.04 and 10.30 ± 0.56 days, respectively (Sampaio et al. 2008).

The sex ratio of *A. colemani* is usually female-biased (Saleh et al. 2014). However, this is affected by environmental factors. In a temperature range of 15 ~ 25°C, sex ratio is female-biased, whereas at temperatures below 10°C or above 30°C, it is male-biased (Zamani et al. 2007). Body size of aphids is another factor affecting *A. colemani* sex allocation. For example, those attacking small aphids (first instar nymphs) produce offspring of a male-biased sex ratio and those parasitising large aphids (third instar nymphs) have offspring of a female-biased sex ratio (Jarošík et al. 2003).

2.5 Host range

Aphidius colemani attacks more than 40 aphid species, which in turn are known to damage more than 90 host plant species (Table 2.2). Green peach aphid *M. persicae* and cotton aphid *A. gossypii* Glover are among the most preferred host species for parasitisation (Ode et al. 2005).

Table 2.2 Aphid species parasitised by *A. colemani* and their host plants.

Aphid Species	Infested Plants	References
Aphis coreopsidis (Thomas)	*Bidens pilosa*, *Emilia sonchifolia*	Starý et al. (2007)
Aphis craccivora Koch	*Abelmoschus esculentus*, *Citrus aurantiifolia*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita pepo*, *Hedera helix*, *Hibiscus esculentus*, *Lycopersicon esculentum*, *Medicago sativa*, *Vicia faba*, *Robinia pseudoacacia*	Ölmez & Ulusoy (2003), Kavallieratos et al. (2004), Starý et al. (2007), Rakhshani et al. (2008), Kavallieratos et al. (2010), Barahoei et al. (2013)
Aphis fabae Scopoli	*A. esculentus*, *Callendula officinalis*, *Chenopodium album*, *C. sativus*, *C. pepo*, *L. esculentum*,	Kavallieratos et al. (2004), Rakhshani et al. (2008), Kavallieratos et al. (2010),

	Solanum melongena, *S. nigrum*, *S. tuberosum*, *Vicia faba*	Rakhshani et al. (2012)
Aphis fabae solanella Theobald	*S. americanum, Sessea brasiliensis, S. nigrum*	Starý et al. (2007), Kavallieratos et al. (2010)
Aphis glycines Matsumura	*Glycine max*	Kaiser et al. (2007)
Aphis gossypii Glover	*Ageratum conyzoides*, *Catalpa bignonjoides*, *Chrysanthemum morifolium*, *Citrus aurantium*, *Cucurbita pepo*, *Cydonia oblonga*, *Cyphomandra arabica*, *Dendranthema grandiflora*, *Dieffenbachia amoena*, *Duranta repens aurea*, *Evonymus* sp., *Gossypium hirsutum*, *Hibiscus rosa-sinensis*, *H. esculentus*, *Ixora macrothyrsa*, *Malva neglecta*, *Pyrus communis*, *Prunus domestica* var. *insititia*, *Schefflera* sp., *Tecomma* sp., *Theobroma cacao*, *Whitania* sp.	Messing & Rabasse (1995), Kavallieratos et al. (2004), Ode et al. (2005), Starý et al. (2007), Kavallieratos et al. (2008), Rakhshani et al. (2008), Kavallieratos et al. (2010)
Aphis hederae Kaltenbach	*Hedera helix*	Starý et al. (2007)
Aphis helianthi Monell	*Helianthus annus*	Elliott et al. (1994)
Aphis illinoisensis Shimer	*Vitis vinifera*	Havelka et al. (2011)
Aphis nasturtii Kaltenbach	*Capsicum annuum*, *C. sativus*, *S. tuberosum*	Kavallieratos et al. (2010)
Aphis nerii Boyer de Fonscolombe	*Asclepias curassavica*, *Helianthus annus*, *Nerium oleander*	Elliott et al. (1994), Starý et al. (2007), Rakhshani et al. (2008)
Aphis punicae Shinji	*Punica granatum*	Kavallieratos et al. (2004), Rakhshani et al. (2008)
Aphis spiraecola Patch	*Baccharis dracunculifolia*,	Kavallieratos et al. (2004),

	Citrus sinensis, *Emilia sonchifolia*, *Erigeron bonariensis*, *Rosa* sp., *Spirea* sp., *Tecomma* sp.	Starý et al. (2007)
Aphis umbrella (Börner)	*Malva neglecta*	Rakhshani et al. (2008)
Brachycaudus amygdalinus (Schouteden)	*Prunus persica*	Barahoei et al. (2013)
Brachycaudus helichrysi (Kaltenbach)	*Ageratum conyzoides*, *Baccharis dracunculifolia*, *Callendula officinalis*, *Erechtites valerianaefolia*, *Erigeron bonariensis*	Starý et al. (2007), Rakhshani et al. (2008)
Brachycaudus cardui (L.)	*Cirsium arvense*	Rakhshani et al. (2008)
Brachycorynella asparagi (Morvilko)	*Asparagus officinalis*	Starý (2002)
Brachycaudus schwartzi (Börner)	*Prunus domestica*, *Prunus persica*	Starý et al. (2007)
Brevicoryne brassicae (L.)	*Lepidium virginicum*, *Brassica rapa*	Starý et al. (2007) Ibarra-Sandate et al. (2016)
Capitophorus eleagni (Del Guercio)	*Eleagnus angustifolia*	Rakhshani et al. (2008)
Cavariella aegopodii (Scopoli)	*Foeniculum vulgare*	Starý et al. (2007)
Diuraphis noxia (Kurdjumov)	*Hordeum vulgare*	Starý (2002)
Dysaphis apiifolia (Theobald)	*Petroselinum sativum*	Starý et al. (2007)
Dysaphis tulipae (Boyer De Fonscolombe)	*Belamcanda chinensis*	Starý et al. (2007)
Eucarazzia elegans (Ferrari)	*Salvia splendens*	Starý et al. (2007)
Hysteroneura setariae	*Eleusine indica*, *Oryza sativa*,	Starý et al. (2007)

(Thomas)	*Rhynchaelitrum roseum*	
Macrosiphum euphorbiae (Thomas)	*Pisum sativum*, *S. melongena*	Messing & Rabasse (1995), Pike et al. (2000)
Metopolophium dirhodum (Walker)	*Triticum aestivum*, *Triticum* sp.	Messing & Rabasse (1995), Adisu et al. (2002), Starý et al. (2007), Alikhani et al. (2013)
Myzus cerasi (F.)	*Prunus avium*	Kavallieratos et al. (2008)
Myzus ornatus Laing	*Duranta repens aurea*, *Erechtites valerianaefolia*, *Erigeron bonariensis*, *Solanum americanum*, *Sonchus oleraceus*	Starý et al. (2007)
Myzus persicae (Sulzer)	*Ageratum conyzoides*, *Bidens pilosa*, *Brassica oleracea*, *Capsicum annuum*, *C. aurantium*, *Cucurbita pepo*, *Datura stramonium*, *Dendranthema grandiflora*, *Duranta repens aurea*, *Emilia sonchifolia*, *Erechtites valerianaefolia*, *Erigeron bonariensis*, *Gossypium herbaceum*, *Lepidium virginicum*, *Malva neglecta*, *Nicotiana tabacum*, *Portulaca oleracea*, *Piper nigrum*, *Prunus padus*,	Messing & Rabasse (1995) Kavallieratos et al. (2004), Ode et al. (2005), Starý et al. (2007), Rakhshani et al. (2008) Kavallieratos et al. (2010)

	P. persica, *Raphanus sativus*, *Spergula arvensis*, *Solanum americanum*	
Nasonovia ribisnigri (Mosley)	*Lactuca sativa*	Starý et al. (2007)
Pentalonia nigronervosa (Coquerel)	*Musa* spp.	Wellings et al. (1994), Stechmann et al. (1996), Rakhshani et al. (2008)
Phorodon humuli (Schrank)	*P. persica*	Rakhshani et al. (2008)
Picturaphis vignaphila Blanchard	*Desmodium* sp.	Starý et al. (2007)
Rhopalosiphum maidis (Fitch)	*Hordeum* sp., *Eleusine indica*, *Oryza sativa*, *Zea mays*	Starý et al. (2007), Ibarra-Sandate et al. (2016)
Rhopalosiphum padi (L.)	*Avena sativa*, *Hordeum vulgare*, *Triticum vulgare*, *T. aestivum*, *Zea mays*	Messing & Rabasse (1995), Adisu et al. (2002), Kavallieratos et al. (2004), Ode et al. (2005), Bilu et al. (2006), Starý et al. (2007), Rakhshani et al. (2008), Bortolotto et al. (2015)
Schizaphis graminum (Rondani)	*Avena sativa*, *H. vulagre*, *Sorghum* sp., *Triticum aestivum*	Ode et al. (2005), Bilu et al. (2006), Starý et al. (2007), Rakhshani et al. (2008), Barahoei et al. (2013)
Sitobion avenae (F.)	*H. vulgare*, *Triticum* sp., *Zea mays*	Messing & Rabasse (1995), Adisu et al. (2002), Rakhshani et al. (2008),

		Barahoei et al. (2013)
Toxoptera aurantii Boyer de Fonscolombe	*Camellia japonica*, *Coffea arabica*, *Citrus aurantium*, *C. deliciosa*, *C. limon*, *Myricaria* sp., *Schefflera arboricola*,	Kavallieratos et al. (2004) Starý et al. (2007)
Toxoptera citricidus (Kirkaldy)	*Citrus* sp., *Zanthoxylum rhoefolium*	Starý et al. (2007)
Uroleucon cordobense (Blanchard), *U. erigeronense* (Thomas)	*Erigeron bonariensis*	Starý et al. (2007)

2.6 *Aphidius* species in biological control

Several species of *Aphidius* have been introduced and released worldwide for aphid pest biological control. In New Zealand, attempts were made to introduce *Aphidius* spp. during 1970s. For example, *A. eadyi* Stary, Gonzales and Hall and *A. ervi* Haliday were introduced and released between 1977 and 1981 in New Zealand (Teulon et al. 2008). These parasitoids have given a good control of blue-green-lucerne aphid *Acyrthosiphon kondoi* (Shinjii) and pea aphid *A. pisum* (Harris) (Teulon et al. 2008). Several other deliberate introductions have also achieved good control of the target pests, for example, *A. rhopalosiphi* De Stefani Perez for rose-grain aphid *M. dirhodum*, *A. sonchi* Marshall for sow thistle aphid *Hyperomyzus lactucae* (L.), and *A. smithi* Sharma & SubbaRao for *A. kondoi* (Teulon et al. 2008). Besides the above deliberate introductions, some other species such as *A. salicis* Haliday and *A. colemani* are self-introduced, contributing significantly to management of several aphid pests (Teulon et al. 2008).

Based on its population growth rate, searching capacity and host preference, *A. colemani* is considered one of the most promising species for aphid control and widely used in biological control programmes (van Steenis 1995). As a result, this species has been introduced between countries or continents. For example, a Chilean strain was introduced to the Czech Republic between 1992 and 2000 to control *Aphis spiraephaga* Müeller on *Spiraea* ornamentals and *D. noxi* on small grains (Starý 2002). During the

early 1990s the species was introduced from Australia into Tonga to control banana aphid, *P. nigronervosa* (Wellings et al. 1994). The parasitoid was later found established on *A. gossypii* on taro, *Colocasia esculenta* (Habitus) (Wellings et al. 1994). *A. colemani* has also successfully controlled *Diuraphis noxia* (Kurdjumov) and other host aphid species in various urban ecosystems, crops and native communities (Wellings et al. 1994). The establishment of *A. colemani* has positively supplemented the native parasitoid guilds in contributing to the overall biodiversity of hymenopteran parasitoids in the cultivated landscape (Starý 2002).

2.7 Host searching behaviour

Host searching behaviour of parasitoids includes three steps: finding the suitable habitat patches with hosts, locating hosts within the habitat, and selection of profitable hosts for offspring production (van Alphen et al. 2003). Different cues are used at different steps (van Alphen et al. 2003). The chemical and visual cues from aphids and their host plants are used by *Aphidius* females to locate and recognise their host patches (Wickremasinghe & van Emden 1992; Powell et al. 1998). Volatiles released from their hosts' host plants provide long range cues for the parasitoids to locate their hosts (Lo Pinto et al. 2004). The response is strongest when the parasitoids uses a combination of aphid and plant cues (Wickremasinghe & van Emden 1992). In close proximity, visual cues play an important role in locating and recognising hosts (Powell et al. 1998). Contact semiochemicals present in the host aphid cuticle and cornicle secretion provide the final cues for host recognition and acceptance (Battaglia et al. 1993; Powell et al. 1998). Furthermore, foraging experience may also affect host searching behaviour in *A. colemani* (Storeck et al. 2000). For example, females with brief exposure to aphid-infested plants parasitise aphids more efficiently than naïve females (Grasswitz 1998). However, such behaviour is not evident when the females are exposed to uninfested plants (Grasswitz 1998).

2.8 Reproductive biology

2.8.1 Emergence

Emergence behaviour is an important component of parasitoid biology, and has significance in the quality control (Reznik & Voinovich 2016). Many insect species

show a distinct daily rhythm of adult emergence (Saunders et al. 2002). The emergence of adult parasitoids from simultaneously parasitised hosts can span for several days and different time of day but the late emerging females are smaller in size, less fecund and have shorter life (Reznik & Voinovich 2016).

Aphidius species also have circadian patterns for emergence and light plays an important role, with most emergence occurring during the first few hours of the photophase (Mackauer & Henkelman 1975; He et al. 2004). However, emergence can become asynchronous in continuous light (Mackauer & Henkelman 1975). So far, emergence behaviour in *A. colemani* is not well understood.

2.8.2 Sex maturation

Many insect species need some time to reach sexual maturity after emergence (Koukidou & Alphey 2014). Hymenopteran species are characterised by haplodiploid sex determination, i.e. haploid males develop from unfertilised eggs and diploid females from fertilised eggs (Biewer et al. 2015). Hence, mating is mandatory for production of female offspring in parasitoid wasps.

In some parasitoid species both males and females can mate immediately after emergence (Hagen 1953) but in many others the wasps may require a period after emergence for sex maturation. For example, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) males can mate 4 days after emergence when sperm are available in the seminal vesicles (Quimio & Walter 2000). Newly emerged males of *A. ervi* fail to show courtship display but newly emerged females are sexually receptive to males, indicating that males need more time than females for sex maturation (He et al. 2004). In *Aphidius matricariae* Haliday, females need around 30 minutes after emergence to become receptive while males are sexually mature and can mate immediately after emergence (Delphine 2012). Similarly, *A. sonchi* males can mate 20 to 40 min after emergence but females reject all copulatory attempts until 2 h after emergence (Liu & Carver 1985). Sex maturation period in *A. colemani* is still not well understood.

2.8.3 Mating

A number of studies have improved our understanding of mating behaviour in *Aphidius* species (McNeil & Brodeur 1995; Battaglia et al. 2002; McClure et al. 2007; He 2008). Female-produced sex pheromones are known to play an important role in regulating mating behaviours of aphidiine species (McNeil & Brodeur 1995; McClure et al. 2007). Female sex pheromones can stimulate upwind flight and elicit close range courtship behaviour by males (McNeil & Brodeur 1995; McClure et al. 2007). Upon detecting females from a distance, males start wing fanning towards females and in close vicinity antennal contact between males and females plays vital role in mate recognition and acceptance (Battaglia et al. 2002).

Aphidius females produce maximal sex pheromones in the morning (McClure et al. 2007), explaining why mating predominantly occurs in the morning. Mated females cease pheromone production to avoid multiple matings but males are active post mating and continue to mate with other virgin females (McClure et al. 2007). A preliminary study on mating behaviour of *A. colemani* shows that the behavioural sequence leading to copulation is similar to that described for other *Aphidius* species (Benelli et al. 2013). Cues that may affect mating behaviour of *A. colemani* have also been explored by Benelli et al. (2014). However, whether and how diel periodicity affects mating success are not well understood in *A. colemani*.

2.8.4 Oviposition

Understanding parasitoid oviposition patterns can help optimise mass-rearing systems and develop strategies for release of mass-reared parasitoids for biological control programmes (Idoine & Ferro 1990). Many studies indicate that lighting is critical for parasitoid oviposition (Idoine & Ferro 1990; Reznik et al. 2009; Khatri 2011; Chen et al. 2017). For example, females of *Coccophagus bartletti* Annecke and Insley (Hymenoptera: Aphelinidae), an aphelinid parasitoid of scale insects, lay most eggs in the early morning (Walter 1988). Furthermore, eggs laid during the oviposition peak may be more female-biased (van Huis & Appiah 1995; Sagarra et al. 2000). For example, most *C. bartletti* eggs laid in the early morning are female-biased (Walter 1988). It is thus suggested that the field release of mass-reared *C. bartletti* should be undertaken in the morning. In other species, such as *Anagyrus kamali* Moursi

(Hymenoptera: Encyrtidae), a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus* Green, the lifetime reproductive fitness is the greatest when reared under consistent dark conditions (Sagarra et al. 2000). Knowledge of this can facilitate laboratory mass-rearing of *A. kamali* with greatly improved energy saving (Sagarra et al. 2000).

However, in some species like *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae), an egg parasitoid of the cowpea bruchid beetle *Callosobruchus maculates* (Fabricius), eggs can be laid throughout 24-h period with most oviposition taking place during the first 12 h after emergence regardless of photoperiod (van Huis & Appiah 1995). In *Eretmocerus warrae* Naumann and Schmidt (Hymenoptera: Aphelinidae), a parasitoid of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), oviposition occurs in both photophase and scotophase (Hanan et al. 2009). *Aphidius* females usually oviposit in the photophase although they can lay some eggs in the scotophase (He et al. 2004). The oviposition behaviour of *A. colemani* is still largely unknown.

2.8.5 Sex allocation and sex ratio

In hymenopteran parasitoids such as *Aphidius* species, mated females can regulate the sex ratio of their offspring depending on the environmental conditions (King 1987; Godfray 1994). Factors such as host size, host density, and parasitoid density and age can affect sex allocation (He 2008). Parasitoids are expected to oviposit more fertilised eggs (daughters) in larger, higher quality hosts and unfertilised eggs (sons) in smaller, lower quality hosts (Charnov et al. 1981). This phenomenon occurs more often in idiobiont parasitoids than in koinobiont parasitoids (King 1989) probably because the hosts of the former cease growth upon parasitisation and their body size at parastitisation is a good indicator of fitness return (Godfray 1994). In koinobiont parasitoids such as *Aphidius* species, however, their hosts continue to grow and develop after parasitisation (Mackauer 1986). Hence, the host size at parasitisation may not be a good indicator of fitness return (Colinet et al. 2005).

Solitary parasitoids of colony forming hosts such as aphids may produce batches of offspring known as quasi-gregarious broods. The condition favours sib-mating on the

natal patch and local mate competition occurs (Assem et al. 1980). Hamilton's local mate theory predicts that mothers will produce only as many as sons needed to fertilise all the females (Hamilton 1967). Since males of solitary parasitioids mate multiple times with a number of females, mothers gain reproductive fitness by producing more female offspring. In the field, parasitoid wasps usually forage with other foundresses on the same host patch, which may influence sex allocation (Li et al. 2015). In addition, maternal age may also alter sex allocation (Gündüz & Gülel 2005).

2.9 Aphid growth and parasitoid release rate

Aphids are "r-strategist' due to their rapid reproductive rate (Rabasse & Steenis 1999). The level of host infestation varies depending on a number of factors such as season, crop varieties, presence of natural enemies, etc. (Vuong et al. 2001). For example, the population size of green peach aphid and cotton aphid on greenhouse vegetable crops is generally low early in the season but increases rapidly to high levels in the summer. During the warm seasons, populations can reach to the maximum level of 3,286 ± 535 and 110 ± 9 individuals per plant for cotton aphid and green peach aphid, respectively (Vuong et al. 2001).

Parasitoid release rate in response to pest density plays an important role in pest population suppression. A review on the impact of release rates of 35 augmentative biological control agents against 42 arthropod pests shows that most release rates fail to control pest populations (Crowder 2007). In addition, timing and method of release have significant impact on the effectiveness of biological control (Petersen et al. 1995; Neuville et al. 2016). The recommended release rate of *Aphidius* species from the commercial insectaries are based on crop area rather than pest density, ranging from 0.15 to 5 mummies/m^2 (Applied Bionomics 2017; Bioforce 2017; Biopest 2017; Evergreen Growers Supply 2017). For example, release of *A. colemani* at the rate of 2 adults or females/m^2 3 times a season for the control of *A. gossypii* in glasshouses can maintain the pest population at < 0.6 aphids/leaf compared to very high density (653.2 aphids/leaf) on the untreated crops (Moon et al. 2011). Similarly, release of six mixed parasitoid species at a ratio of 1.2 mummies/m^2 once every two weeks can give a good

control of aphid populations on the sweet peppers in glass tunnels (Dassonville et al. 2012).

2.10 Effect of parasitism on aphid reproduction

Aphid population is age-structured, consisting of individuals of different age and morph (Sequeira & Mackauer 1988). Aphid age at the time of parasitisation can significantly affect its reproduction and population growth (Mackauer & Kambhampati 1984; Hågvar & Hofsvang 1986; Islam et al. 1997; He et al. 2003). It is widely reported that aphids attacked at their early stages fail to reach reproductive maturity whereas those parasitised at late stages can still produce variable number of offspring although contributing little to the population growth (Mackauer & Kambhampati 1984; Sequeira & Mackauer 1988; Tang & Yokomi 1996; Islam et al. 1997; Tsai & Wang 2002; Mitsunaga et al. 2016). A number of studies on the impact of parasitism in the *Aphidius*-aphid systems have been carried out (e.g., Liu & Hughes 1984; He et al. 2003; He et al. 2005b). However, little is known about the *Aphidius colemani*-*Myzus persicae* system so far. Understanding how parasitism by *A. colemani* affects reproduction of *M. persicae* is critical to estimating the efficacy of the parasitoid in biological control of green peach aphid (Khatri et al. 2017).

2.11 Factors affecting parasitoid reproductive fitness

2.11.1 Host size and host preference

In the field, aphidiine females can encounter aphids of different sizes/stages in a host patch. They may prefer certain instars to others for parasitisation to gain optimal fitness (Sequeira & Mackauer 1994; Colinet et al. 2005; Henry et al. 2005; He et al. 2005a). However, literature shows that the fitness-related traits of aphidiine parasitoids do not increase linearly with the host size or age in which they develop because host quality is optimal at intermediate host ages, and hence the female wasps prefer to parasitise these hosts (Sequeira & Mackauer 1994; Colinet et al. 2005).

Host size preference by *A. colemani* is not univocally supported. For example, it prefers to parasitise young instars of *M. persicae* and *A. gossypii* (Goh et al. 2001;

Martinou & Wright 2007), particularly the first and second instars (Lykouressis et al. 2009). However, in the soybean aphid, *A. glycines*, *A. colemani* attacks larger hosts more frequently (2003). Although larger female wasps are generally capable of producing a higher total number of mummies than small females (Lykouressis et al. 2009), knowledge on host size or age preference behaviour of *A. colemani* and its fitness consequences is lacking.

2.11.2 Parasitoid body size and fitness

Knowledge of the relationship between body size and fitness is essential for the understanding of parasitoid evolutionary ecology (Rivero & West 2002). Body size is an important determinant of parasitoid fitness because it is related with many fitness components. For example, larger females may have longer longevity (Visser 1994; Ueno 1999; He & Wang 2006), better male attraction through sex pheromones (Cloutier et al. 2000), higher dispersal and host searching ability (Visser 1994; Ellers et al. 1998; He & Wang 2006), greater fecundity (Bai et al. 1992; Visser 1994; He & Wang 2006) and oviposition success (Ueno 1999), and higher innate capacity for population increase (Cloutier et al. 2000). Larger males may also live longer (Ode et al. 1996; He & Wang 2006) and gain advantage in competition for mate access and thus have higher insemination opportunity (Ode et al. 1996; He & Wang 2006). In *A. colemani*, larger females have more eggs in their ovarioles (Sampaio et al. 2008) and are capable of producing a higher total number of offspring than small ones (Lykouressis et al. 2009). However, the size-fitness relationships are still not well understood for *A. colemani*.

Parasitoid body size is also known to affect the sex ratio of offspring. For example, in *Anaphes nitens* Girault (Hymenoptera: Mymaridae), larger females produce more female offspring, compared to the smaller ones (Serena Santolamazza-Carbone 2007). Similarly, larger males of *A. ervi* result in the production of a female-biased population for longer periods than smaller males (He & Wang 2006). However, larger body size may not always be advantageous to parasitoids. For example, the fitness gain from being large may be subject to a cost, such as prolonged developmental time or increased juvenile mortality (Sequeira & Mackauer 1992; Harvey et al. 1994). Therefore, some endoparasitoids may favour rapid development over size while others favour size over

rapid development (Harvey & Strand 2002). Further studies are necessary to examine how parents' body size affects offspring sex ratio in *A. colemani*.

2.11.3 Host and parasitoid density

Host and parasitoid density forms an integral component of population dynamic models (Fernández-arhex & Corley 2003; Uçkan et al. 2004; Lopes et al. 2009) and affects the functional response (relationship between host density and the number of hosts parasitised) and demographic response (changes in the parasitoids' reproductive output) of insect parasitoids (Mills & Lacan 2004; Luo et al. 2014). Functional response describes the per capita response of a parasitoid to changing host density (Holling 1959). Determination of the type of functional response is essential to estimating the efficiency of biological control agents. Practitioners of inundative biological control may use this information to determine the number of biological control agents to be released in order to bring about an immediate reduction in pest numbers (Mills & Lacan 2004; Luo et al. 2014).

In the field, foraging parasitoids often encounter other parasitoids, either from the same or different species. Hence, there may be competitive interactions among foraging parasitoids. This may cause parasitoids to cease host searching and leave the area, triggering reduction in per capita searching and attacking efficiency, a phenomenon known as mutual interference (Cronin & Strong 1993; Elliott 2003; Luo et al. 2014). For example, mutual interference in parasitoid *Anagyrus* spp. may result in lower parasitism rate and parasitoid reproductive output (Chong & Oetting 2006).

Host and parasitoid density also affects sex allocation by parasitoids. For example, in *Gonatocerus* spp. (Hymenoptera: Mymaridae), an egg parasitoid of glassy-winged sharpshooter *Homalodisca coagulate* (Germar), if a female forages singly, it produces offspring with a strongly female-biased sex ratio but if 3 females forage together, the offspring sex ratio becomes highly male-biased (Irvin & Hoddle 2006). Therefore, density of both hosts and parasitoids should be included in analysis to reveal the functional response of parasitoids (Chong & Oetting 2006).

Functional response of *A. colemani* to host density has been studied by several authors (Jones et al. 2003; Zamani et al. 2007; Byeon et al. 2011). Some suggest that *A. colemani* has a type III functional response (Jones et al. 2003) but others predict that it has a type II functional response (Sampaio et al. 2001; Zamani et al. 2006). This may be caused by variations of methodologies adopted (Byeon et al. 2011). Effects of parasitoid density and maternal age on functional response behaviour are not known for this species.

Chapter Three

General Methodology

3.1 Introduction

This chapter describes the general methodology applied throughout this research. The materials, procedures, environmental conditions and definitions detailed in this section are used throughout the thesis.

3.2 General materials and methods

3.2.1 Insects

A breeding colony of *A. colemani* and *M. persicae* started from mummies and live aphids feeding on tobacco plants, supplied by BioForce Ltd, Auckland, New Zealand in May 2012. Aphids were reared on the Chinese cabbage plants *Brassica rapa* var *pekinensis* (Michihili Jade Pagoda, F1 Hybrid, Egmont Seed Company, New Plymouth, NZ) for five generations before experiments.

3.2.2 Host plant

Chinese cabbage plants were grown in the glasshouse before bringing them for experimental use. Seedlings were raised in the corrugated plastic trays filled with potting mix in a glasshouse (Figure 3.1) in the Plant Growth Unit, Massey University, Palmerston North. The potting mix consisted of 150 gm of short term fertiliser mix and 150 gm Dolomite in 100 L soil). About 20 to 25 seedlings of Chinese cabbage were raised per tray. Plants were watered daily in summer and every two or four days in other seasons, and used for experiments when they were 6 to 8 weeks old.

Figure 3.1 Chinese cabbage plants grown in a glasshouse.

3.2.3 Colony of *M. persicae*

Myzus persicae colony (n ≈ 2,000) was maintained in a wooden cage (65.0 cm in length × 44.0 cm in width × 42.0 cm in height) with fine metal screen (aperture = 0.25 mm) on the back and both sides and Perspex on the top and front and wooden base on the bottom (Figure 3.2) in a walk-in climate room in the Entomology and IPM Laboratory. To maintain the colony, 10 6- to 8-week-old potted Chinese cabbage plants (Figure 3.2) were supplied to aphids every two weeks.

To obtain the aphids of desired age for experiments, 50 aphid adults were placed on a leaf cut, enclosed within a plastic cylinder (8.0 cm in diameter × 20.0 cm in height) (Figure 3.3) for 4 hours, after which time the adults were transferred to another fresh leaf cut using a fine paint brush, and the date of nymph birth was labelled.

The experimental cylinder consisted of two identical transparent containers (8.0 cm in diameter × 10.0 cm in height) (Figure 3.3). The bottom one was filled with water and covered by a lid with a small hole (0.5 cm in diameter) at the centre, through which a fresh cabbage leaf cut was inserted so that the lower petiole was immersed into the

water. The space between the petiole and hole was covered with cotton wool. The top container had two opposite holes (3 cm in diameter) on the wall and a hole (3 cm in diameter) on the top covered with metal meshes (aperture = 0.25 mm) for ventilation, and was then placed on the lid of the bottom container. A piece of parafilm (30.0 cm in length × 1.0 cm in width; Parafilm®, USA) was used to hold those two containers together.

Figure 3.2 Wooden cage used for insect rearing.

Figure 3.3 Experimental cylinder.

3.2.4 Colony of *A. colemani*

To establish the breeding colony of *A. colemani*, 150 mummies supplied by BioForce Ltd were individually placed in 2-ml micro-centrifuge tubes (EzMicro™, Bio-Rad Laboratories, USA) until emergence (Figure 3.4). The tubes were perforated with a fine needle to allow ventilation. Newly emerged parasitoids were released into a wooden cage (Figure 3.2) maintaining 10 plants each infested with about 100 second instar nymphs in another walk-in climate room. Most adult parasitoids emerge within 15 days after oviposition, thus the 'old' plants were replaced with 10 plants each infested by about 100 second instar nymphs every two weeks. This procedure was repeated to maintain the parasitoid colony.

Parasitoid adults were provided with a 10% honey solution filled in a plastic tube (7.5 cm in length × 1.0 cm in diameter) fitted with cotton wool wick (3.5 cm in length × 1 cm in diameter). The opposite end of the plastic tube was hung in the inner top surface of the cage by using adhesive. Honey solution in the plastic tube was changed every two days.

Figure 3.4 Micro-centrifuge tubes.

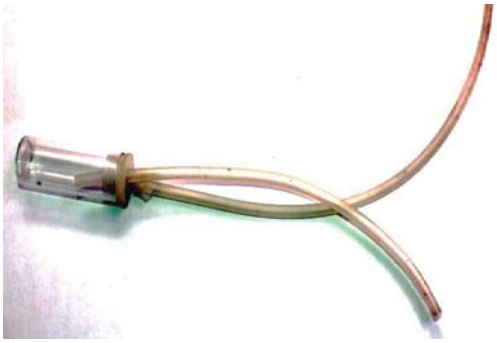

Figure 3.5 Aspirator.

3.2.5 Experimental parasitoids

Parasitoids used for experiments were obtained by allowing 2-d-old *M. persicae* nymphs to be parasitised. Using an aspirator (Figure 3.5), a naïve 1-d-old mated female parasitoid was introduced into a Petri dish (5.5 cm in diameter × 1.2 cm in height) which contained 20 2-d-old *M. persicae* nymphs feeding on a leaf disc (3 cm in diameter) placed upside down over a moistened filter paper (Figure 3.6). Twenty-four hours later, the parastisoid was removed using the aspirator. The parasitoid-exposed aphids were allowed to settle on the same leaf disc for two days. This helped reduce the possibility of mortality of aphids due to frequent handling. Thereafter, the aphids were transferred to a fresh leaf cut maintained in a plastic container (6.5 cm in diameter × 17.0 cm in height) (Figure 3.7) and maintained until mummification (Figure 3.8). Mummies were carefully removed by a fine needle, individually transferred into the micro-centrifuge tubes using a fine paint brush, and maintained until emergence (Figure 3.9). Upon emergence, adults were sexed based on the pointed abdomen of females and round abdomen of males (Figure 2.1). Newly emerged adults were supplied with 10% honey solution soaked in a cotton wool ball (5 mm in diameter) placed in the inner cavity of the lid (Figures 3.4 and 3.9).

To obtain mated females for experiments, newly emerged males and females (< 12 h) were paired in the 2-ml micro-centrifuge tubes (Figure 3.10). Female parasitoids that mated in 20 minutes were used for experiments.

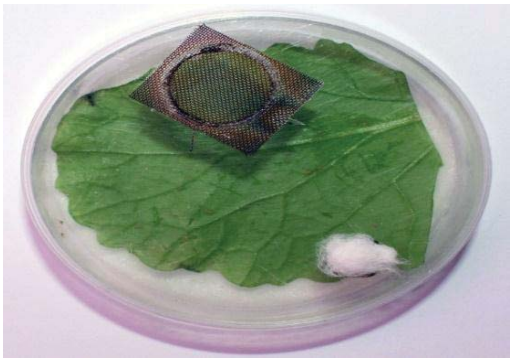

Figure 3.6 *A. colemani* oviposition.

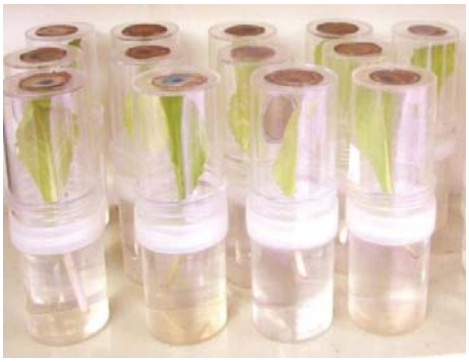

Figure 3.7 Maintenance of parasitoid-exposed aphids.

Figure 3.8 *A. colemani* mummification.

Figure 3.9 Mummies in tubes.

Figure 3.10 Pairing parasitoids in a tube.

3.2.6 Environmental conditions

The colonies were maintained and experiments were carried out in climate rooms at 22 ± 1°C and RH 50-60% with a photoperiod of 16:8 hour (L:D). Lighting was provided by high frequency broad-spectrum biolux tubes (36 Watt, Osram, Germany).

3.2.7 Dissection and measurement

Dissection of parasitoids and aphids was done under a stereomicroscope (Leica MZ12, Germany) (Figure 3.11). Aphids were dissected to determine the fecundity, and parasitism and superparasitism rate. To determine the body size of parasitoids, the body length, head width and hind tibia length were measured using an image system (CellSens GS-ST-V1.7, Olympus, Germany) connected to a digital camera (Olympus SC30, Germany) attached to the above stereomicroscope (Figure 3.11).

Figure 3.11 Stereomicroscope and image system.

Figure 3.12 Behavioural recording.

3.2.8 Behavioural recording

A digital camcorder (Sony Handycam DCR-SR85, Japan) was used to record the behaviour of parasitoid oviposition and mating (Figure 3.12). Behavioural events were quantified by transferring the videos to a desktop computer and re-playing those videos using the VLC media player installed in the computer.

3.3 Key definitions

The key definitions applied in this study are listed as below:

Fecundity: the total number of eggs laid by a female parasitoid during her lifetime;

Fertility rate: the proportion of female offspring produced by a female parasitoid;

Parasitism: a female parasitoid lays egg(s) in an aphid regardless of egg number;

Superparasitism: a female parasitoid lays more than one egg in an aphid.

3.4 Statistical analysis

All data were analysed using SAS software (SAS 9.4, SAS Institute Inc., NC, USA). Rejection level was set at $\alpha < 0.05$. Values were reported as means ± SE.

Chapter Four

General Biology of *Aphidius colemani*

4.1 Introduction

Organisms have evolved to adapt to the cycling environment and developed circadian rhythms. Circadian rhythms help organisms save energy, reduce competition for food, define habitat niche, regulate physiological and biochemical machinery, and/or coordinate mating behaviour (Nation 2016). For example, insects synchronise their daily physiological and behavioural processes with the circadian cycles (Sheeba et al. 1999; Lear & Allada 2001; Vaze & Sharma 2013; Vaze et al. 2014), including emergence (He et al. 2004; Greenberg et al. 2006; Karpova 2006; Xu et al. 2008), mating and oviposition (Sakai & Ishida 2001; Wang & Shi 2004; Rymer et al. 2007; Xu et al. 2008).

Circadian activity patterns have been studied in some parasitic hymenopterans (Vogt & Nechols 1991; Armstrong et al. 1996; Couch et al. 1997; He et al. 2004). For example, oviposition of many parasitic wasps occurs in the morning (Vogt & Nechols 1991; Quicke 1997) and that of others takes place according to circadian rhythms of their hosts (Armstrong et al. 1996; Couch et al. 1997). Knowledge of parasitoids' emergence, mating and oviposition patterns is vital to the understanding of the ecology and evolution of their reproductive strategies, which in turn contributes to the development and implementation of biological control programmes (He 2008). However, so far little is known about the circadian rhythms of *A. colemani*, making it difficult to improve its efficiency in biological control of aphids.

After emergence, many insect species need some time to become sexually mature, i.e. capable of reproducing (Ramadan et al. 1991; Perezlachaud & Campan 1994; Lima & Howse 1997; Baker et al. 2003). In haplodiploid parasitoids, such as *Aphidius* species, mating is essential to producing female progeny (Quicke 1997). Sexually mature females usually produce sex pheromones to attract males from a long distance and to evoke male courtship within a short distance (Marchand & McNeil 2000; McClure et al. 2007). However, sex maturation period of males and females in *A.*

colemnai is still poorly understood, information of which is important for its laboratory handling, mass-rearing and field release.

A number of factors such as parasitoid age (McClure et al. 2007), food access (Benelli et al. 2017) and mating space (Kimani & Overholt 1995) may affect mating success in parasitoids. For example, female sex pheromone production and male responsiveness to female sex pheromones may decline with parasitoid age (McClure et al. 2007). Lack of food at the adult stage has negative effects on adult longevity (Charles & Paine 2016), male courting, female responsiveness, and mating duration (Scharf 2016). Adams & Morse (2014) report that the space in which mating occurs can strongly influence the mating behaviour of both sexes. So far, whether and how parasitoid age, adult food and mating space affect mating behaviour of *A. colemani* are unknown, knowledge of which is also useful for its laboratory handling, mass-rearing and field release.

In this chapter I carried out a series of experiments to provide new information on *A. colemani*, including circadian rhythms of emergence, mating and oviposition, sex maturation period, general mating behaviour and factors that affected mating success. Knowledge generated here is essential for the design and conduction of experiments in the following chapters and may contribute to the development of efficient techniques for mass-rearing and release of the parasitoid in aphid biological control programmes.

4.2 Materials and methods

4.2.1 Circadian rhythms of emergence, mating and oviposition

Insects used for experiments were obtained as described in Sections 3.2.3-3.2.5. Two climate rooms were set up to study the circadian rhythms of *A. colemani*. The photophase in one room was set from 0800~2400 hours (normal-light regime) and in the other room the scotophase was between 0900~1700 hours (reverse-light regime). A red light (36 Watt, Osram, Germany) was used for behavioural observation during the scotophase in the reverse-light room. Other environmental conditions were the same for these rooms (Section 3.2.6).

To determine whether most *A. colemani* adults emerged during the photophase or scotophase, I allowed 2-d-old nymphs of *M. persicae* to be parasitised within two hours after lights on and reared them in the normal-light room for mummification. I then collected 515 pupae, and individually transferred them into the 2-ml micro-centrifuge tubes which were maintained in the same room. Emergence of adult parasitoids was monitored twice a day during the photophase (immediately after lights on and immediately before lights off, respectively). The adults recorded immediately after lights on were considered to have emerged during the scotophase and those recorded just before lights off during the photophase. Newly emerged adults were sexed. Developmental time from eggs to adults was calculated for both sexes. Because the above experiment shows that most adults emerged during the photophase, I then observed 375 pupae for hourly emergence rhythm. I recorded the number of adults that merged once every hour for the entire photophase and sexed newly emerged adults in each hour.

I set up a total of 159 and 80 mating pairs to determine circadian mating rhythm in the photophase and scotophase, respectively. All parasitoids used for the experiment were 1 d old and virgin without any oviposition experience. Parasitoids were paired in the 2-ml micro-centrifuge tubes for two hours every two hours (Figure 3.10), resulting in 8 and 4 mating bouts during the photophase and scotophase, respectively. They were fed with 10% honey solution soaked in a cotton wool ball and placed in the inner cavity of the tube lid (Figure 3.10). I recorded the premounting, mounting and mating duration using a stop watch. The percentage of males that courted as well as successfully mated were calculated for each mating bout.

I performed 16 and 20 replicates to investigate oviposition rhythm of *A. colemani* during the photophase and scotophase, respectively. For each replicate, I released a naïve, 1-d-old mated female into a Petri dish (3.5 cm in diameter × 1.0 cm in height) containing 20 2-d-old *M. persicae* nymphs feeding on a leaf disc and allowed her to stay for 2 h (one oviposition bout), after which time, I transferred the female to another Petri dish containing the same number of *M. persicae* nymphs for 2 h, etc., until the end of the photophase or scotophase. The parasitoid was provided with 10% honey solution soaked in a cotton wool ball inserted through the opening made on the upper lid of the Petri dish. The parasitoid-exposed aphids from each oviposition bout were kept on the

same leaf disc for two days and then transferred to a leaf cut maintained in a plastic cylinder (Figure 3.3). I randomly selected 10 aphids from each oviposition bout and dissected them under the stereomicroscope four days after parasitisation to determine parasitism and superparasitism. Superparasitism was counted when more than one larva was found in an aphid (Bueno et al. 1993). The remaining aphids were reared on the same leaf cut until mummification. Mummies were maintained individually in 2-ml micro-centrifuge tubes until emergence. Newly emerged adults were sexed.

4.2.2 Sex maturation period of both sexes

I determined sex maturation period of sexes in *A. colemani* by pairing (1) a 24-h-old female with a 0- (newly emerged), 2-, 4- or 6-h-old male, with 20 to 22 individuals tested for each male age (Figure 4.4), and (2) a 24-h-old male with a 0-, 2-, 4- or 6-h-old female, with 20 to 24 individuals tested for each female age (Figure 4.5). For each replicate, insects were paired in a clear 2-ml micro-centrifuge tube with 10% honey solution soaked in cotton wool ball serving as food. I recorded durations of precourting, courting, mounting and mating for 10 minutes for each pair using a stop watch as the background information for the next experiment (see Section 4.2.3).

4.2.3 General mating behaviour

Mating behaviour was observed and recorded using 1-d-old parasitoids. I randomly selected 60 virgin males and 60 virgin females and set up 60 pairs, each in a 2-ml centrifuge tube. Because mating usually occurs within 10 minutes after pairing, I recorded mating behaviour for 10 minutes using a digital camcorder (Figure 3.12). The following behavioural parameters were quantified:

(1) Precourting duration: period required by a male to start wing fanning after pairing;
(2) Courtship: wing fanning with mounting attempt by a male;
(3) Courting duration: period between initiation of male wing fanning and mating;
(4) Mounting duration: period between start of mounting and genitalia connection;
(5) Antennation duration: period of antennation on a female by a male during mounting and copulation;

(6) Mating duration: period between genitalia connection and disconnection.

4.2.4 Factors affecting mating behaviour

To determine whether and how parasitoid age had any effect on mating success, I paired parasitoids of different ages (1, 3, and 5 d old), each in a 2-ml micro-centrifuge tube, with seven different combination treatments: (1) 1-d-old male × 1-d-old female (n = 23), (2) 1-d-old male × 3-d-old female (n = 20), (3) 1-d-old male × 5-d-old female (n = 21), (4) 1-d-old female × 3-d-old male (n = 20), (5) 1-d-old female × 5-d-old male (n = 20), (6) 3-d-old male × 3-d-old female (n = 22), and (7) 5-d-old male × 5-d-old female (n = 22). All parasitoids used for experiment were individually maintained, virgin and naïve. Observation time and method were described in Section 4.2.3 but no food was provided for parasitoids during experiment.

I carried out an experiment to examine whether and how food availability during the adult stage affected mating behaviour. Parasitoids were either fed or not fed with 10% honey solution for 24 hours before paired. I set up three treatments: (1) fed male × unfed female (n = 23), (2) fed female × unfed male (n = 20), and (3) both sexes fed (n = 23). The parasitoids were paired in the 2-ml micro-centrifuge tubes without food supply during experiment. Mating behaviour was recorded as described Section 4.2.3.

In addition, I examined whether and how pairing arena affected mating success. For this experiment, I tested three types of containers with different sizes: (1) glass vials [small (3.5 cm in length × 1.0 cm in diameter, n = 23), medium (5.0 cm in length ×1.8 cm in diameter, n = 24) and large (7.0 cm in length × 2.4 cm in diameter, n = 26)]; (2) Petri dishes [small (3.5 cm in diameter × 1.1 cm in height, n = 23), medium (5.5 cm in diameter ×1.2 cm in height, n = 25) and large (9.0 cm in diameter × 1.5 cm in height, n = 25)], and (3) cylinders [small (6.0 cm in height × 4.5 cm in diameter, n = 20), medium (8.5 cm in height × 6.5 cm in diameter, n = 20) and large (10.5 cm in height ×8.5 cm in diameter, n = 25)]. All parasitoids used in the experiment were 1 d old, virgin without any oviposition experience. Mating behaviours were recorded as Section 4.2.3. Parasitoids were fed with 10% honey solution before experiment but not during experiment.

4.2.5 Statistical analysis

A goodness-of-fit test (Shapiro-Wilk test) was used to test the distribution of data before mean comparisons. Data on the developmental duration from egg to adult, hourly emergence during the photophase, and superparasitism rate were not normally distributed and thus analysed using a non-parametric ANOVA followed by a Bonferroni (Dunn) tests (ANOVA, Glm Procedure). Data on the number of adult emergence during the photophase and scotophase were subject to Chi-square test. A likelihood rate test (LR, Genmod Procedure) was used to compare the difference in the proportion of male courting and mating success between treatments.

Data on precourting, courting, mounting and mating duration were subject to natural logarithmic transformation and the proportion of female progeny produced to arcsine square-root transformation, and data on the number of hosts parasitised were normally distributed. These transformed and normal data were analysed by ANOVA, followed by a Tukey's studentised range test (ANOVA, Glm Procedure).

A quadratic linear regression (Glm Procedure) was applied to determine the relationships of the premounting, mounting and mating duration over parasitoid age; these data were natural logarithmic [ln(x)] transformed before regression. The regression model included the linear and quadratic parameters of age of both sexes and their interactions. However, only the significant parameter(s) were retained in the final model.

4.3 Results

4.3.1 Circadian rhythms of emergence, mating and oviposition

My results indicate that significantly more adults emerged in the photophase than in the scotophase (280 versus 6 and 192 versus 5 for males and females, respectively) (Chi-square test: x_1^2 = 262.50 and 177.51 for males and females, respectively; $P < 0.0001$). Males developed significantly faster than did females (mean ± SE = 14.55 ± 0.16 and 15.40 ± 0.15 days for males and females, respectively) (Non-parametric ANOVA: $F_{1,481}$ = 18.06, $P < 0.0001$; Figure 4.1A). On hourly basis during the photophase, adult emergence peaked during first two hours after lights on with males emerging significantly earlier than females (mean ± SE = 2.12 ± 0.13 and 3.48 ± 0.28 h

after lights on for males and females, respectively) (Non-parametric ANOVA: $F_{1,349}$ = 21.93, P < 0.0001) (Figure 4.1B).

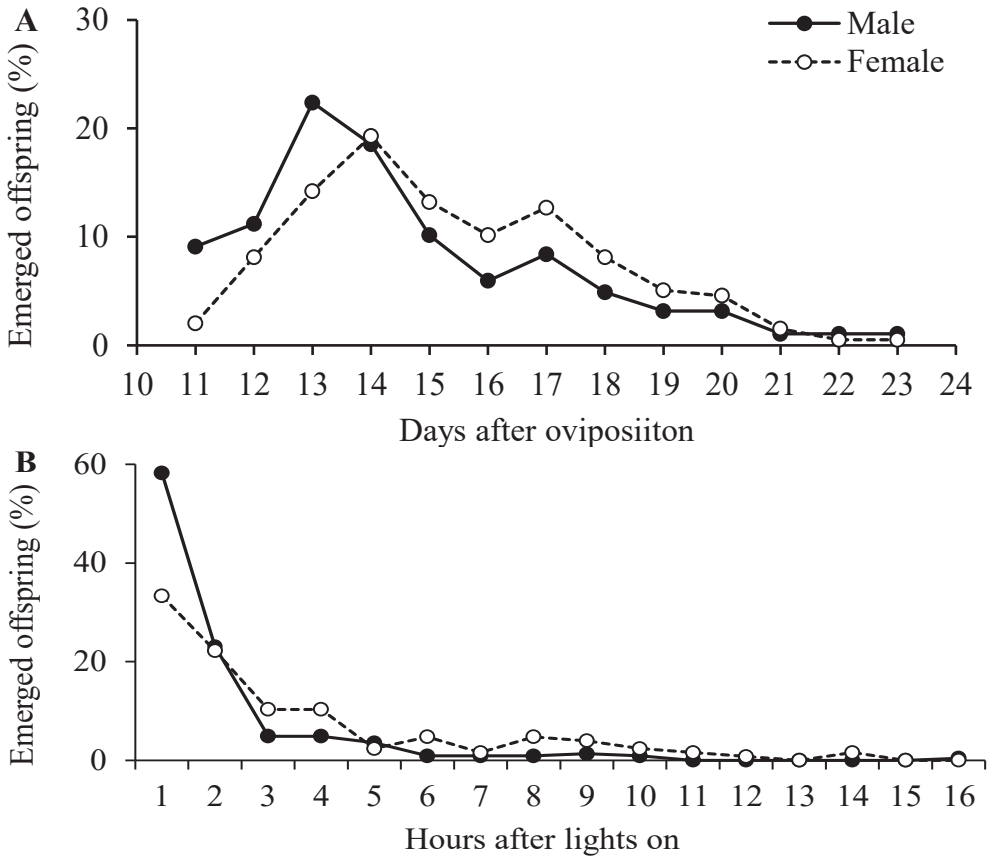

Figure 4.1 Emergence rhythms of *A. colemani*: (**A**) daily emergence, and (**B**) hourly emergence.

More than 80% of males courted throughout the photophase with no significant difference between any 2-h observations, however, male courting significantly decreased during the first four hours of the scotophase and then significantly increased (LR: x_{11}^2 = 7161, P < 0.0001) (Figure 4.2A). Mating success followed the trend similar to courting during the photophase (LR: x_{11}^2 = 66.96, P < 0.0001) but not during the scotophase (Figure 4.2B).

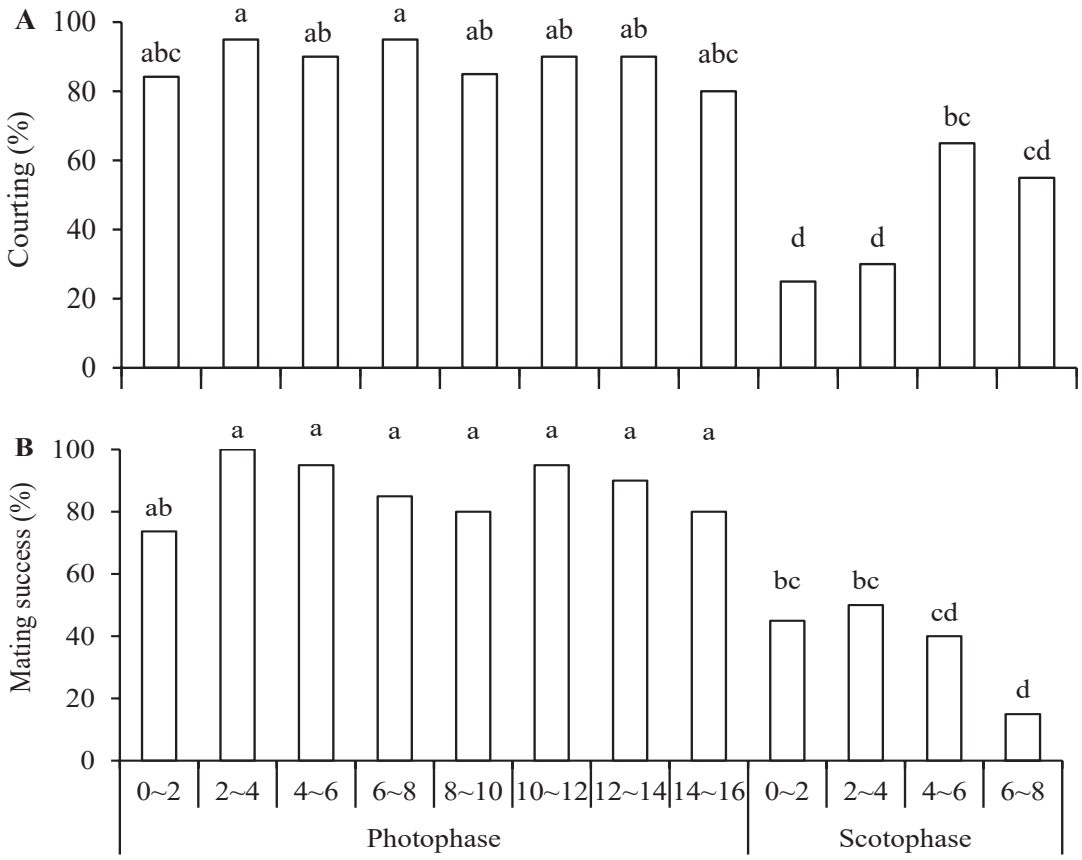

Figure 4.2 Proportion of male courting (**A**) and mating success of *A. colemani* (**B**) during the 24-hour cycle. Bars with the same letters are not significantly different ($P > 0.05$).

There was no significant difference in premounting and mounting duration between any bihourly bouts throughout the 24-h cycle (Table 4.1). Mating duration was similar in most bihourly bouts but was significantly longer during the last two bihourly bouts of the scotophase and the first bihourly bout of the photophase (Table 4.1).

Table 4.1 Premounting, mounting and mating duration (seconds) of *A. colemani* during bihourly bouts after lights on in photophase and lights off in scotophase.

Light condition	Premounting	Mounting	Mating
Photophase			
0~2	80.36 ± 27.02 a	9.50 ± 1.25 a	44.79 ± 2.28 a
2~4	110.65 ± 21.42 a	11.20 ± 1.20 a	38.05 ± 1.23 ab
4~6	127.26 ± 31.58 a	10.68 ± 1.25 a	39.42 ± 1.47 ab
6~8	107.77 ± 38.62 a	13.53 ± 2.03 a	35.82 ± 0.92 b
8~10	136.56 ± 37.39 a	10.69 ± 1.58 a	34.94 ± 1.17 b
10~12	108.16 ± 24.44 a	14.89 ± 2.13 a	36.89 ± 1.29 b
12~14	140.61 ± 39.18 a	14.94 ± 2.43 a	40.61 ± 1.45 ab
14~16	111.13 ± 40.69 a	13.25 ± 2.24 a	38.38 ± 0.97 ab
Scotophase			
0~2	167.22 ± 43.87 a	12.78 ± 2.99 a	36.78 ± 1.95 ab
2~4	77.70 ± 11.38 a	14.10 ± 2.05 a	42.90 ± 2.36 ab
4~6	211.86 ± 68.07 a	11.14 ± 2.16 a	54.88 ± 6.70 a
6~8	42.67 ± 13.68 a	13.33 ± 4.06 a	51.00 ± 7.02 a
$F_{(df)}$	$1.07_{(11,154)}$	$0.83_{(11,156)}$	$4.12_{(11,157)}$
P	0.3916	0.6097	< 0.0001

Means (± SE) with the same letters in columns are not significantly different (P > 0.05).

As Figure 4.3 shows, oviposition occurred throughout the 24-h cycle. However, the mean number of hosts parasitised per oviposition bout was significantly greater in the photophase (mean ± SE = 9.36 ± 1.08) than in the scotophase (mean ± SE = 3.94 ± 0.31) (ANOVA: $F_{1,206} = 49.60$, $P < 0.0001$). During the photophase, the number of hosts parasitised was significantly higher in the first few oviposition bouts (ANOVA: $F_{7,120} = 6.40$, $P < 0.0001$; Figure 4.3). In the scotophase the number of hosts parasitised was consistently low with no significant difference between oviposition bouts (ANOVA: $F_{3,76} = 0.28$, $P = 0.8390$; Figure 4.3).

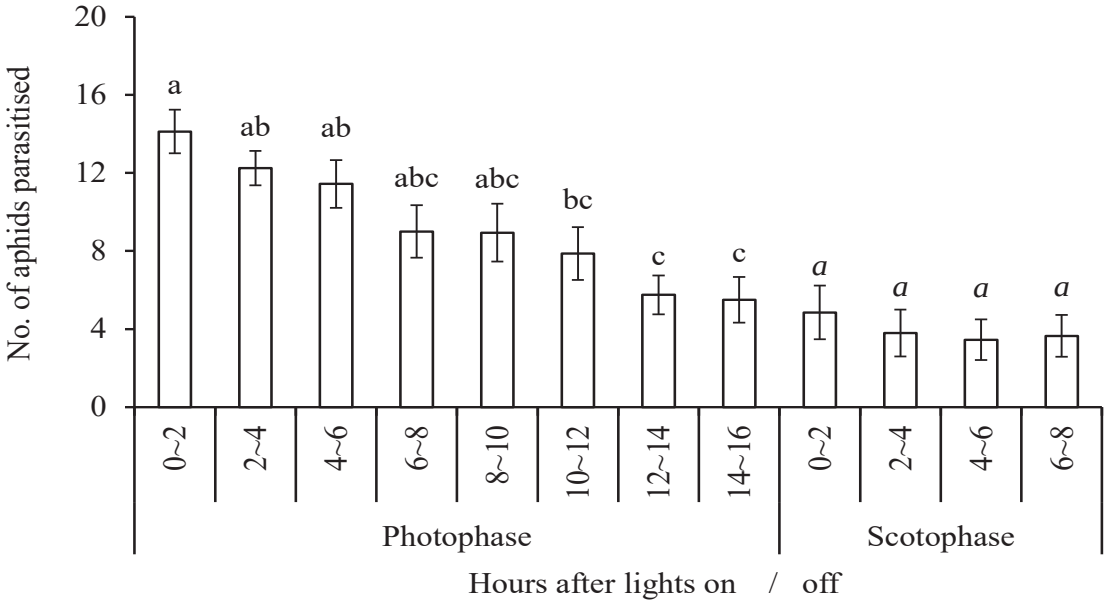

Figure 4.3 Number of hosts parasitised by *A. colemani* in the photophase and scotophase. Columns with the same letters in the photophase or scotophase are not significantly different (P > 0.05).

The possibility of hosts superparasitised by a parasitoid per oviposition bout was low with no significant difference between the photophase (mean ± SE = 3.82 ± 0.66%) and scotophase (mean ± SE = 3.75 ± 1.06%) (Non-parametric ANOVA: $F_{1,161} = 0.06$, P = 0.8139).

The proportion of female progeny produced was not significantly different between oviposition bouts during the photophase (between 50 and 70%) and the scotophase (between 40 and 70%) (ANOVA: $F_{7,103} = 0.63$, P = 0.7281 for photophase; $F_{3,33} = 2.47$, P = 0.0789 for scotophase). The overall proportion of female progeny was also not significantly different between the photophase and scotophase (mean ± SE = 59.26 ± 2.54% and 53.76 ± 5.30%, respectively) (ANOVA: $F_{1,164} = 1.09$, P = 0.2979).

4.3.2 Sex maturation period of both sexes

About 60% of newly emerged males and ≥ 90% of 4- and 6-h-old males courted and mated (LR: x_3^2 = 9.84 and 8.73 for courting and mating, respectively, P < 0.05) (Figure 4.4). When paired with 1-d-old males, > 80% females, regardless of their age,

elicited male's courting (LR: x_3^2 = 3.32, P = 0.3453) (Figure 4.5A). However, mating success was significantly lower in newly emerged females than in 4- and 6-h-old females (LR: x_3^2 = 7.80, P = 0.05) (Figure 4.5B).

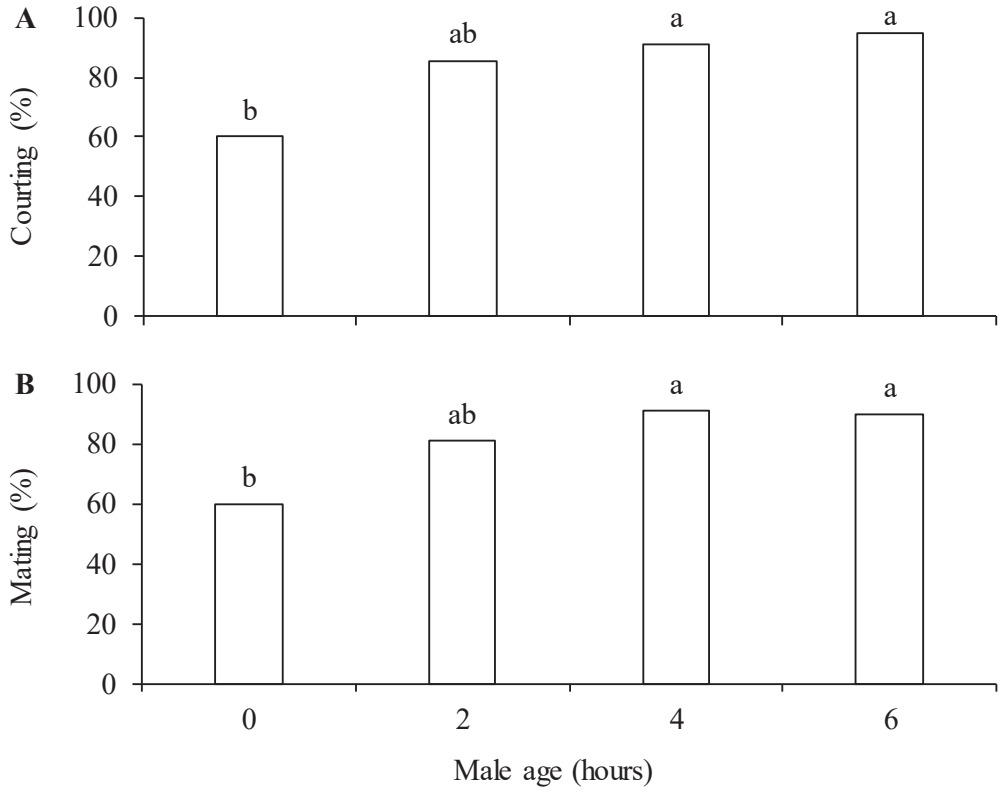

Figure 4.4 Proportion of males of different age courted (**A**) and mated (**B**) with 1-d-old females in *A. colemani*. Bars with the same letters are not significantly different (P > 0.05).

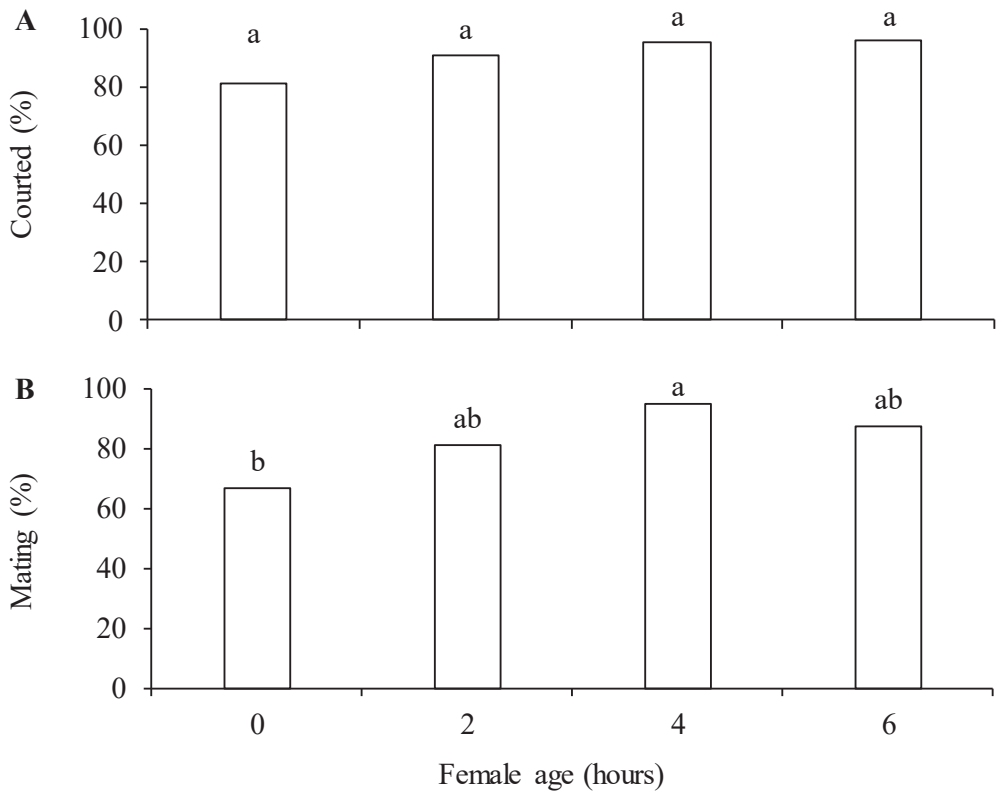

Figure 4.5 Proportion of females of different age courted by (**A**) and mated with (**B**) 1-d-old males in *A. colemani*. Bars with the same letters are not significantly different (P > 0.05).

Although newly emerged and young males (0 and 2 hours old) took relatively longer time to respond to females compared to older ones (4 and 6 hours old), no significant difference in precourting duration was found between males of different age ($F_{3,54}$ = 2.07, P = 0.1151) (Figure 4.6A). Similarly, there was no significant difference in courting and mounting durations between males of different age ($F_{3,55}$ = 1.95 and 1.46 for courting and mounting, respectively; P > 0.05) (Figure 4.6B and 4.6C). However, mating duration of newly emerged males was significantly longer than that of 4- and 6-h-old males ($F_{3,63}$ = 3.64, P = 0.0174) (Figure 4.6D).

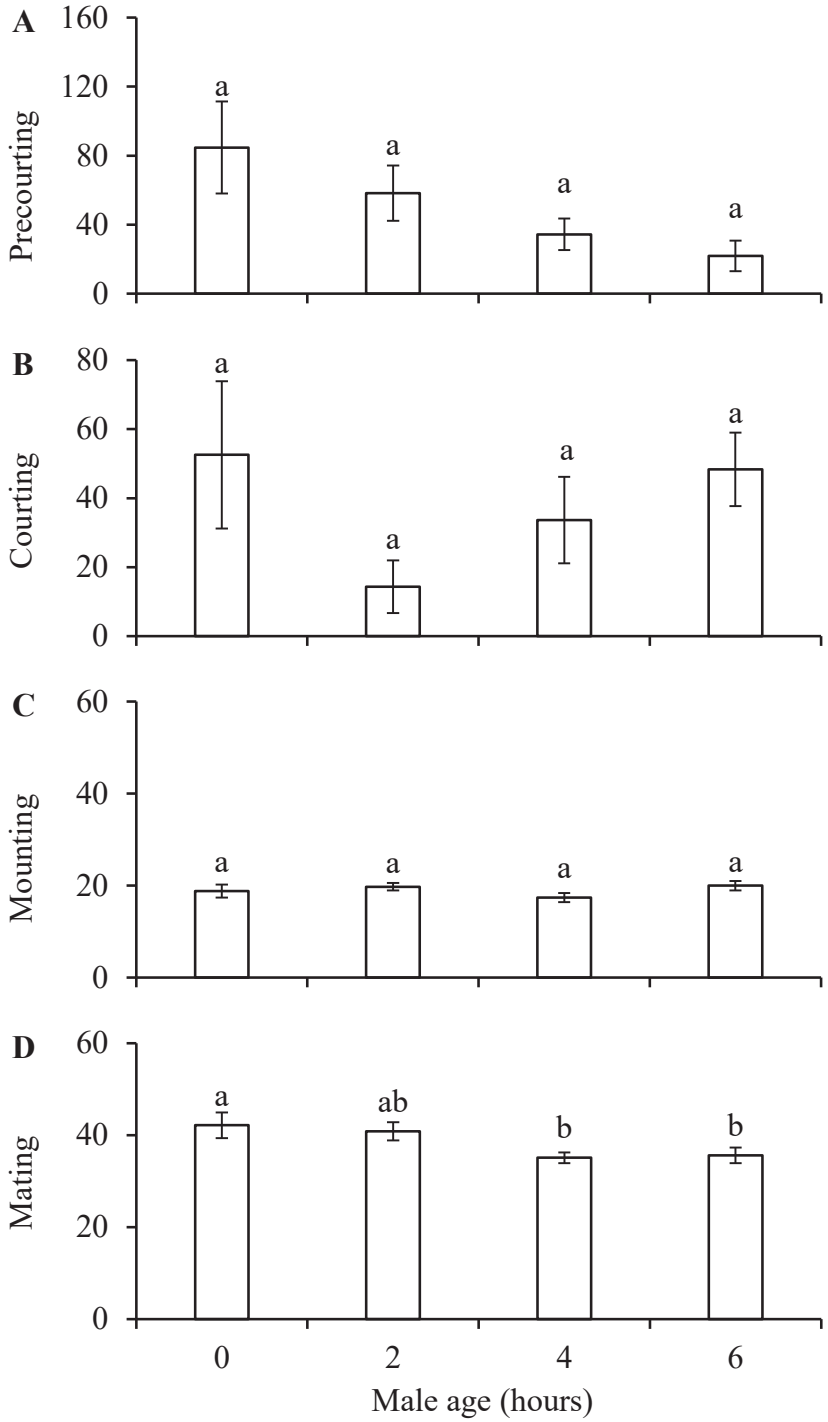

Figure 4.6 Duration (seconds) of precourting (**A**), courting (**B**), mounting (**C**) and mating (**D**) of males of different age when paired with 1-d-old females in *A. colemani*. Bars with the same letters are not significantly different (P > 0.05).

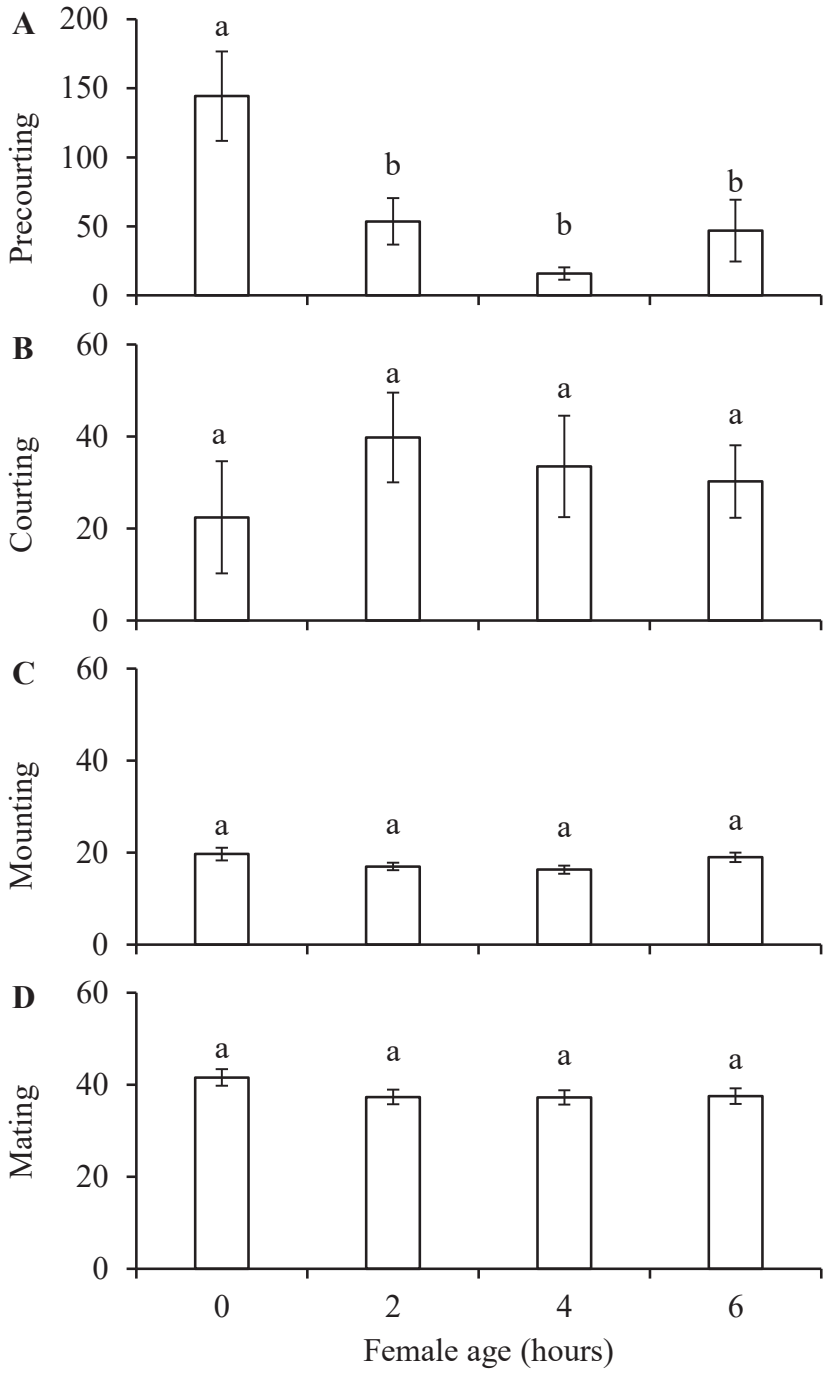

Figure 4.7 Duration (seconds) of precourting (**A**), courting (**B**), mounting (**C**) and mating (**D**) of 1-d-old males when paired with females of different age in *A. colemani*. Bars with the same letters are not significantly different ($P > 0.05$).

My results show that 1-d-old males spent significantly longer time to respond to the newly emerged females than to 2-, 4- and 6-h-old females ($F_{3,61} = 5.72$, $P = 0.0016$) (Figure 4.7A). However, durations for courting, mounting and mating were not significantly affected by female age ($F_{3,55} = 0.83$, 2.46 and 1.76 for courting, mounting and mating, respectively; $P > 0.05$) (Figure 4.7B-D).

4.3.3 General mating behaviour

The general mating behaviour is summarised in Table 4.2 and Figure 4.10. Among 60 pairs, 52 (86.7%) mated in 10 minutes. After released into the tube, the male walked randomly. Upon encountering the female nearby, he often slowed down walking, started fanning his wings, approached and attempted to mount her (Figure 4.8). The courting duration was usually < 100 seconds. The male mounted the female from the back or side and aligned along her body axis (Figure 4.8). If she was receptive, mating occurred (Figure 4.9). If the female was not receptive, she would run or fly away.

Figure 4.8 Mate mounting.

Figure 4.9 Mating pair.

First mounting usually resulted in successful mating (94.2%) (Figure 4.10). Mate mounting usually lasted 20 seconds. During copulation, the female held her antennae backward, whereas the male held his antennae forward just above the female's antennae. If she attempted to move during mating, he would tap her antennae with his, which seemed to calm her down. Mating duration was < 40 seconds. Male terminated the copulation (90.38%) by moving backward, dismounting and walking away. After dismounting, nearly 50% of females remained stationery for a while and others started

walking immediately. After mating, nearly one fourth of mated males (12 of 52 males) performed courtship display to females again and two of them (2 of 12) were able to mount the females but remating did not occur.

Table 4.2 Mating behavioural parameters in *A. colemani* (n = 52).

Parameter	**Mean ± SE**	**Range**
Precourting duration (s)	48.56 ± 14.96	0 - 493
Courting duration (s)	96.42 ± 17.02	13 - 573
No. of Courtship display	2.88 ± 0.32	0 - 12
Mounting duration (s)	20.67 ± 4.49	5 - 241
Antennating duration (s)	36.77 ± 4.69	18 - 268
Mating duration (s)	36.75 ± 1.12	24 - 73

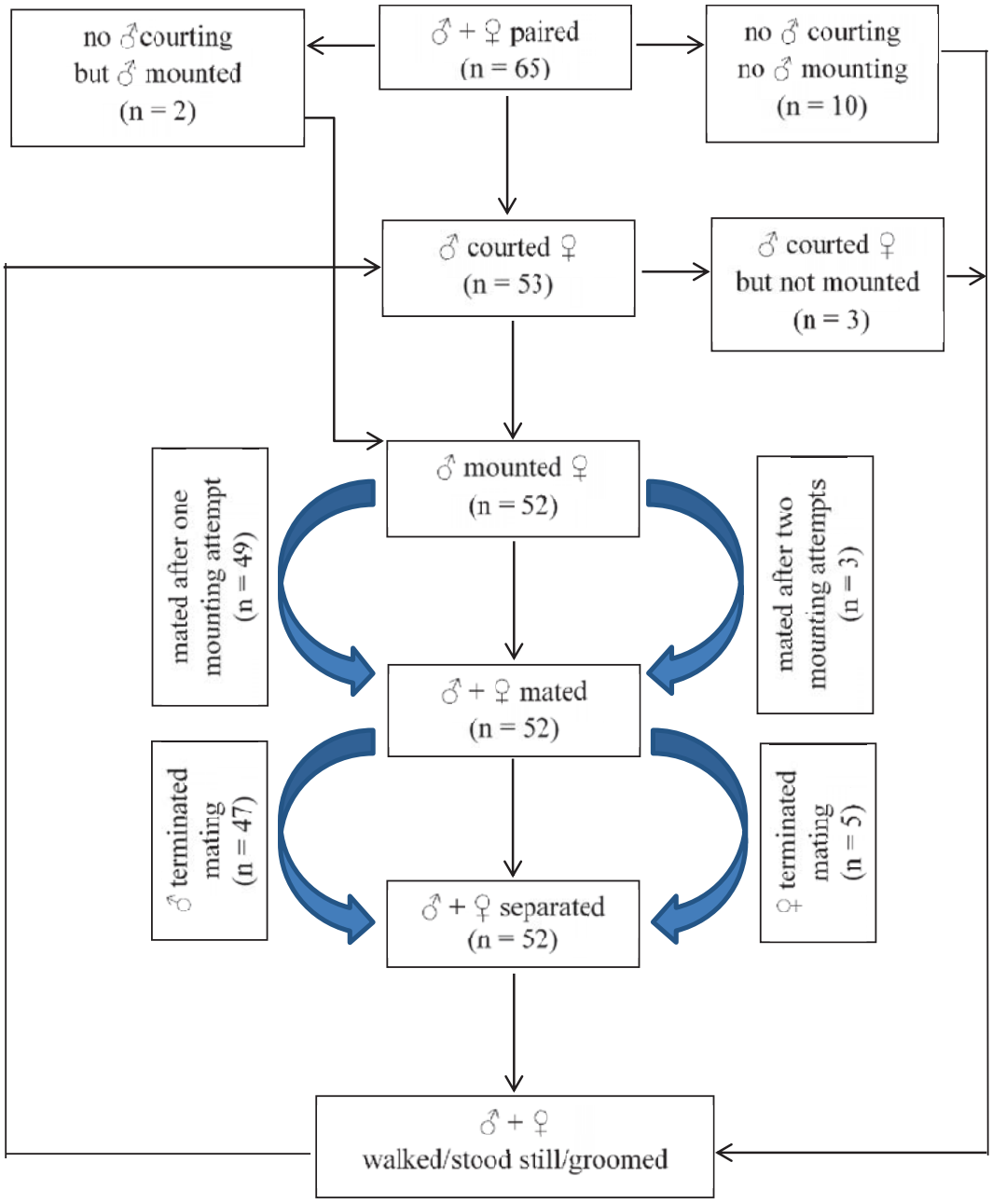

Figure 4.10 Ethogram of mating behavioural events shown by *A. colemani* within 10 minutes of pairing.

4.3.4 Factors affecting mating behaviour

As shown in Figure 4.11A, age of sexually mature adults had no significant effect on mating success in both sexes of *A. colemani* (LR: x_6^2 = 9.33, P = 0.1556), although mating success was only 77.27% when both sexes were 5 days old, lower than that in other treatments (≥ 95%). Premounting duration significantly increased with mate age ($F_{1,134}$ = 4.11 and 4.98 for male and female, respectively; P < 0.05) (Figure 4.11B). Mounting duration significantly increased with female age ($F_{1,135}$ = 39.27, P < 0.0001) but male age had no significant effect ($F_{1,133}$ = 1.82, P = 0.1798) (Figure 4.11C). Mating duration increased significantly with male age ($F_{1,135}$ = 13.36, P = 0.0003) but was not affected by female age ($F_{1,133}$ = 0.14, P = 0.7079) (Figure 4.11D). The interaction between male and female age had no significant effect on premounting, mounting and mating durations ($F_{1,133}$ = 0.36, 1.78 and 1.08 for premounting mounting and mating, respectively; P > 0.05).

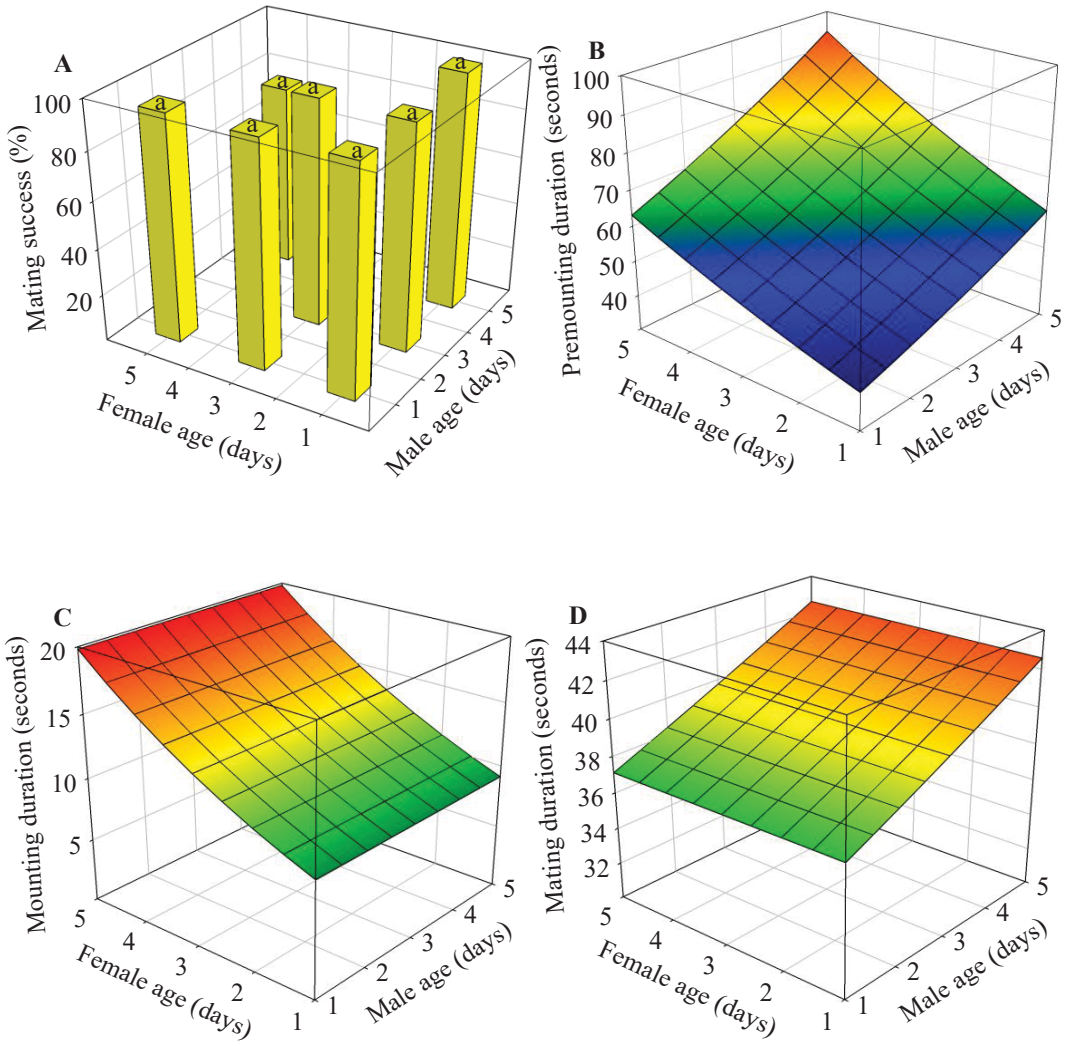

Figure 4.11 Effect of male (Mage) and female age (Fage) on mating success (**A**), premounting duration (**B**), mounting duration (**C**) and mating duration (**D**) in *A. colemani*. (**A**) Columns with the same letters are not significantly different ($P > 0.05$); (**B**) premounting duration = exp (3.4881 + 0.1007Mage + 0.1110Fage), $R^2 = 0.0604$, $F_{2,134} = 4.31$, $P = 0.0154$; (**C**) mounting duration = exp (2.0143 + 0.1945Fage), $R^2 = 0.2253$, $F_{1,135} = 39.27$, $P < 0.0001$; (**D**) mating duration = exp (3.5796 + 0.0343Mage), $R^2 = 0.0917$, $F_{1,135} = 13.63$, $P = 0.0003$.

Mating success was significantly lower in pairs where females were not fed, as compared to other treatments (LR: x_2^2 = 18.68, P < 0.0001) (Figure 4.12A). However, food supply had no significant impact on premounting, mounting and mating duration ($F_{2,41}$ = 0.95, 1.70 and 2.80 for premounting mounting and mating duration, respectively; P > 0.05) (Figure 4.12B-D). In unmated pairs (n = 13) where females were deprived from food, 85% (n = 11) of males did not perform any courtship display.

For a given mating space type, mating success was significantly higher in small space than in medium and large space (LR: x_2^2 = 6.27, 13.27 and 9.96 for vial, Petri dish and cylinder, respectively; P < 0.05) (Figure 4.13A). The premounting period was not significantly affected by the size of Petri dish and cylinder ($F_{2,22}$ = 1.69, P = 0.2081 for Petri dish; $F_{2,10}$ = 2.17, P = 0.1144 for cylinder) but significantly longer in large vial than in small vial ($F_{2,56}$ = 3.43, P = 0.0392) (Figure 4.13B). Mounting period was significantly longer in medium and large vials than in small ones, and in small cylinder than in large ones ($F_{2,55}$ = 4.52, P = 0.0192 for vial; $F_{2,10}$ = 6.64, P = 0.0146 for cylinder), with no significant difference between cylinders of different sizes ($F_{2,20}$ = 2.11, P = 0.1473) (Figure 4.13C). Space had no significant impact on mating duration ($F_{2,55}$ = 0.14, P = 0.8708 for vial; $F_{2,20}$ = 0.28, P = 0.7614 for Petri dish; $F_{2,10}$ = 0.04, P = 0.9613 for cylinder) (Figure 4.13D).

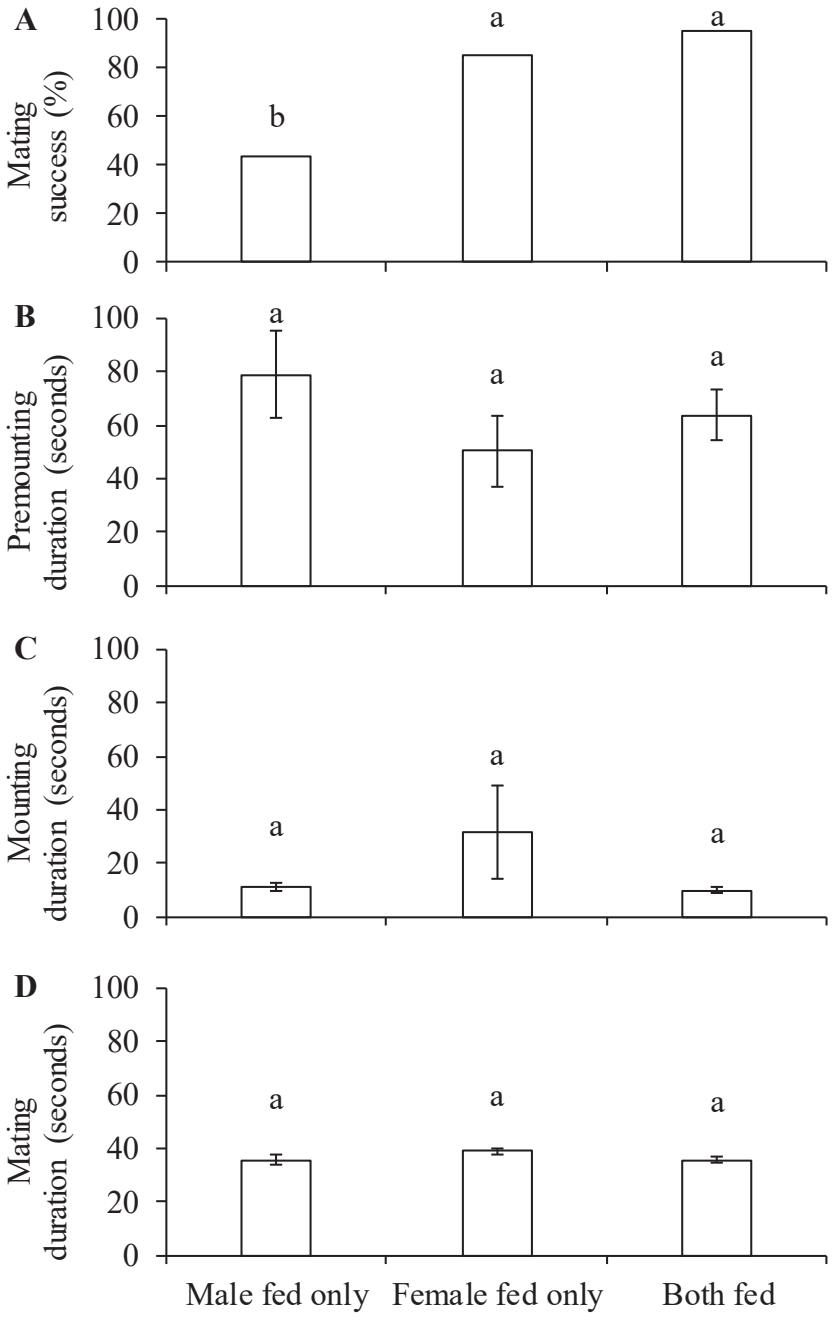

Figure 4.12 Effect of food supply on mating success (**A**), premounting period (**B**), mounting period (**C**) and mating period (**D**) in *A. colemani*. Columns with the same letters are not significantly different ($P > 0.05$).

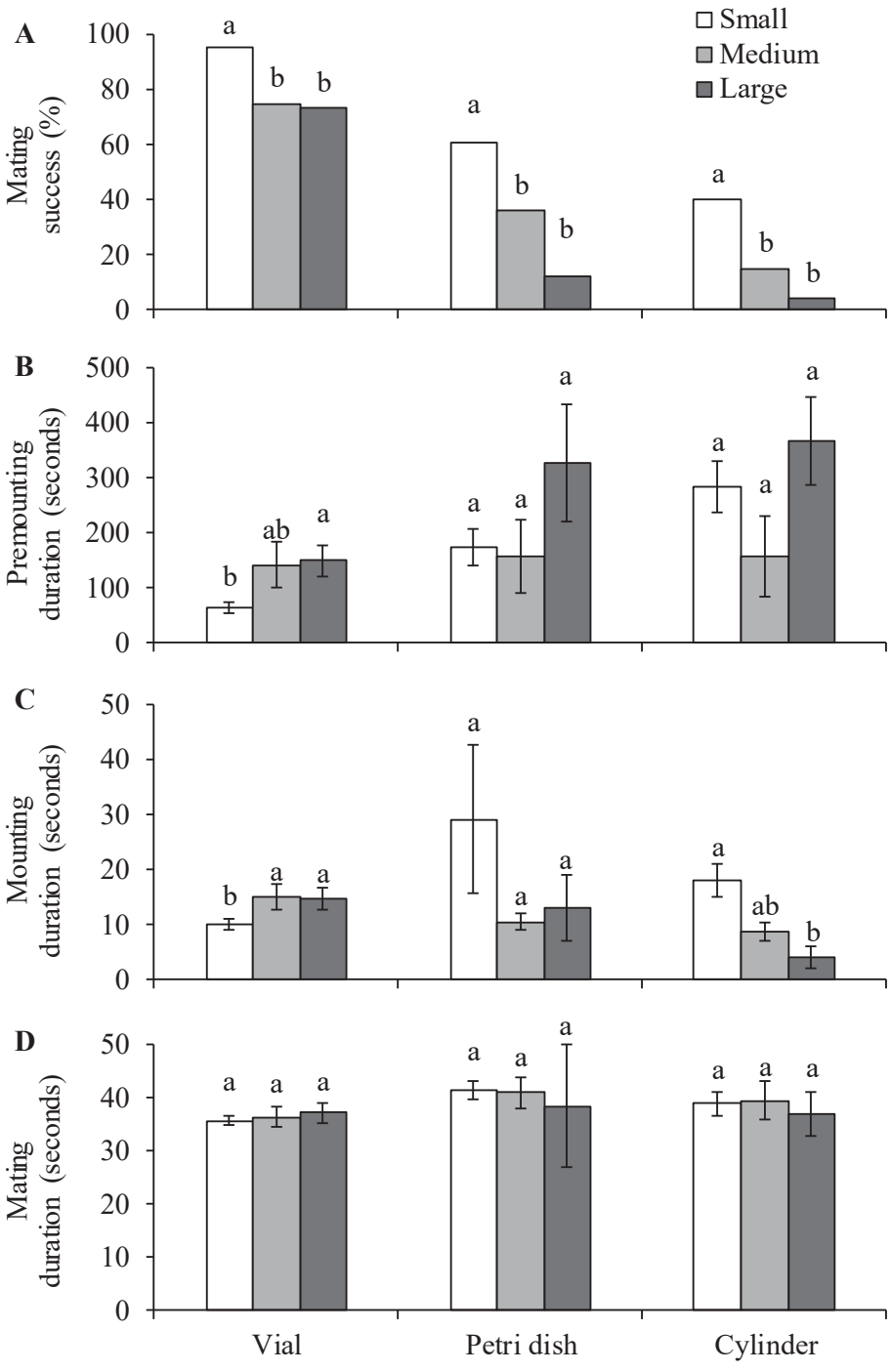

Figure 4.13 Effect of space on mating success (**A**), premounting duration (**B**), mounting duration (**C**) and mating duration (**D**) in *A. colemani*. For each space category, columns with the same letters are not significantly different ($P > 0.05$).

4.4 Discussion

My results show that more than 95% *A. colemani* adults emerged in the photophase and emergence peaked soon after lights on (Figure 4.1B), suggesting that the onset of light may act as a signal that triggers emergence. The occurrence of adult emergence in the morning is common in many parasitoid species, such as *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (van Lenteren et al. 1992), several *Trichogramma* species (Hymenoptera: Trichogrammatidae) (Corrigan et al. 1995; Pompanon et al. 1995; Reznik et al. 2008), *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) (Fantinou et al. 1998) and *A. ervi* Haliday (He et al. 2004). This could be attributed to previous findings that parasitoid females release sex pheromones mostly in the morning, during which time, the environmental conditions, such as light, little wind, cool temperature and right relative humidity, are favourable for males to locate females (Marchand & McNeil 2000; McClure et al. 2007). Therefore, emergence early in the morning may be advantageous to both sexes in mate location and mating success before female oviposition. My current study also indicates that the developmental period from egg to adult was significantly shorter in males than in females, and as a result, males emerged significantly earlier than did females (Figure 4.1A). The observed emergence pattern in *A. colemani* may be a life history strategy for maximal mating success in both sexes (Morbey & Ydenberg 2001).

As Figure 4.2 shows, probabilities of courting and mating were significantly higher in the photophase than in the scotophase, generally supporting the previous findings that maximal sex pheromone release by *Aphidius* females occurs in the morning (McNeil & Brodeur 1995; Marchand & McNeil 2000; McClure et al. 2007; Benelli et al. 2013). The non-significant difference in probabilities of courting and mating between eight 2-h bouts in the photophase may be attributed to small space (2 ml) provided for pairs, which may weaken the effect of sex pheromones. In addition, male courting usually resulted in successful mating during the photophase but not the scotophase (Figure 4.2). This could be related to pheromone release, female receptivity, and male mating attempt, details of which warrant further investigations.

The current study demonstrates that *A. colemani* females laid eggs throughout the 24-h cycle but the number of aphids parasitised decreased since the onset of lighting (Figure 4.3), generally supporting the previous findings in *C. bartetti* (Walter 1988),

Monoctonus pseudoplatani (Marshall) (Hymenoptera: Braconidae) (Collins & Dixon 1986), and *A. ervi* (He et al. 2004). The significantly higher parasitism per oviposition bout in the photophase than in the scotophase may be because visual cues play an important role in host searching, recognition and location of parasitoids (Michaud & Mackauer 1994) and lighting is critical to motivate locomotor activity of parasitoids (Abe et al. 2014). Furthermore, some previous studies reveal that female parasitoids may alter sex allocation patterns of their offspring in response to photoperiod, for example, *U. lariophaga* females produce more female offspring in the photophase than in the scotophase (van Huis & Appiah 1995; Sagarra et al. 2000). In the present study, however, the sex ratio was female-biased (between 50 and 70% of offspring were females) and not significantly different between any oviposition bouts in both light phases, suggesting that neither light phase nor oviposition rate has any impact on *A. colemani* sex allocation.

My findings indicate that both sexes *A. colemani* require ≈ 2 hours for sex maturation before reproduction (Figures 4.4-4.7). My observations on mating behaviour show that the sequence of events leading to copulation in *A. colemani* (Figures 4.8-4.10) was similar to that in many other braconid parasitoids where males exhibit courtship display such as wing fanning, antennation and mounting on females before copulation (Kimani & Overholt 1995; De Freitas et al. 2004; Benelli et al. 2014; Avila et al. 2016). The mating duration of *A. colemani* was 36.8 seconds on average (Table 4.2), falling into the range (< 1 min) reported for many other parasitic wasps (Tagawa et al. 1985; Quicke 1997; Liu et al. 2001; De Freitas et al. 2004; Al-Wahaibi et al. 2007; Avila et al. 2016).

Although age of neither sex had a significant impact on mating success in *A. colemani* (Figure 4.11A), premounting duration increased with age of both sexes (Figure 4.11B), older females increased mounting duration (Figure 4.11C) and older males prolonged mating duration (Figure 4.11D). It is suggested that older couples need more time before initiation of sex contact, older females demand longer time to become receptive after mounting, and older males require more time to transfer sperm to females.

The adult diet of parasitoids could play a role in mating success (Benelli et al. 2017). My results indicate that food access had different effects on males and females in

A. colemani (Figure 4.12). If females were deprived from food supply, mating success was significantly lower as compared to other treatments; however, males achieved similar mating success regardless of whether they were fed or not (Figure 4.12A). Furthermore, most males (≈ 83%) did not display any courtship behaviour to food-deprived females. These results suggest that females deprived from food may reduce or even stop pheromone production and release.

My results reveal that the area size for mating had significant effect on mating behaviour with higher mating success occurring in smaller space (Figure 4.13). These may be attributed to easier detection and location of mates by males via chemical (Marchand & McNeil 2000; McClure et al. 2007) and visual cues (Michaud & Mackauer 1994). Smaller space promoting mating success has been demonstrated in other studies (Adams & Morse 2014).

In summary, most emergence and reproductive activities of *A. colemani* occur during the photophase. After emergence, both sexes need about 2 hours for sex maturation, but once sexually mature, age of neither sex has a significant effect on mating success. Mating duration is brief, falling into the range (< 1 min) reported for many other parasitic wasps. The mating behavioural sequence of this parasitoid species is similar to that of many other braconid parasitoids. Furthermore, food supply to adult females is essential to mating success in *A. colemani*. Overall, this study has provided basic knowledge on the biology of *A. colemani*, which can be used for design of further experiments and methods for laboratory handling, rearing, and field release.

Chapter Five

Life History Strategies of *Aphidius colemani* and *Myzus persicae*

5.1 Introduction

The solitary and koinobiont endoparasitoid, *A. colemani*, is produced commercially for biological control of *M. persicae* and cotton aphid *A. gossypii* around the world (Fernandez & Nentwig 1997; Starý 2002; van Lenteren 2003; Vásquez et al. 2006; Teulon et al. 2008; van Driesche et al. 2008). However, several features in parasitoid-aphid systems may limit the effectiveness of parasitoids in suppressing aphid pests. For example, aphids parasitised at the later instars can still produce offspring (Tang & Yokomi 1996; Lin & Ives 2003; He et al. 2005b), and parasitoids may take longer than their hosts to complete lifecycles (Adly et al. 2006; He 2008; He & Wang 2011), prefer to attack hosts of certain age (Lykouressis et al. 2009; He et al. 2011), and have lower intrinsic rate of increase (r_m) than their hosts (van Steenis & El-Khawass 1995a; Torres et al. 2007). In practice, *A. colemani* has failed to control aphid pests on some occasions, e.g. *A. gossypii* (Burgio et al. 1997; Jacobson & Croft 1998) and soybean aphid, *A. glycines* (Gariepy et al. 2015). Nevertheless, the parasitoid has successfully suppressed aphid populations on other occasions, e.g. *A. gossypii* (Vásquez et al. 2006; Torres et al. 2007) and *M. persicae* (Herzog et al. 2007; Shah et al. 2013). So far, the mechanisms behind the success and failure of parasitoids in biological control of aphids are largely unknown probably due to the lack of knowledge on life history strategies of both parasitoids and their hosts.

Essential information for evaluation of biological control potential of a parasitoid can be obtained from direct measurement of life history parameters for both parasitoid and its host (Bellows et al. 1992; van Driesche & Bellows 1998; Bellows & van Driesche 1999; van Lenteren 2009; Lins et al. 2011) and subsequent construction and comparison of their life tables (Bellows et al. 1992; Bellows & van Driesche 1999; van Lenteren 2009; Lins et al. 2011). However, most previous studies have not given

complete pictures for both parasitoid and host species, telling only one side of the story. For example, in evaluation of the efficiency of *A. colemani* in suppressing *A. gossypii* populations, van Steenis (1993) and Torres et al. (2007) investigated the life table of *A. colemani* without considering that of *A. gossypii*. In contrast, van Steenis and El-Khawass (1995a) and Vásquez et al. (2006) estimated the population growth of *A. gossypii* but did not compare it with that of *A. colemani*. Although Chi and Su (2006) analysed the life table parameters of both *A. gifuensis* and *M. persicae*, they did not take parasitised aphids of different ages into consideration. The lack of complete data sets for both parasitoids and their aphid hosts makes it difficult to evaluate parasitoids' biological control potential and estimate their release rate in augmentation programmes. To date, little is known about life history strategies and life tables of the *A. colemani*-*M. persicae* system.

Mass-production of *A. colemani* is still expensive with a cost of US$ 0.07 per adult (van Driesche et al. 2008). With a normal population sex ratio of ≈ 1:1 (Khatri et al. 2016) production of one *A. colemani* female adult costs US$ 0.14. In New Zealand 100 mummies of *A. colemani* are sold for NZ$10 plus GST and freight (Bioforce 2017). In biological control programmes using *A. colemani*, the parasitoid release rate is usually based on the area of crops (Burgio et al. 1997; Jacobson & Croft 1998; Vásquez et al. 2006; van Driesche et al. 2008; Bioforce 2017). According to Vásquez et al. (2006) biological control of *A. gossypii* using *A. colemani* is ≥ 4.5 times more expensive than the application of the standard pesticide, imidacloprid. The understanding of life history strategies and life table parameters of insects involved, including parasitoids and parasitised and unparasitised aphids, may provide critical knowledge for development of a cost effective biological control programme using *A. colemani* and for estimation of parasitoid release rate based on aphid population density.

The present study aimed to generate essential information for evaluation of the potential of *A. colemani* to control *M. persicae* and for estimation of optimal parasitoid release rate and time. The study measured survival and reproduction of the parasitoid and parasitised and unparasitised aphids, examined parasitism rate in different host stages by the parasitoid, and analysed population growth of both species. Findings of this study may provide a scheme to assist in development of cost effective biological control programmes for *M. persicae* using *A. colemani*.

5.2 Materials and methods

5.2.1 Insects

The maintenance of breeding colonies of *M. persicae* and *A. colemani* was described in Sections 3.2.3 and 3.2.4, respectively. Parasitoids used for experiments were obtained by parasitising 2-d-old *M. persicae* nymphs as mentioned in the Section 3.2.5.

5.2.2 Survival and reproduction of parasitoids and parasitised and unparasitised aphids

To determine survival and parasitism potential of *A. colemani*, I released a mated female (< 12 hour old) into a Petri dish (5.5 cm in diameter × 1.0 cm in height, Figure 3.6) containing a fresh Chinese cabbage leaf disc (3 cm in diameter) infested with 50 2-d-old *M. persicae* nymphs and allowed her to stay for 24 hours. The leaf disc was placed upside down on a moistened filter paper. The parasitoid was then carefully transferred into another Petri dish with 50 2-d-old *M. persicae* nymphs on a fresh leaf disc for 24 hours. The process was repeated until the parasitoid died. Sixteen parasitoid females were tested in this experiment. The parasitoid-exposed aphids were maintained on the same leaf disc for three days, after which time, they were transferred to a plastic cylinder with a fresh leaf cut (Figure 3.3). I examined aphids in each cylinder once a day, and counted mummies. I then moved mummies individually to 2-ml micro-centrifuge vials and recorded parasitoid emergence and developmental period. Emerged adults were sexed.

To investigate how parasitisation affected life history performance of aphids of different ages, *A. colemani* was allowed to parasitise *M. persicae* of four different ages (1, 3, 5, and 7 d old; 1-d-old = 1st instar, 3-d-old = 2nd instar, 5-d-old = 3rd instar, and 7-d-old = late 4th instar-adult). The instar-age relationships under our experimental conditions were determined through direct observations on molting (unpubl. data DK). For each aphid age class, one naïve 1-d-old mated female parasitoid was released into a Petri dish with 10 aphids of the same age feeding on a fresh leaf disc as above. The parasitoid was allowed to stay in the Petri dish for two hours and then discarded. The

parasitoid-exposed aphids were individually transferred to a fresh leaf disc placed in a Petri dish as above. A total of 64 parasitoid-exposed aphids were confirmed to be parasitised. Data on the survival and reproduction of the parasitised aphids were recorded once every 24 hours until death. Data on survival and reproduction of 48 healthy (unparasitised) adults were also recorded in 48 above mentioned Petri dishes once every 24 hours until death.

5.2.3 Parasitism rate in relation to host stage

This experiment examined whether the parasitoids preferred aphids of particular age for parasitsation when aphids of all life stages were present in the vicinity. One 1-d-old mated parasitoid female was released into a Petri dish containing 60 aphids of different ages (15 aphids for each of 1, 3, 5 or 7 d old) on a fresh leaf disc as mentioned above. The parasitoid was allowed to stay in the dish for four hours, after which time, she was removed and discarded. The parasitoid-exposed aphids were then separated according to their age. All aphids of each age were transferred onto a fresh leaf cut in an above-mentioned plastic cylinder. Aphids were maintained in the cylinders until mummification. The parasitism rate was calculated as the number of mummies divided by 15 for each aphid age. Twenty-seven parasitoid females were tested.

5.2.4 Population growth of parasitoids and parasitised and unparasitised aphids

To estimate relative population growth of the aphid and its parasitoid and provide knowledge for augmentation programmes, their life table parameters were calculated and compared using data collected from the above experiments on survival and reproductive potential of parasitoids and parasitised and unparasitised aphids. Life tables used to study population dynamics are organized presentations of the number of individuals surviving to fixed points in the life cycle, together with their reproductive output at those points (Bellows & van Driesche 1999; Jervis et al. 2005).

The daily survival and reproduction were compiled to assess the population growth using Jervis et al.'s (2005) method. The intrinsic rate of increase (r_m, females/female/day) was estimated by solving the Lotka-Euler equation, $\sum e^{-r_m x} l_x m_x = 1$, where x is the pivotal age, l_x is the proportion of the females surviving to age x, and m_x is the number of female offspring produced per female at age x. Other life table

parameters included the net reproductive rate ($R_0 = \sum l_x m_x$, females/females/generation), mean generation time [$T = \log_e(R_0)/r_m$, days] and doubling time [$Dt = \log_e(2)/r_m$, days]. For each treatment, a jackknife method (Caswell 2001) was used to estimate the life table parameters for each aphid or parasitoid female.

5.2.5 Statistical analysis

The distribution of data was tested using a goodness-of-fit test (Shapiro-Wilk test, UNIVARIATE Procedure) before analysis. Data on the parasitism rate were normally distributed after being arsine square-root transformed and thus analysed by an analysis of variance (ANOVA, GLM Procedure) followed by a Tukey's studentized range test. Data on survival, reproduction and life table parameters of both aphids and parasitoids were not normally distributed even after transformation, and hence analysed using nonparametric ANOVA followed by Bonferroni (Dunn) t Tests for multiple comparisons.

Nonlinear regression models were developed using the methods described by Archontoulis and Miguez (2015). The nonlinear exponential decay and peak models (NLIN Procedure) were applied to fit the data on the daily number of aphids parasitised by *A. colemani* and that of offspring produced by *M. persicae* over time, respectively. A nonlinear exponential rise model was used to estimate the accumulative number (%) of aphids parasitised and produced as below:

Number of aphids parasitised = $a + b \exp(-c \text{ age})$, where a is a constant, b is the maximum number of parasitism, and c is the decreasing rate of parasitism.

Number of aphid offspring produced = $a/\{1 + [(\text{age} - b)/c]2\}$, where a is the maximum number of offspring produced, b is the age when maximum number of offspring is produced, and c is the decreasing rate of offspring produced.

Accumulative number (%) = $a + b\,[1 - \exp(-c \text{ age})]$, where a is a constant, b is the maximum proportion of accumulative number, and c is the decreasing rate of accumulation.

All analyses were performed using SAS software (SAS 9.4, SAS Institute Inc., NC, USA).

5.3 Results

5.3.1 Survival and reproduction of parasitoids and parasitised and unparasitised aphids

Results show that parasitisation significantly reduced overall fitness of aphids regardless of aphid age at parasitisation (Figure 5.1). Parasitised aphids lived about four times shorter than healthy (unparasitised) adult aphids ($F_{5,122}$= 149.04, $P < 0.0001$; Figure 5.1A). Aphids parasitised at 1 and 3 d old died before reaching adult stage and those parasitised at 5 and 7 d old reproduced for only one or two days ($F_{3,78}$ = 87.36, $P < 0.0001$; Figure 5.1B) and produced very few offspring ($F_{3,78}$ = 853.17, $P < 0.0001$; Figure 5.1C).

Although the parasitoid adult female had significantly shorter longevity and reproductive period than healthy aphid adult (Figure 5.1A-B), she parasitised 3.5 times more aphids in her lifetime (218.88 ± 18.93) than the number of offspring a healthy aphid adult produced in her lifetime (62.99 ± 2.91). When only female offspring were considered, a parasitoid produced twice as many offspring as a healthy aphid adult did (Figure 5.1C).

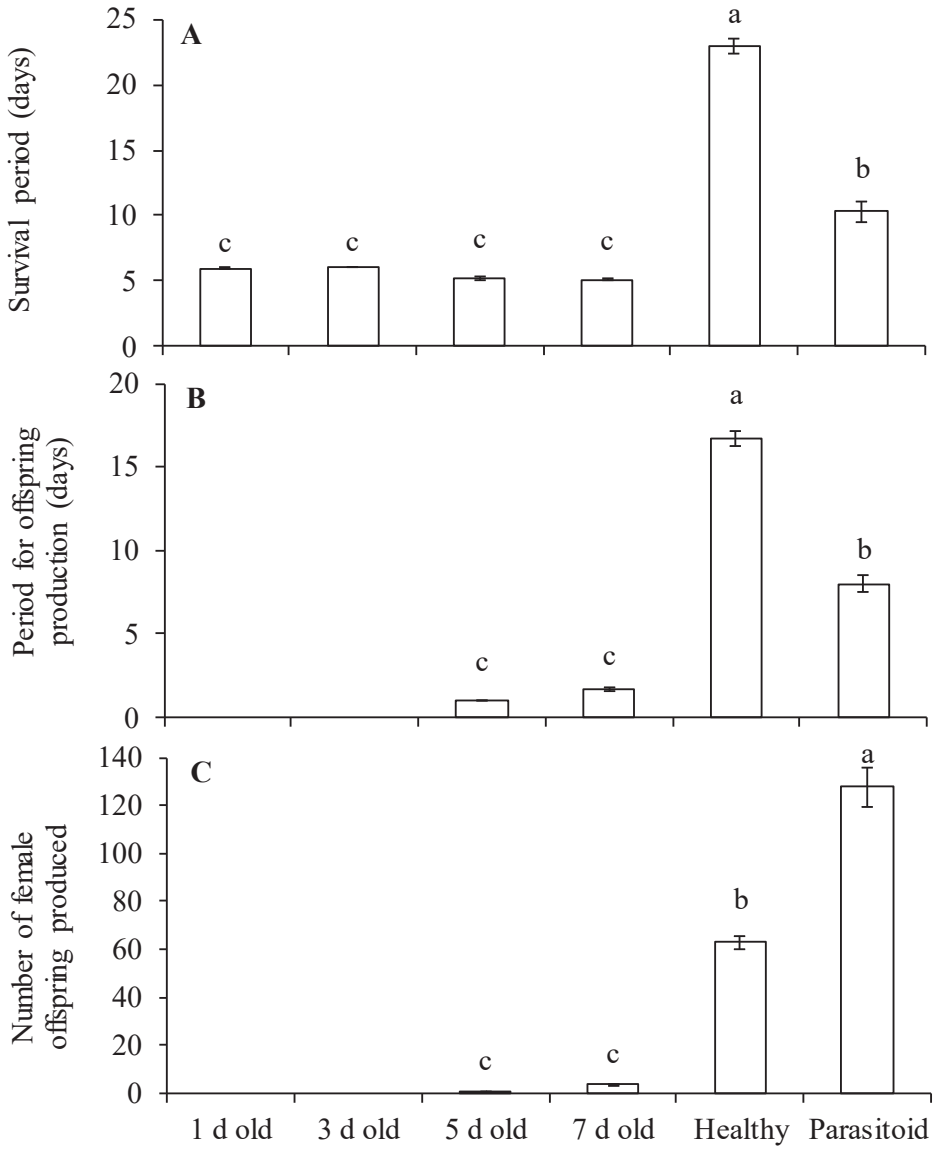

Figure 5.1 Survival and reproduction of parasitoids, parasitised aphids of different ages, and unparasitised aphid (healthy) adults. Bars (mean ± SE) with the same letters are not significantly different (P > 0.05).

A comparison of reproductive patterns between *A. colemani* and healthy *M. persicae* indicates that the parasitoids reached 50% and 90% of their lifetime parasitism potential two and six days after emergence, respectively (Figure 5.2A) while aphids produced 50% and 90% of their lifetime offspring seven and sixteen days after emergence, respectively (Figure 5.2B).

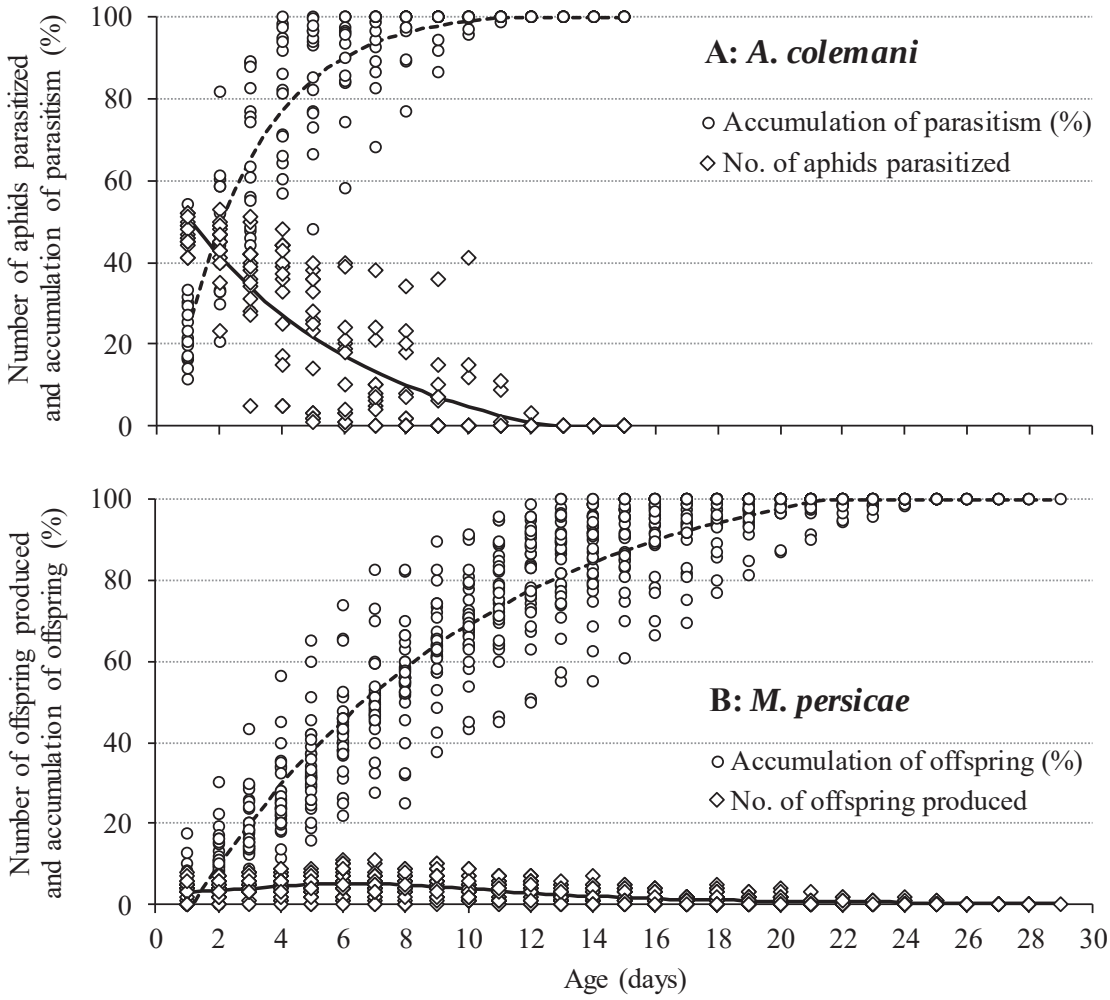

Figure 5.2 Reproduction of *A. colemani* females (**A**) and healthy *M. persicae* adults (**B**) in relation to their age after emergence. (**A**) Number of aphids parasitised = -8.51+70.09exp(-0.17age), R^2 = 0.7248, $F_{2,182}$ = 239.67, P < 0.0001; accumulative parasitism (%) = -13.10+114.50[1-exp(-0.38age)], R^2 = 0.8231, $F_{2,182}$ = 423.50, P < 0.0001. (**B**) Number of aphid offspring produced = 5.26/{1+[(age-6.41)/6.12]^2}, R^2 = 0.4644, $F_{3,780}$ = 735.98, P < 0.0001; accumulative number of aphid offspring produced (%) = -15.09+128.0[1-exp(-0.11age)], R^2 = 0.9144, $F_{2,780}$ = 4163.69, P < 0.0001.

5.3.2 Parasitism rate in relation to host stage

My results show that when the parasitoids were given choice of aphids of all ages for oviposition, they parasitised about 80% of aphids within four hours without any significant difference between aphid ages ($F_{3,104}$ = 0.27, P = 0.8458; Figure 5.3).

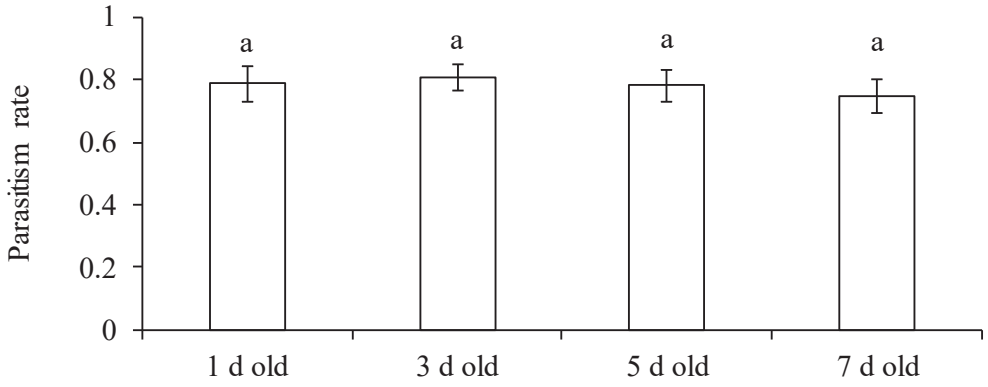

Figure 5.3 Parasitism rate of *A. colemani* in relation to host age. Bars (mean ± SE) with the same letters are not significantly different (P > 0.05).

5.3.3 Population growth of parasitoids and parasitised and unparasitised aphids

Compared to the healthy aphids, the parasitoids had significantly lower intrinsic rate of increase (r_m) and significantly longer generation time (T), and took significantly longer time to double the population (Dt) (Table 5.1). This may be because the developmental period of parasitoid females (15.37 ± 0.18 days from egg to adult) was significantly longer than that of aphids (7.38 ± 0.09 days from neonate nymph to adult) ($F_{1,244}$ = 222.05, P < 0.0001). However, the net population growth rate (R_0) of parasitoids was twice as great as healthy aphids (Table 5.1).

Table 5.1 shows that parasitisation significantly suppressed the aphid population growth. For example, aphids parasitised at 5 d old or younger age (Figure 5.1C) contributed none or little to the next generation. Although aphids parasitised at 7 d old still produced some offspring (Figure 5.1C), they had significantly lower r_m and R_0, shorter T and longer Dt than healthy aphids (Table 5.1).

Table 5.1 Life table parameters of *A. colemani* and parasitised and unparasitised *M. persicae*.

Insect	r_m^*	R_0	T	Dt
Aphids parasitized at 5 d old	-0.1495±0.0000 c	0.33±0.02 c	7.46±0.45 c	-4.64±0.00 d
Aphids parasitized at 7 d old	0.1484±0.0011 c	3.36±0.03 c	8.16±0.03 c	4.67±0.04 a
Unparasitized aphid adults	0.3606±0.0000 a	62.98±0.06 b	11.49±0.00 b	1.92±0.00 c
A. colemani	0.2838±0.0010 b	126.48±0.92 a	17.05±0.02 a	2.44±0.01 b
$F_{(df)}$	$127.98_{(3,83)}$	$127.25_{(3,83)}$	$117.45_{(3,83)}$	$127.98_{(3,83)}$
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

* According to life table theory (Lotka 1913, Lewis 1942), if $R_0 < 1$, then $r_m < 0$, resulting in a negative Dt. Means (± SE) followed by different letters in columns are significantly different ($P < 0.05$).

5.4 Discussion

The success of aphid biological control using parasitoids largely depends on life history strategies of both parasitoids and hosts. Therefore, in evaluation of efficiency of a parasitoid as an aphid biological control agent, life history strategy parameters of insects involved should be measured (Bellows et al. 1992; van Driesche & Bellows 1998; Bellows & van Driesche 1999; van Lenteren 2009; Lins et al. 2011). However, prior to the present study these parameters of *M. persicae* and *A. colemani* had not been measured simultaneously and compared (van Steenis 1993; Vásquez et al. 2006; Torres et al. 2007), making it difficult to assess the effectiveness and estimate the release rate of the parasitoid for the control of *M. persicae*.

One of the potential constraints that may limit parasitoids in controlling aphid pests is the ability of the parasitised late instar nymphs and adults to continue reproducing (Tang & Yokomi 1996; Lin & Ives 2003; He et al. 2005b). For example, parasitised blackcurrant-sowthistle aphid *H. lactucae* (Liu & Hughes 1984), pea aphid *A. pisum* (Sequeira & Mackauer 1988; He et al. 2005b) and green citrus aphid *A. spiraecola* (Tang & Yokomi 1996) can still produce substantial number of offspring, reducing the effectiveness of their parasitoids to suppress their populations. The present study, however, shows that young and mid-aged *M. persicae* nymphs parasitised by *A.*

colemani died before reaching adults and parasitised late instar nymphs and adults produced only very few offspring (Figure 5.1B-C). Furthermore, parasitised aphids had significantly lower intrinsic rate of increase and net population growth rate, longer doubling time (Table 5.1) and shorter survival time (Figure 5.1A) than unparasitised aphids. These results indicate that *M. persicae* parasitised by *A. colemani* can contribute little to their population growth and make limited damage to plants.

In some parasitoid-aphid systems, parasitoids may be considered less effective in controlling aphid pests because they have longer lifecycle (Adly et al. 2006; He & Wang 2011) and lower intrinsic rate of increase than their hosts (van Steenis & El-Khawass 1995a; Torres et al. 2007). In the *A. colemani-M. persicae* system, although the parasitoid had about 21% lower intrinsic rate of increase and 33% longer lifecycle than unparasitised *M. persicae*, the former produced twice as many female offspring (Figure 5.1C) and had twice as great net population growth rate (Table 5.1) as the latter did. The parasitoid's overwhelmingly higher reproductive output and faster population growth may overcome the constraints of its longer lifecycle and lower intrinsic rate of increase and potentially make it an effective biological control agent of *M. persicae*. The results may also explain why the relatively lower intrinsic rate of increase in *A. colemani* than in *A. gossypii* (Torres et al. 2007) does not compromise the biological control efficiency for *A. gossypii* using *A. colemani* (van Steenis & El-Khawass 1995b; Vásquez et al. 2006; Prado et al. 2015).

Aphid pests usually appear on field crops in early spring and their population grows fast if not in check by biological control agents (Ro & Long 1999). For example, *M. persicae* are first observed on potato plants in early spring and their population quickly increases to the peak in about three weeks (Saljoqi 2009). Depending on environmental conditions and crops on which *M. persicae* feed, the peak population size can range from very high (750 aphids/leaf) (Athanassiou et al. 2003) to extremely high (> 2500 aphids/leaf) (Kavallieratos et al. 2005), which is difficult or very expensive to suppress through release of *A. colemani*. Therefore, the first three weeks after the onset of aphid populations are the critical period for the practice of augmentation. For example, in the control of *A. gossypii* in glasshouses release of *A. colemani* at the rate of 2 mummies/m^2 three times in the early season suppresses the pest population to < 0.6 aphids/leaf compared to 653.2 aphids/leaf in the untreated crops (Moon et al. 2011). For

the control of several aphid pests such as *A. gossypii* and *M. persicae,* Bioforce (2017) recommends release rates of 0.1-0.2 mummies/m^2 before the pest onset and 1-5 mummies/m^2 after the pest onset over several weeks. However, none of the above-mentioned release rates is based on aphid density at the time of release.

A comparison of reproductive patterns of the two insects (Figure 5.2A-B) indicates that *A. colemani* reached 50% and 90% of their lifetime reproductive potential five and ten days earlier than unparasitised *M. persicae*, respectively. This suggests that *A. colemani* has high potential to suppress the aphid population quickly if it is released at the right time. Similar to *A. pisum* (Tenhumberg et al. 2009; Tenhumberg 2010), about 85% of the spring *M. persicae* population are < 4 instars (Hughes 1972). During the first week of the appearance of aphids the population probably consists of even higher proportion of young aphids (Schowalter 2000). Given that one *A. colemani* female parasitised ≈ 220 *M. persicae* in a week (Figure 5.2A) without any preference for aphid age (Figure 5.3), and parasitised aphids contributed little to population growth (Figure 5.1 and Table 5.1), it is estimated that a small release rate of ≈1:220 (*A. colemani* female:*M. persicae*) at a weekly interval during the first three weeks after the onset of *M. persicae* population could quickly suppress the aphid population growth and effectively control the pest. However, this hypothesis needs testing in the field. Moreover, to achieve successful and cost-effective control of *M. persicae* by release of *A. colemani*, a close monitoring of the onset of the aphid population early in the season is required.

In summary, *A. colemani* is an effective biological control agent of *M. persicae* because reproductive outputs of the parasitoid are twice as high as the aphid, the parasitoid reaches the maximum lifetime reproductive potential about a week earlier than the aphid, and parasitised aphids contribute little to their population growth and make limited damage to plants. Successful and cost-effective control of *M. persicae* by augmentative release of *A. colemani* can be achieved if the parasitoids are released immediately upon the first appearance of the aphids when the aphid population density is low. Overall, the success of biological control of aphids using parasitoids largely depends on life history strategies of both insects involved and time of the season when they meet.

Chapter Six

Trade-off between Fitness Gain and Cost in *Aphidius colemani*

6.1 Introduction

Reproductive fitness and biological control potential of aphidiine parasitoids may vary depending on aphids of different ages or sizes available in the vicinity (Hågvar & Hofsvang 1991; He et al. 2005a; He et al. 2005b). Larger hosts usually contain more resources than smaller ones (Liu 1985; Mackauer 1986). Consequently, females that parasitise larger hosts produce progeny of larger body size (Liu 1985; He et al. 2005a; Henry et al. 2009), more female-biased sex ratio (Henry et al. 2005; He et al. 2005a), higher egg load at emergence (He et al. 2005a), and shorter developmental time with lower premature mortality (Jenner & Kuhlmann 2006).

However, unlike idiobiont parasitoids that attack hosts with fixed resources, hosts of koinobiont species continue to grow and develop after being parasitised and thus their properties continue to change during the course of parasitoid progeny development (Mackauer 1986; Brough et al. 1990; Lin & Ives 2003). Furthermore, larger aphids may be able to defend themselves more effectively than smaller ones against parasitoid attack, costing more to parasitoids in terms of time and energy for handling (Henry et al. 2006; Wyckhuys et al. 2008; He et al. 2011). Therefore, aphidiine females may have developed strategies to accurately assess the suitability of hosts for their progeny before oviposition (Colinet et al. 2005; Henry et al. 2005) and to balance their fitness return and oviposition cost (Chau & Mackauer 2001; Henry et al. 2009). Although the mechanisms may be different, strategic selection of hosts of certain age for oviposition for maximum progeny fitness has been reported in other parasitoid-host systems (Hanan et al. 2015).

Several studies on the host stage preference of *A. colemani* in different parasitoid-host systems have reached varied conclusions. For example, Lin and Ives (2003) demonstrate that the females prefer to attack older nymphs of *A. glycines* while Perdikis et al. (2004) and Lykouressis et al. (2009) show that they prefer earlier to later instar

nymphs of *M. persicae* and *A. gossypii* for oviposition. In non-choice and paired-choice experiments Barrette et al. (2009) indicate that the parasitoid gains more from large aphids but fitness gain rate is higher from the second instar. However, none of these studies has quantified host defence behaviours. Therefore, the underlying behavioural mechanisms behind, and fitness consequences of, host stage preference are still poorly understood for *A. colemani* on *M. persicae*, making it difficult to evaluate host-parasitoid interactions in greenhouse crops.

To provide knowledge for the evaluation of parasitoid-host interactions in greenhouse crops and development of effective mass rearing programmes, this study investigated how and why host age or size affected fitness gain in *A. colemani* on green peach aphid. Behavioural interactions between parasitoids of the same age and hosts of different ages upon encounter were recorded and quantified. Moreover, the effect of host age at parasitisation on the parasitoid progeny fitness was determined. This study shows that there was a clear trade-off between fitness gain and cost in relation to host age and that parasitising mid-aged green peach aphid nymphs was most profitable for *A. colemani*.

6.2 Materials and methods

6.2.1 Insects

The breeding colonies of *M. persicae* and *A. colemani* was established as discussed in Sections 3.2.3 and 3.2.4.

Parasitoids used for experiments were obtained by parasitising 2-d-old *M. persicae* nymphs as mentioned in the Section 3.2.5. To obtain aphids of different ages (1, 3, 5 and 7 d old, which corresponded to 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instars, respectively), 50 aphid adults were collected randomly from the above aphid colony and transferred them onto a fresh leaf cut in a plastic cylinder (Figure 3.3). The aphid adults were allowed to stay in the cylinder for 4 hours. Eight such cylinders were established. Newly born nymphs were allowed to develop on the same leaf cut until desired ages were achieved.

6.2.2 Parasitisation, host defensive behaviour and fitness gain rate

Newly emerged virgin females were individually paired with a 1-d-old virgin male in a micro-centrifuge tube for 20 minutes to allow mating to occur (Figure 3.10). Upon the termination of copulation, the males were removed. Insects who did not mate within 20 minutes were discarded. The mated females were fed with 10% honey solution soaked in cotton wool balls for about 24 hours before experiments. Four nymphs of different ages (one individual from each age) were transferred into a Petri dish (3.5 cm in diameter × 0.8 cm in height). One mated 1-d-old parasitoid female was released into the Petri dish, and behaviours were recorded of both the parasitoid and aphids using a digital camcorder (Figure 3.12) for 20 minutes. Forty-two parasitoid females were tested in 42 dishes.

The following behavioural events were recorded:

(a) encounter - the number of times the parasitoid chased an aphid or stopped walking when 0.5~1.0 cm away from a stationary aphid;

(b) attack attempt - the number of times the parasitoid curved inwards its abdomen attempting to probe an aphid of a particular age with its ovipositor upon encountering;

(c) ovipositor probing - the number of times the parasitoid probed an aphid of a particular age with its ovipositor;

(d) oviposition - eggs confirmed in aphids detected by dissecting (see below);

(e) handling time - the duration a parasitoid spent between the start of encountering a host and completion of attack attempt, ovipositor probing and/or oviposition.

Aphid defensive behaviours were recorded as:

(a) escape - an aphid walked away to avoid being attacked by a parasitoid;

(b) shaking - an aphid raised its legs and swayed its abdomen abruptly to avoid being attacked by a parasitoid.

After the above behavioural recording, parasitoid-exposed aphids were separated according to their age/size and transferred each to a fresh leaf disc (3 cm in diameter) on a moistened filter paper in a Petri dish (5.5 cm in diameter × 1 cm in height). Four days

after exposure to parasitoids all aphids were dissected under a stereomicroscope (Figure 3.11) to determine whether oviposition occurred. Parasitoid fitness gain rate in relation to host age was calculated as: number of aphids parasitised/handling time for each host age.

6.2.3 Effect of host age on parasitism, and progeny development and body size

The experimental setting was similar to that mentioned above and 27 replicates were performed for this experiment. For each replicate one 1-d-old mated female parasitoid was released into a Petri dish (5.5 cm in diameter × 1 cm in height) containing 60 aphids of different ages (15 aphids from each of four ages) on a fresh leaf disc on a moistened filter paper (Figure 3.6). The parasitoid was allowed to stay in the dish for four hours, after which time, she was removed. Aphids exposed to the parasitoid were then separated according to their age. All aphids of each age (n = 15) were transferred onto a fresh leaf cut in an above mentioned plastic cylinder. Four days after exposure to the parasitoid, five aphids from each age category were randomly selected and dissected to count the number of eggs laid as mentioned above. The remaining aphids were maintained on the same leaf cut until mummification. Mummies were individually placed in the micro-centrifuge tubes until adult emergence. Newly emerged adults were sexed and the secondary sex ratio was calculated. For both sexes, the developmental period from oviposition to adult emergence was recorded, and emergence rate was estimated as the number of emerged adults divided by total number of pupae. The number of parasitism was the sum of the number of parasitised aphids determined by dissecting and that of mummies. The hind tibial length of all parasitoids that emerged from hosts of different ages when parasitised was measured as an index of body size using the image system (Figure 3.11).

6.2.4 Effect of female parasitoid body size on reproductive potential

Because the above experiment shows that the nymph age when parasitised significantly affected the body size of parasitoid progeny (Figure 6.5B), this experiment was designed to determine whether and how the body size of female parasitoids influenced their reproductive potential. From the above experiment, 13 parasitoid females that newly emerged from aphids parasitised when 1 d old and 15 from those

parasitised when 3 d old were obtained. Females were then individually paired with 1-d-old virgin males that developed from aphids parasitised when 2 d old. Each pair was maintained in a micro-centrifuge tube for 20 minutes for mating. Each mated female was released into a Petri dish (5.5 cm in diameter × 1 cm in height) with a cabbage leaf disc (3 cm in diameter) infested by 50 2-d-old nymphs and placed on moistened filter paper, and allowed to stay in the dish for 24 hours. The parasitoid was then transferred into another Petri dish containing the same number and age of aphids infested on a leaf disc and allowed to stay for 24 hours. The process was repeated until the parasitoid died.

The parasitoid-exposed aphids were maintained on the same leaf disc for two days and then transferred to an above mentioned plastic cylinder. The aphids were monitored for mummification to record the number of parasitism.

6.2.5 Statistical analysis

A goodness-of-fit test (Shapiro-Wilk test) was used to test the distribution of data before analysis. Data on host handling time, parasitism rate, progeny body size, parasitism and fitness gain rate were analysed using an analysis of variance (ANOVA), followed by a Tukey's studentised range test. However, data on parasitism rate and fitness gain rate were arsine square-root transformed and host handling time was $\log_e(x)$ transformed before analysis. Data on number of encounters and aphid defensive behaviours, progeny developmental period, emergence rate and proportion of female progeny were not normally distributed even after transformation. Therefore, these data were analyzed using a nonparametric ANOVA followed by Bonferroni (Dunn) t Tests for multiple comparison.

Data on relationships between the number of encounters and parasitisation behavioral events were analyzed with a linear regression. Slopes of regression lines were compared using an analysis of covariance (ANCOVA). All analyses were done using SAS (SAS 9.4, SAS Institute Inc., NC, USA).

6.3 Results

6.3.1 Parasitisation, host defensive behaviour and fitness gain rate

Results show that older aphids were significantly more likely to be encountered by parasitoids ($F_{3,164} = 11.32$, $P < 0.0001$; Figure 6.1). With the increase of encounters the number of attack attempts, ovipositor probings and ovipositions significantly increased (Figure 6.2). However, ANCOVA shows that with the increase of encounters the number of ovipositions increased significantly slower than that of ovipositor probings and attack attempts, and the number of ovipositor probings increased significantly slower than that of attack attempts ($F_{2,437} = 32.25$, $P < 0.0001$; Figure 6.2).

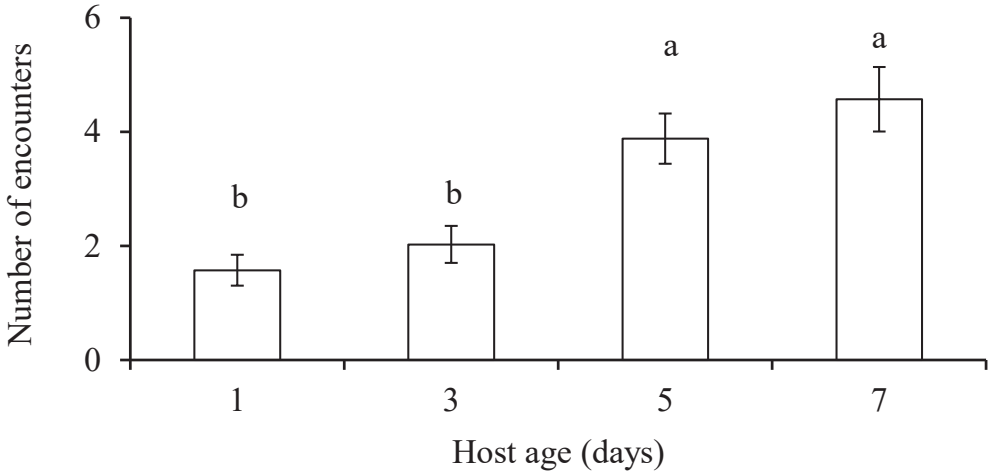

Figure 6.1 Mean (± SE) number of aphids of different ages encountered by *A. colemani* females. Columns with the same letters are not significantly different ($P > 0.05$).

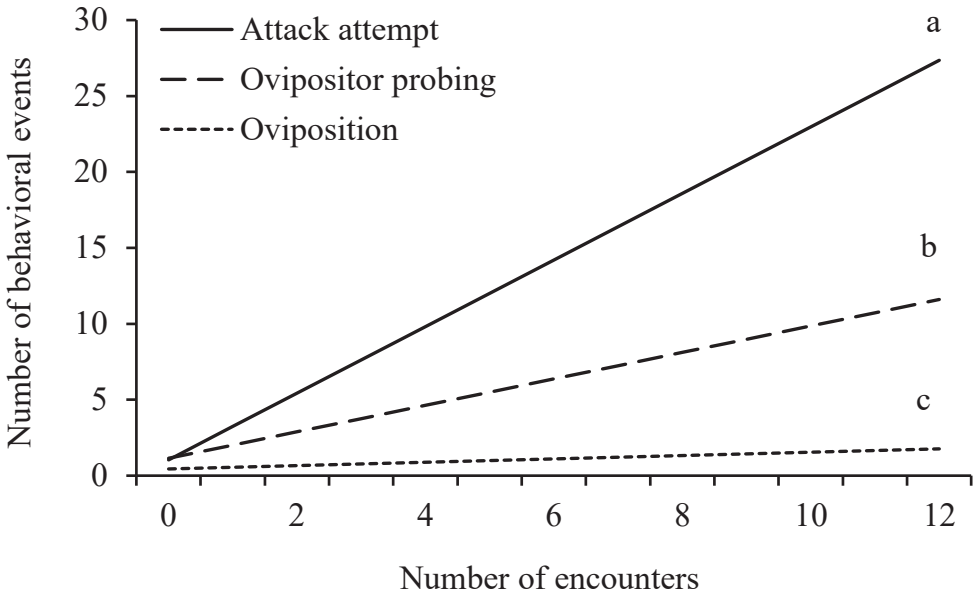

Figure 6.2 Oviposition behaviours of *A. colemani* females after encountering the hosts: **attack attempt** = 1.05 + 2.19encounter (R^2 = 0.3499, $F_{1,166}$ = 89.34, P < 0.0001); **ovipositor probing** = 1.14 + 0.87encounter (R^2 = 0.2153, $F_{1,166}$ = 45.54, P < 0.0001), and **oviposition** = 0.44 + 0.11encounter (R^2 = 0.1413, $F_{1,105}$ = 17.28, P < 0.0001). Slopes of lines with the same letters do not differ significantly (ANCOVA: P > 0.05).

The defensive ability of 7-d-old aphids against parasitoid attacks was significantly greater than that of 1- and 3-d-old aphids ($F_{3,131}$ = 10.38, P < 0.0001 for escaping; $F_{3,129}$ = 15.91, P < 0.0001 for body shaking; Figure 6.3A-B). *A. colemani* females spent significantly more time when handling older hosts ($F_{3,130}$ = 20.94, P < 0.0001; Figure 6.3C).

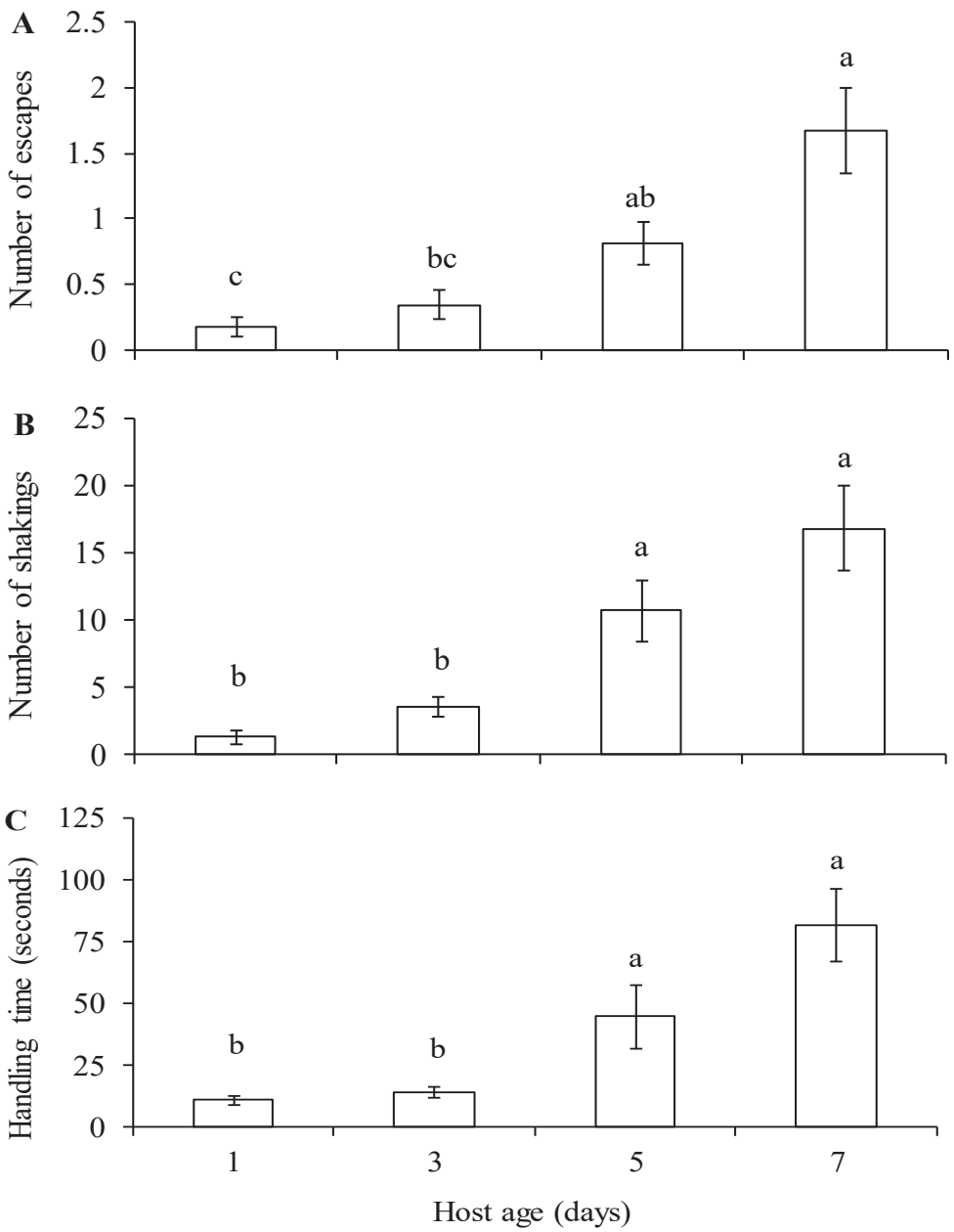

Figure 6.3 Defensive behaviours of aphids of different age and handling time of *A. colemani* females for attacking aphids: (**A**) aphid escaping, (**B**) aphid body shaking, and (**C**) handling time of *A. colemani* females. Columns with the same letters in each category are not significantly different ($P > 0.05$).

The parasitoid had significantly higher fitness gain rate when attacking 1- and 3-d-old aphids than when attacking 5- and 7-d-old ones ($F_{3,104} = 4.67$, $P = 0.0042$; Figure 6.4).

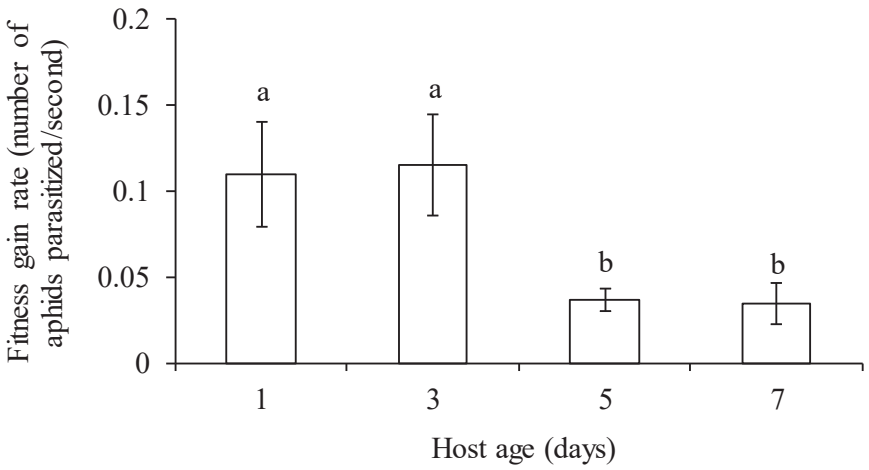

Figure 6.4 Fitness gain rate estimated from parasitism and handling time in *A. colemani*. Columns with the same letters are not significantly different (P > 0.05).

6.3.2 Effect of host age on parasitism, progeny development, body size and reproductive potential

The study shows that the host age at parasitisation had no significant effect on parasitism rate (ranged from 48% to 52%, $F_{3,104} = 0.27$, P = 0.8458), emergence rates (ranged from 88% to 95%; $F_{3,101} = 0.89$, P = 0.4062) and progeny sex ratio (ranged from 1♂:1.08♀ to 1♂:1.14♀; $F_{3,97} = 0.04$, P = 0.9910).

Parasitoid progeny of both sexes developed significantly faster in older aphids ($F_{3,188} = 12.49$, P < 0.0001 for males; $F_{3,233} = 3.65$, P = 0.0133 for females; Figure 6.5A). The progeny that developed from mid-aged aphids were significantly larger than those from younger ones ($F_{3,89} = 3.97$, P = 0.0105 for males; $F_{3,101} = 3.71$, P = 0.0140 for females; Figure 6.5B). Results show that larger parasitoids parasitised significantly more aphids than small ones ($F_{1,26} = 5.79$, P = 0.0235; Figure 6.6).

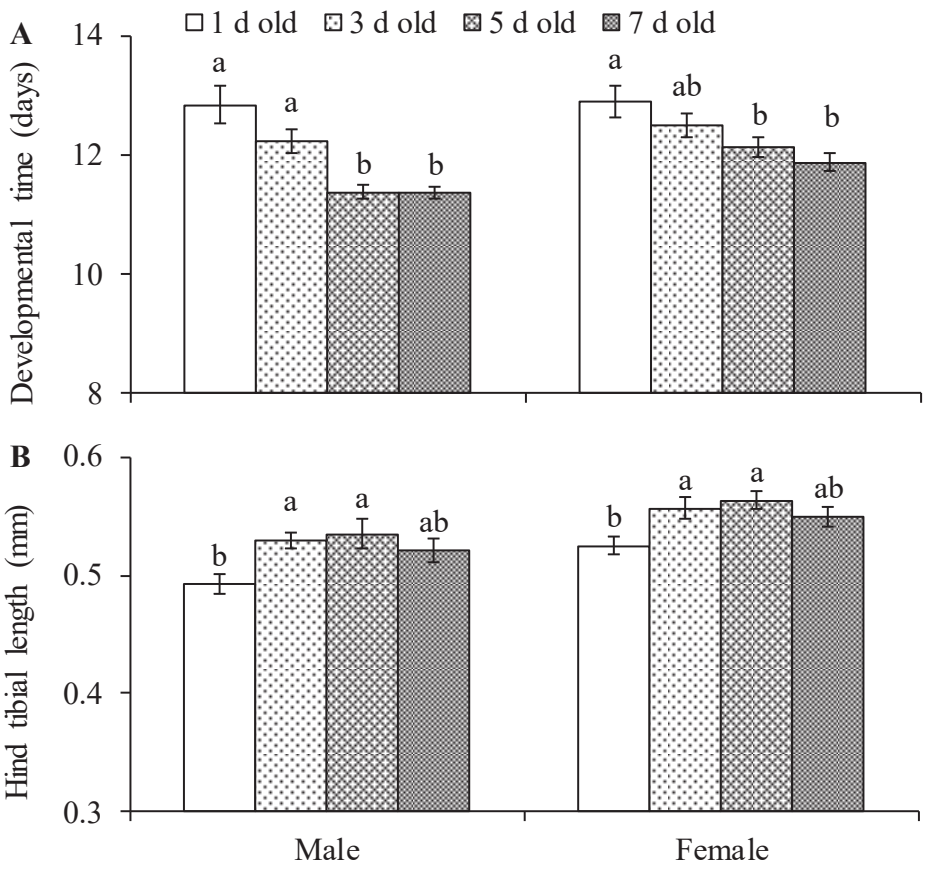

Figure 6.5 Developmental duration (**A**) and body size (**B**) of *A. colemani* progeny developing from aphids parasitised at different ages. In each sex, columns with the same letters are not significantly different (P > 0.05).

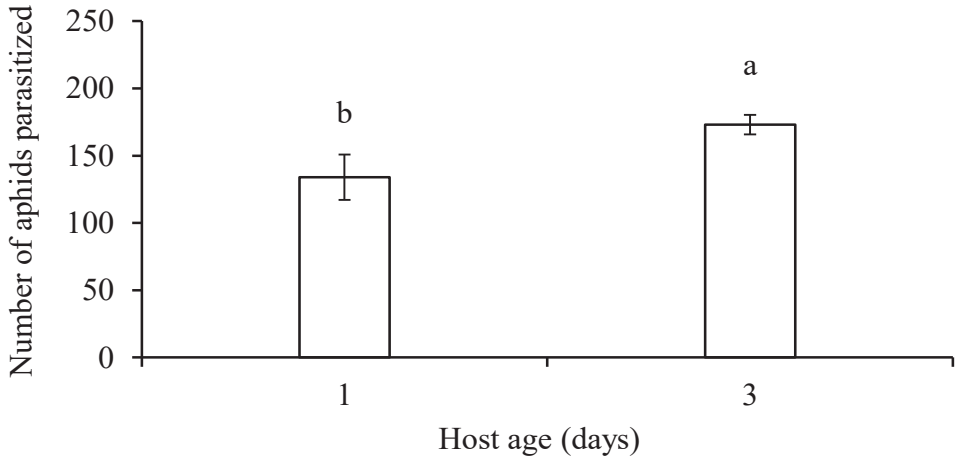

Figure 6.6 Parasitism of *A. colemani* females produced from *M. persicae* when parasitised at 1 and 3 d old. Columns with the same letters are not significantly different (P > 0.05).

6.4 Discussion

The success of natural enemies in biological control of pests largely depends on their ability to locate, recognise, select and capture their hosts and the subsequent fitness of their progeny. Unlike insect predators that need to consume many prey items before reaching maturity, the fitness of parasitoid progeny is determined entirely by the resources of a single host (van Alphen & Visser 1990). As a result, parasitising larger hosts is expected to be more profitable than smaller ones in terms of parasitoid fitness (He et al. 2005a; Wyckhuys et al. 2008; Henry et al. 2009) because larger hosts contain more resources (Liu 1985; Opp & Luck 1986). However, larger hosts at the parasitisation may have better defence ability such as kicking and escaping (Wyckhuys et al. 2008; He et al. 2011) and the hosts of koinobiont parasitoids continue to change in resources after being parasitised (Brough et al. 1990; Lin & Ives 2003).

Similar to previous findings in other parasitoid-aphid systems such as *Monoctonus paulensis* (Ashmead) (Hymenoptera: Braconidae) (Chau & Mackauer 2000) and *A. ervi* Haliday (He et al. 2011) on *A. pisum*, *A. colemani* females were significantly more likely to encounter their hosts with the increase of host size (Figure 6.1). This phenomenon could be attributed to stronger chemical (Battaglia et al. 1993; Battaglia et al. 2000) or physical (Battaglia et al. 1995; Michaud & Mackauer 1995; Mackauer et al. 1996) cues from larger hosts. Although the encounter of a host by *A. colemani* almost always triggered an attack attempt, the attempt did not proportionally translate into an ovipositor probing, and the ovipositor probing did not proportionally turn to an oviposition (Figure 6.2). The allometric patterns may be caused by the fact that larger aphids had greater ability to defend themselves (Figure 6.3A-B) and the parasitoid spent more time in handling larger aphids (Figure 6.3C), which is reflected in the fitness gain rate of parasitoids (Figure 6.4). Furthermore, my findings show that *A. colemani* had the same parasitism rate in hosts of all ages. These results suggest that *A. colemani* females prefer to attack larger aphids but the counterbalance between the parasitoid preference and host defence makes any host age preference pattern undetectable.

Previous studies on various parasitoid-host systems demonstrate that by parasitising larger hosts a parasitoid produces progeny of larger size (He et al. 2005a; Henry et al. 2009), more female-biased sex ratio (Ueno 1999; Henry et al. 2005;

Wyckhuys et al. 2008), shorter developmental time (Petitt & Wietlisbach 1993; Jenner & Kuhlmann 2006), and lower premature mortality (Jenner & Kuhlmann 2006). However, results from the current study support only a fraction of the above notions. My study shows that *A. colemani* obtained maximum fitness in terms of shorter progeny developmental period when parasitising 5-d-old hosts and had no further gain by parasitising hosts > 5-d-old (Figure 6.5A). Similarly, the progeny gained maximum body size if their mother parasitised ≥ 3-d-old hosts (Figure 6.5B). These results suggest that the parasitoid gains maximum fitness in progeny body size and developmental period by parasitising mid-age hosts.

Contradictory to previous findings, the host age at parasitisation had no effect on progeny sex ratio and emergence rates in the current study, suggesting that in the *A. colemani-M. persicae* system pupae have similar survival rate regardless of host size at parasitisation. The similar sex ratio of progeny that emerged from hosts of different ages at parasitisation may result from the fact that (1) the parasitoid females do not adjust sex allocations depending on host age, (2) the progeny of sexes have similar survival rate during the larval stage regardless of host size, or both (1) and (2).

Several authors reveal that the larger progeny produced from larger hosts at parasitisation carry more eggs at emergence (Chau & Mackauer 2001; He et al. 2005a). However, they have not tested whether these larger progenies have higher lifetime parasitism. The present study indicates that larger parasitoids that were produced from 3-d-old aphids at parasitisation parasitised significantly more aphids than smaller ones that emerged from 1-d-old hosts at parasitisation (Figure 6.6). These findings demonstrate that larger progenies produced from larger hosts at parasitisation are more fecund and probably more effective in the control of aphids.

In conclusion, the preference for larger *M. persicae* by *A. colemani* for parasitisation is counterbalanced by greater defensive ability of larger hosts, resulting in similar parasitism rate on hosts of all ages. Parasitising mid-aged hosts allows *A. colemani* females to gain maximum fitness in developmental period, body size and parasitism of their progenies. The parasitoid progenies have similar sex ratio and survival rate regardless of host size. Taking all findings together, parasitising mid-aged

green peach aphid nymphs is most profitable for *A. colemani*. It is suggested that in *A. colemani* mass rearing programmes for the control of *M. persicae*, using aphids of mid-ages as hosts may substantially increase the production of parasitoids.

Chapter Seven

Functional Response of *Aphidius colemani*

7.1 Introduction

In nature, damage to plants by herbivores is usually maintained at an acceptable level by naturally occurring enemies such as parasitoids, predators and pathogens (DeBach & Rosen 1991). The success of biological control of insect pests using parasitoids usually depends on their functional and demographic responses to host density and the mutual interference between searching parasitoids (Kidd & Jervis 2005; He & Wang 2014). Functional response is defined as per capita response of a parasitoid to the variation in host density (Solomon 1949) whereas demographic response refers to the change in parasitoid density as a function of change in host density (Solomon 1949; Hassell 1978). These parameters are widely used to predict the potential success of biological control programmes (Fernández-arhex & Corley 2003; He & Wang 2014; Hanan et al. 2017).

Holling (1959) developed three functional response curves to describe the responses of natural enemies to the changing host density: (1) Type I functional response – the relationship between the number of parasitised hosts and host density is linear; (2) Type II functional response - the proportion of hosts parasitised by a parasitoid decreases exponentially as host density increases, and (3) Type III functional response - there are an initial increase and a subsequent decrease in the proportion of hosts parasitised with increasing host density. Most parasitoid species appear to have a Type II response (Fernández-arhex & Corley 2003). However, these functional response curves are determined based on host density only, neglecting other factors that may also influence the host-parasitoid dynamics, for example, parasitoid age (Tazerouni et al. 2016) and density (Montoya et al. 2000; Chong & Oetting 2006; He & Wang 2014; Luo et al. 2014). As a result, it is often difficult to establish the relationship between the curve shape and biological control success (Fernández-arhex & Corley 2003).

Various studies reveal that the age of parasitoids affects how they respond to host density (Bellows 1985; Völkl & Mackauer 1990; Asadi et al. 2012; Nikbin et al. 2014; Pasandideh et al. 2015; Tazerouni et al. 2016). For example, in *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) (Nikbin et al. 2014) and *A. matricarie* (Tazerouni et al. 2016), with the increase of female age their type of functional response shifts from Type III to Type II, whereas in *Paron volucre* (Haliday) (Hymenoptera: Braconidae), female age does not significantly affect the the type of functional response (Tazerouni et al. 2016). Furthermore, foraging parasitoids may encounter each other on the same host patch (Chong & Oetting 2006; He & Wang 2014), which may cause mutual interference, reducing biological control efficiency (Hassell & Varley 1969; Cronin & Strong 1993; He & Wang 2014). For example, mutual interference in *Anagyrus* spp can result in lower parasitism rate and progeny production (Chong & Oetting 2006). In *Gonatocerus* spp., singly foraging females produce highly female-biased offspring while those in group on the same host patch yield male-biased offspring (Irvin & Hoddle 2006).

Aspects of functional response in *A. colemani* have been studied by several groups (van Steenis & El-Khawass 1995a; Jones et al. 2003; Byeon et al. 2011). However, the experimental designs of those studies keep parasitoid density constant (n =1) and only allow host density to vary. In the present study I carried out a series of experiments to investigate how age of adult female *A. colemani* and density of both *A. colemani* and *M. persicae* affected host searching and parasitism. Information generated from this study is critical to estimating the potential impact of *A. colemani* on *M. persicae* population dynamics and improving the implementation of biological control programmes.

7.2 Materials and methods

7.2.1 Insects

Parasitoids used for the experiments were obtained as described in Sections 3.2.3-3.2.5. Female parasitoids mated at 1 d old were used for experiments.

7.2.2 Effect of parasitoid age and host density on parasitoid fitness

This experiment was to test the functional response of *A. colemani* of different ages (1, 3 and 5 d old) to 2-d-old *M. persicae* nymphs of five densities (10, 20, 30, 40 and 50 aphids) with 15 combined treatments. For each treatment, a mated female parasitoid of a desired age was released into a Petri dish (5.5 cm in diameter × 1.0 cm in height) containing hosts of a test density feeding on a leaf disc (3 cm in diameter). The female was allowed to forage and oviposit for four hours, after which time, it was removed using an aspirator (Figure 3.5). The parasitoid-exposed aphids were allowed to feed on same leaf disc for two days and then transferred to a plastic cylinder (Figure 3.3). Four days after exposure, 50% of aphids from each plastic cylinder were removed and dissected under a stereomicroscope (Figure 3.11) to determine parasitism (and superparasitism). The remaining aphids were reared on the leaf disc until mummification. Mummies were collected and individually maintained in 2-ml micro-centrifuge tubes until emergence. Emerged adults were sexed and the proportion of female offspring was calculated. The total number of parasitised aphids was the sum of the number of parasitism determined by dissecting and that of mummies. The parasitism rate was estimated as the number of parasitised aphids divided by host density, and the number of female offspring produced as the total number of parasitised aphids multiplied by the proportion of female offspring. There were 13 to 21 replicates for each treatment.

7.2.3 Effect of parasitoid and host density on parasitoid fitness

In this experiment I investigated the functional response of *A. colemani* of different densities (1, 3 and 5 females) to five host densities (10, 20, 30, 40 and 50 aphids) with 15 combined treatments. All parasitoids used for experiment were 1 d old and aphids were 2 d old. The experimental procedures and data recording were the same as the above experiment. I set up 13 to 20 for each treatment.

7.2.4 Statistical analysis

7.2.4.1 Functional response

A cubic logistic regression model was used to determine its functional response (Type II or Type III) by taking into consideration the proportion of parasitised hosts (N_a/N_o) as a function of the initial density of hosts (N_o) (Julious 2001):

$$N_a/N_o = \exp(P_0+P_1N_o+P_2N_o^2+P_3N_o^3)/[1+ \exp(P_0+P_1N_o+P_2N_o^2+P_3N_o^3)]$$

where N_a is the number of parasitised hosts, N_o the number of hosts, and P_0, P_1, P_2, and P_3 the intercept, linear, quadratic and cubic parameters, respectively. The sign of the linear parameter estimated by the logistic regression can be used to distinguish Type II and Type III functional responses (De Clercq et al. 2000; Julious 2001). A linear coefficient of $P_1 = 0$ indicates a Type I functional response; if $P_1 < 0$, the proportion of parasitised host declines monotonically with the initial number of hosts offered, describing a Type II functional response; if $P_1 > 0$, the proportion of parasitised hosts is positively density-dependent, describing a Type III functional response (Julious 2001). However, a quartic logistic regression fitting those data was required (by adding the quartic term, $P_4N_o^4$ to the model) (Julious 2001) to confirm the results of the cubic regressions (Table 7.1).

In this study, the type of functional responses was determined to be Type II (see Results). For Type II functional response, the searching efficiency (a) and the handling time (T_h) were estimated using both disc model (Holling 1959) and random model (Rogers 1972) as follows:

$$N_a = (aTN_oP_t)/(1+aT_hN_o) \qquad \text{(Holling 1959)}$$

$$N_a = N_o[1-\exp(-aTP_t/(1+aT_hN_o))] \qquad \text{(Rogers 1972)}$$

where P_t is the number of parasitoids (P_t = 1 for the parasitoid age experiment, and P_t = 1, 3 and 5 for the parasitoid density experiment), and T is the total time (4 h) available for the parasitoid. Holling's model assumes that the density of available hosts decreases over time while Rogers' model considers that the host density is constant (i.e., parasitised hosts are re-encountered) (Hassell 1978). Both Holling's and Rogers' models were used to test how our data fitted in with these theoretic predictions. The searching efficiency (a) and the handling time (T_h) were estimated separately for each

parastoid age and each parastoid density, and then estimated for the overall parasitoid density.

7.2.4.2 Demographic response

To analyse parasitoid demographic response in the parasitoid density experiment, a model was developed (Waage & Hassell 1982; Hassell & Waage 1984):

$$P_{t+1} = SCN_o[1\text{-}\exp(\text{-}aP_t)]$$

where P_t and P_{t+1} are the number of female parasitoids searching in a host patch and the subsequent number of female progeny produced, respectively; S is the proportion of female progeny produced, and C is the average number of adult parasitoids emerging from each parasitised host ($C = 1$ for solitary parasitoids such as *A. colemani*; $C > 1$ for gregarious parasitoids). The proportion of female progeny produced was estimated as:

$$S = P_{t+1}/\{CN_o[1\text{-}\exp(\text{-}aP_t)]\}.$$

7.2.4.3 Mutual interference

To detect mutual interference among the foraging *A. colemani* females, a model developed by Hassell and Varley (1969) was used:

$$\log a = \log Q - m\log P_t \text{ or } a = QP_t^{-m}$$

where P_t is the number of parasitoids; Q is the constant and the searching efficiency (a) equals Q if only a single parasitoid is present; m is the mutual interference constant. The searching efficiency (a) for each host patch was defined by Hassell (1978) as: $a = (1/P_tT)\ln[N_o/(N_o\text{-}N_a)]$, where T is the duration (4 h) of experiment.

The mutual interference constant m can be used to estimate the contribution of mutual interference to the stability of the parasitoid-host system. In general, the higher m is, the more stable the interaction between parasitoids and hosts becomes (Hassell 2000; Kidd & Jervis 2005; Price et al. 2011). According to Hassell and May (1973), the host populations approach equilibrium when:

$$1 > m > 1 - (F\text{-}1)/(F\ln F)$$

where F is the rate of increase of host population (if the mortality rate is 90%, then $F = 10$).

7.2.4.4 Data computing

The logistic regression model of Julious (2001) was performed using PROC CATMOD to determine the type of functional responses. Nonlinear least square regression (PROC NLIN) was used to estimate the searching efficiency (a) and handling time (T_h) by fitting the data in the functional and demographic response equations. The estimated parameters in the nonlinear least square regressions were significantly different from 0 if the 95% confidence interval (CI) did not include 0 (Julious 2001). The coefficient of determination (R^2) for nonlinear least square regressions was calculated as: 1 – (residual sum of square/corrected total sum of square) (Tahriri et al. 2007). The difference in the mean searching efficiency or handling time between Holling's (1959) and Rogers' (1972) models (Tables 7.5 and 7.6) was compared according to Julious (2004). If the 83.4% CI overlaps, then there is no significant difference in these means between the two models (Julious 2004). The mutual interference constant (m) was estimated by determining the linear relationship between log$a$ and logP_t.

A generalised logistic linear model (PROC CATMOD) was applied to compare the difference between parasitoid age and host density or between parasitoid density and host density:

$$N_a \text{ or } N_f = \text{Exp}(e + b_1P + b_2P^2 + c_1N_o + c_2N_o^2 + dPN_o)$$

where N_f is the number of female offspring producted, P is the parasitoid age (P_a) or density (P_t), and e, b_1, b_2, c_1, c_2 and d are the estimated constant parameters of the model. Only significant parameters were included in the final model. The level of this generalised logistic linear model to fit my data was compared with that of demographic response (see Section 7.3.3) through comparing the coefficients of determination (R^2) of the models according to Littell et al. (2002).

7.3 Results

7.3.1 Functional response

Results of functional response are shown in Tables 7.1 and 7.2 for the effect of parasitoid age and density, respectively. The response of *A. colemani* of different ages or different densities fitted a Type II functional response because (1) the linear parameters of regressions were negative for 3-d-old parasitoids (Table 7.1) and for

parasitoid density of five individuals (Table 7.2) and (2) the positive effect of linear parameters detected in the cubic logistic regression for 1- and 5-d-old parasitoids (Table 7.1) and for parasitoid density of one and three individuals (Table 7.2) were not consistent in the quartic regression. The Type II functional response was then confirmed by the significant negative effect of host density on the parasitism rate (Figures 1B and 2B)

Table 7.1 Logistic regression analysis of functional responses of *A. colemani* parasitising *M. persicae*: effect of parasitoid age.

Parasitoid age	Parameters	Estimate	SE	x^2	P
One day old	*Cubic regression*				
	P_0	-4.09	0.92	19.68	< 0.0001
	P_1	0.30	0.99×10^{-1}	9.28	0.0023
	P_2	-0.78×10^{-2}	0.33×10^{-2}	5.80	0.0160
	P_3	0.67×10^{-4}	0.33×10^{-4}	4.09	0.0431
	Quartic regression				
	P_0	-3.33	0.80	17.54	< 0.0001
	P_1	-0.17	0.75×10^{-1}	4.89	0.0270
	P_2	0.39×10^{-4}	0.18×10^{-2}	0.00	0.9833
	P_3	-0.12×10^{-3}	0.44×10^{-4}	13.09	0.0003
	P_4	0.15×10^{-7}	0.27×10^{-8}	31.15	< 0.0001
Three day old	*Cubic regression*				
	P_0	-1.65	0.82	3.98	0.0461
	P_1	-0.05	0.91×10^{-1}	0.34	0.5615
	P_2	0.35×10^{-2}	0.31×10^{-2}	1.35	0.2447
	P_3	-0.40×10^{-4}	0.31×10^{-4}	1.91	0.1668
	Quartic regression				
	P_0	-5.70	2.19	6.78	0.0092
	P_1	0.66	0.37	3.26	0.0711
	P_2	-0.38×10^{-1}	0.20×10^{-1}	3.31	0.0690
	P_3	0.92×10^{-3}	0.48×10^{-3}	3.66	0.0556
	P_4	-0.78×10^{-7}	0.39×10^{-7}	4.03	0.0448

Table 7.1 (continue) Logistic regression analysis of functional responses of *A. colemani* parasitising *M. persicae*: effect of parasitoid age.

Parasitoid age	Parameters	Estimate	SE	x^2	P
Five day old	*Cubic regression*				
	P_0	-5.29	0.99	28.23	< 0.0001
	P_1	0.39	0.10	13.94	0.0002
	P_2	-0.11×10^{-1}	0.34×10^{-2}	11.00	0.0009
	P_3	0.11×10^{-3}	0.34×10^{-4}	9.91	0.0016
	Quartic regression				
	P_0	-2.42	2.26	1.14	0.2849
	P_1	-0.11	0.37	0.09	0.7659
	P_2	0.18×10^{-1}	0.21×10^{-1}	0.71	0.4007
	P_3	-0.56×10^{-3}	0.48×10^{-3}	1.36	0.2430
	P_4	0.54×10^{-7}	0.37×10^{-7}	1.95	0.1631

Table 7.2 Logistic regression analysis of functional responses of *A. colemani* parasitising *M. persicae*: effect of parasitoid density.

Parasitoid density	Parameter	Estimate	SE	x^2	P
One female	*Cubic regression*				
	P_0	-4.09	0.92	19.68	< 0.0001
	P_1	0.30	0.99×10^{-1}	9.28	0.0023
	P_2	-0.78×10^{-2}	0.33×10^{-2}	5.80	0.0160
	P_3	0.67×10^{-4}	0.33×10^{-4}	4.09	0.0431
	Quartic regression				
	P_0	-3.33	0.80	17.54	< 0.0001
	P_1	-0.17	0.75×10^{-1}	4.89	0.0270
	P_2	0.39×10^{-4}	0.18×10^{-2}	0.00	0.9833
	P_3	-0.12×10^{-3}	0.44×10^{-4}	13.09	0.0003
	P_4	0.15×10^{-7}	0.27×10^{-8}	31.15	< 0.0001

Table 7.2 (continue) Logistic regression analysis of functional responses of *A. colemani* parasitising *M. persicae*: effect of parasitoid density.

Parasitoid density	Parameter	Estimate	SE	x^2	P
Three females	*Cubic regression*				
	P_0	-2.74	0.96	8.06	0.0045
	P_1	0.74×10^{-1}	0.11	0.48	0.4898
	P_2	-0.24×10^{-2}	0.36×10^{-2}	0.45	0.5047
	P_3	0.29×10^{-4}	0.37×10^{-4}	0.64	0.4230
	Quartic regression				
	P_0	2.99	2.56	1.36	0.2430
	P_1	-0.95	0.44	4.68	0.0305
	P_2	0.58×10^{-1}	0.25×10^{-1}	5.24	0.0221
	P_3	-0.14×10^{-2}	0.58×10^{-3}	5.56	0.0183
	P_4	0.11×10^{-4}	0.47×10^{-7}	5.85	0.0156
Five females	*Cubic regression*				
	P_0	-2.38	1.06	5.08	0.0242
	P_1	-0.53×10^{-1}	0.12	0.20	0.6549
	P_2	0.35×10^{-2}	0.39×10^{-2}	0.81	0.3692
	P_3	-0.50×10^{-4}	0.40×10^{-4}	1.26	0.2615
	Quartic regression				
	P_0	-2.61	0.36	52.48	< 0.0001
	P_1	-0.32×10^{-1}	0.14×10^{-1}	5.47	0.0193
	P_2	0.33×10^{-2}	0.65×10^{-3}	21.12	< 0.0001
	P_3	-0.60×10^{-4}	0.50×10^{-4}	4.07	0.0436
	P_4	0.20×10^{-8}	0.62×10^{-9}	10.27	0.0014

As shown in Tables 7.3 and 7.4, both Holling's and Rogers' models fitted parameters for each parasitoid age and density indicated by similar R^2 values. However, for the overall parasitoid density, Rogers' model fitted my data better than did Holling's model (R^2 = 0.8804 vs 0.3427) (Table 7.4).

Table 7.3 Statistical results of modelling Type II functional response of *A. colemani* parasitising *M. persicae*: effect of parasitoid age.

Parasitoid age	Model	R^2	F	df	P
One day old	Holling	0.5614	317.67	2,60	< 0.0001
	Rogers	0.5611	317.49	2,60	< 0.0001
Three day old	Holling	0.7432	1001.67	2,88	< 0.0001
	Rogers	0.7430	1000.96	2,88	< 0.0001
Five day old	Holling	0.6607	590.18	2,73	< 0.0001
	Rogers	0.6606	589.88	2,73	< 0.0001

Table 7.4 Statistical results of modelling Type II functional response of *A. colemani* parasitising *M. persicae*: effect of parasitoid density.

Parasitoid density	Model	R^2	F	df	P
One female	Holling	0.5614	317.67	2,60	< 0.0001
	Rogers	0.5611	317.49	2,60	< 0.0001
Two females	Holling	0.8775	2109.15	2,86	< 0.0001
	Rogers	0.8778	2113.74	2,86	< 0.0001
Five females	Holling	0.8967	2374.99	2,88	< 0.0001
	Rogers	0.8967	2374.03	2,88	< 0.0001
Overall density	Holling	0.3427	826.49	2,238	< 0.0001
	Rogers	0.8004	2995.01	2,238	< 0.0001

The estimated searching efficiency (a) and handling time (T_h) for parasitoids of different age and density are shown in Table 7.5 and 7.6, respectively. In 3- and 5-d-old parasitoids the searching efficiency (a) estimated by Rogers' model was significantly higher than that by Holling's model (83.4% CL did not overlap) while for 1-d-old parasitoids, a estimated by both models was similar (83.4% CL overlapped) (Table 7.5). The handling time (T_h) estimated by Rogers' model was slightly longer than that by

Holling's model but was not significantly different between the two models (83.4% CL overlapped) (Table 7.5).

For Rogers' model, the searching efficiency (a) of 3- and 5-d-old parasitoids was higher than that of 1-day-old ones, and the handling time (T_h) of 3-d-old parasitoids was shorter than that of 1- and 5-d-old ones; however, no significant difference was detected between parasitoids of different age (83.4% CL overlapped) (Table 7.5).

Table 7.5 Searching efficiency (a, h^{-1}) and handling time (T_h, h) and their confidence limits (CL) for Type II functional response of *A. colemani* parasitising *M. persicae*: effect of parasitoid age.

Parasitoid age	Model	Parameter	Estimate	SE	95% CL		83.4% CL	
One day old	Holling	a	0.1891	0.0344	0.1204	0.2578	0.1409	0.2372
		T_h	0.0406	0.0238	-0.0069	0.0882	0.0073	0.0740
	Rogers	a	0.3436	0.1245	0.0945	0.5927	0.1690	0.5182
		T_h	0.0429	0.0259	-0.0089	0.0947	0.0066	0.0792
Three day old	Holling	a	0.2311	0.024	0.1834	0.2789	0.1975	0.2647
		T_h	0.0296	0.0111	0.0076	0.0516	0.0142	0.0450
	Rogers	a	0.5851	0.1906	0.2064	0.9639	0.3189	0.8513
		T_h	0.0344	0.0134	0.0077	0.0611	0.0156	0.0532
Five day old	Holling	a	0.2366	0.0316	0.1937	0.2795	0.2124	0.2608
		T_h	0.0460	0.0146	0.0170	0.0750	0.0256	0.0664
	Rogers	a	0.6091	0.2516	0.1277	1.1305	0.2771	0.9411
		T_h	0.0516	0.0171	0.0175	0.0857	0.0277	0.0755

For parasitoid density of one female the searching efficiency (a) was not significantly different between Holling's and Rogers' models (83.4% CL overlapped); however, for parasitoid densities of three and five females and overall parasitoid density, the searching efficiency (a) estimated by Rogers' model was significantly higher than that by Holling's model (83.4% CL did not overlap) (Table 7.6). The handling time (T_h) estimated by Rogers' model was longer than that by Holling's model but was not significantly different between the two models (83.4% CL overlapped) (Table 7.6).

For Rogers model, the searching efficiency (a) decreased with increasing parasitoid density, and the handling time (T_h) for parasitoid densities of one and five females was shorter than that for parasitoid density of three females; however, no significant difference was detected between different parasitoid densities (83.4% CL overlapped) (Table 7.6).

Table 7.6 Searching efficiency (a, h^{-1}) and handling time (T_h, h) and their confidence limits (CL) for Type II functional response of *A. colemani* parasitising *M. persicae*: effect of parasitoid density.

Parasitiod density	Model	Parameter	Estimate	SE	95% CL		83.4% CL	
One female	Holling	a	0.1891	0.0344	0.1204	0.2578	0.1409	0.2372
		T_h	0.0406	0.0238	-0.0069	0.0882	0.0073	0.0740
	Rogers	a	0.3436	0.1245	0.0945	0.5927	0.1690	0.5182
		T_h	0.0429	0.0259	-0.0089	0.0947	0.0066	0.0792
Three females	Holling	a	0.0815	0.0056	0.0704	0.0926	0.0737	0.0893
		T_h	0.0540	0.0206	0.0131	0.0949	0.0253	0.0828
	Rogers	a	0.2688	0.0889	0.0921	0.4455	0.1447	0.3930
		T_h	0.0619	0.0292	0.0037	0.1200	0.0210	0.1027
Five females	Holling	a	0.0462	0.0030	0.0403	0.0521	0.0421	0.0503
		T_h	0.0350	0.0327	-0.0300	0.0999	-0.0107	0.0806
	Rogers	a	0.1256	0.0320	0.0621	0.1892	0.0810	0.1703
		T_h	0.0477	0.0473	-0.0463	0.1418	-0.0184	0.1139
Overall density	Holling	a	0.0590	0.0065	0.0463	0.0718	0.0500	0.0680
		T_h	0.0574	0.0447	-0.0307	0.1455	-0.0048	0.1195
	Rogers	a	0.3978	0.1403	0.1213	0.6743	0.2028	0.5928
		T_h	0.0855	0.0217	0.0428	0.1281	0.0554	0.1156

7.3.2 Effect of parasitoid age and density and host density on parasitoid fitness

My results show that the number of aphids parasitised significantly increased with increasing parasitoid age ($F_{1,222} = 14.07$, $P = 0.0002$) and host density ($F_{1,222} = 76.42$, $P < 0.0001$) and then significantly decreased ($F_{1,222} = 11.15$, $P = 0.0008$ for parasitoid age;

$F_{1,222}$ = 22.29, P < 0.0001 for host density) (Figure 7.1A). Parasitoid age had significantly more effect than did host density, i.e., the number of aphids parasitised increased and decreased significantly faster with increasing parasitoid age than with increasing host density (83.4% CL did not overlap) (Figure 7.1A). Host density had significantly negative effect on parasitism rate ($F_{1,223}$ = 34.63, P < 0.0001), and with the increase of parasitoid age, parasitism rate significantly increased ($F_{1,223}$ = 13.74, P = 0.0002) and then decreased ($F_{1,223}$ = 10.90, P = 0.0010) (Figure 7.1B).

As Figure 7.1C shows, the number of female offspring produced significantly increased with increasing parasitoid age ($F_{1,210}$ = 7.63, P = 0.0058) and host density ($F_{1,210}$ = 37.89, P < 0.0001) and then significantly decreased ($F_{1,210}$ = 6.03, P = 0.0141 for parasitoid age; $F_{1,210}$ = 14.60, P < 0.0001 for host density). Parasitoid age had significantly more effect than did host density, i.e., the number of female offspring produced increased and decreased significantly faster with increasing parasitoid age than with increasing host density (83.4% CL did not overlap) (Figure 7.1C). The proportion of female offspring was not significantly affected by parasitoid age ($F_{1,218}$ = 0.09, P = 0.7670) or host density ($F_{1,218}$ = 3.23, P = 0.0723) (Figure 7.1D).

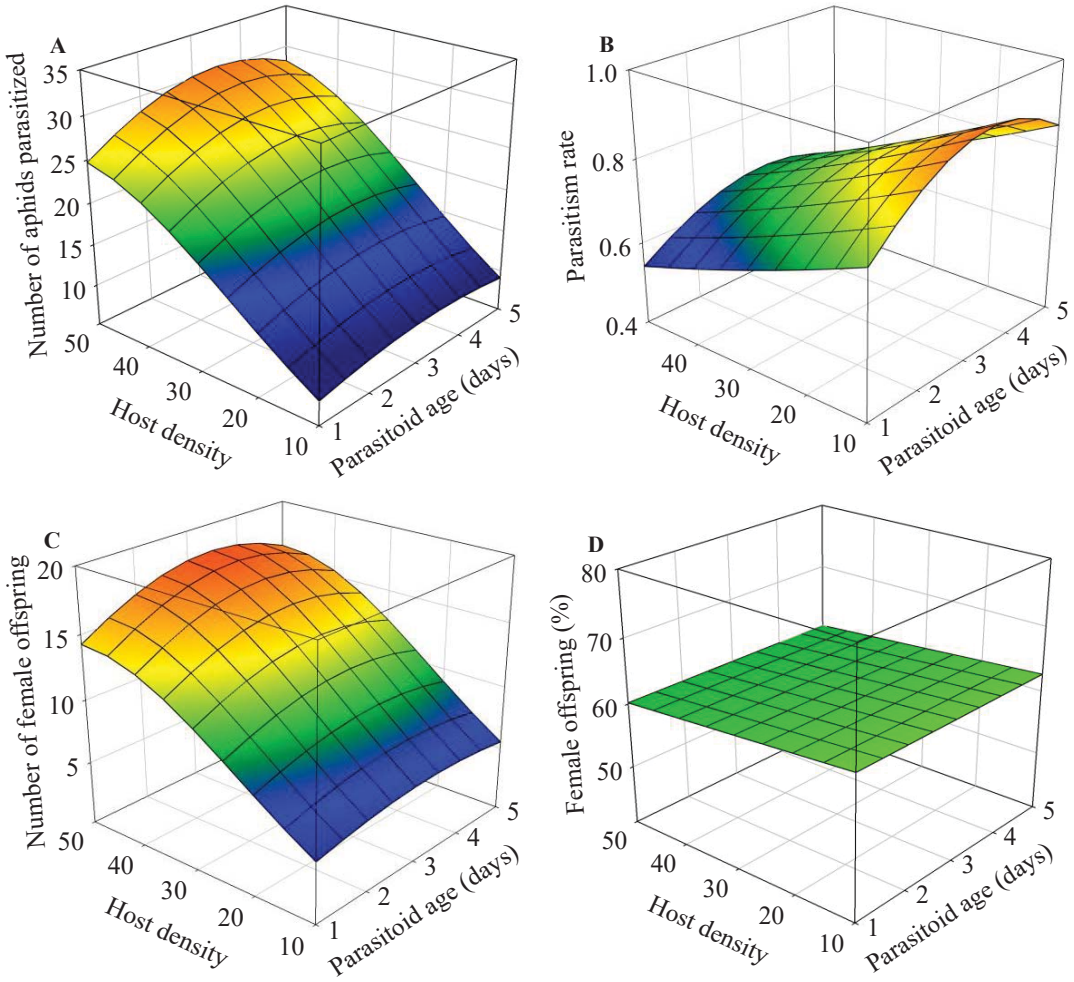

Figure 7.1 Reproductive fitness of *A. colemani* of different age (*Pa*) in response to host density (*No*). (**A**) Number of hosts parasitised (N_a): $N_a = \exp(1.2917 + 0.2344Pa - 0.0366Pa^2 + 0.0643N_o - 0.0006N_o^2)$ ($F_{4,222} = 116.35$, $P < 0.0001$, $R^2 = 0.6772$); (**B**) Parasitism rate (N_a/N_o): $N_a/N_o = \exp(-0.4065 + 0.2317Pa - 0.0333Pa^2 - 0.0081N_o)$ ($F_{3,223} = 16.39$, $P < 0.0001$, $R^2 = 0.1806$); (**C**) Number of female offspring (N_f): $N_f = \exp(1.4881 + 0.1048Pa - 0.0011Pa^2 + 0.0072N_o - 0.0006PaN_o)$ ($F_{4,222} = 90.75$, $P < 0.0001$, $R^2 = 0.3048$); (**D**) Proportion of female offspring [N_f(%)]: $N_f(\%) = \exp(4.1833 - 0.0060Pa - 0.0031N_o)$ ($F_{2,218} = 1.66$, $P = 0.1930$, $R^2 = 0.0150$).

With the increase of parasitoid and host density, the number of aphids parasitised significantly increased ($F_{1,234} = 20.35$ and 31.90 for parasitoid and host, respectively; $P < 0.0001$) and then significantly decreased ($F_{1,234} = 19.87$ and 53.56 for parasitoid and host, respectively; $P < 0.0001$) (Figure 7.2A). Parasitoid density had significantly more

effect than did host density, i.e., the number of aphids parasitised increased and decreased significantly faster with increasing parasitoid density than with increasing host density (83.4% CL did not overlap) (Figure 7.2A). There was a significantly positive interaction between parasitoid and host densities for parasitism ($F_{1,234} = 7.78$, $P = 0.0053$). Host density had significantly negative effect on the parasitism rate ($F_{1,235} = 20.21$, $P < 0.0001$), and with the increase of parasitoid density, parasitism rate significantly increased ($F_{1,235} = 20.40$, $P < 0.0001$) and then significantly decreased ($F_{1,235} = 19.71$, < 0.0001) (Figure 7.2B). A significantly positive interaction between parasitoid and host densities was also detected for parasitism rate ($F_{1,235} = 7.94$, $P = 0.0048$).

The number of female offspring produced significantly increased with increasing parasitoid and host densities ($F_{1,223} = 28.51$ and 43.43 for parasitoid and host density, respectively; $P < 0.0001$) and then significantly decreased ($F_{1,223} = 20.59$, $P < 0.0001$ for parasitoid density; $F_{1,223} = 9.96$, $P = 0.0016$ for host density) (Figure 7.2C). Parasitoid density had significantly more effect than did host density, i.e., the number of female offspring produced increased or decreased significantly faster with increasing parasitoid density than with increasing host density (83.4% CL did not overlap) (Figure 7.2C). With increasing parasitoid density, the proportion of female offspring significantly increased ($F_{1,233} = 4.55$, $P = 0.0329$) and then significantly decreased ($F_{1,233} = 5.57$, $P = 0.0182$), and the proportion of female offspring significantly decreased at lower host density ($F_{1,233} = 4.43$, $P = 0.0353$) and then significantly increased at higher host density ($F_{1,233} = 4.39$, $P = 0.0362$) (Figure 7.2D).

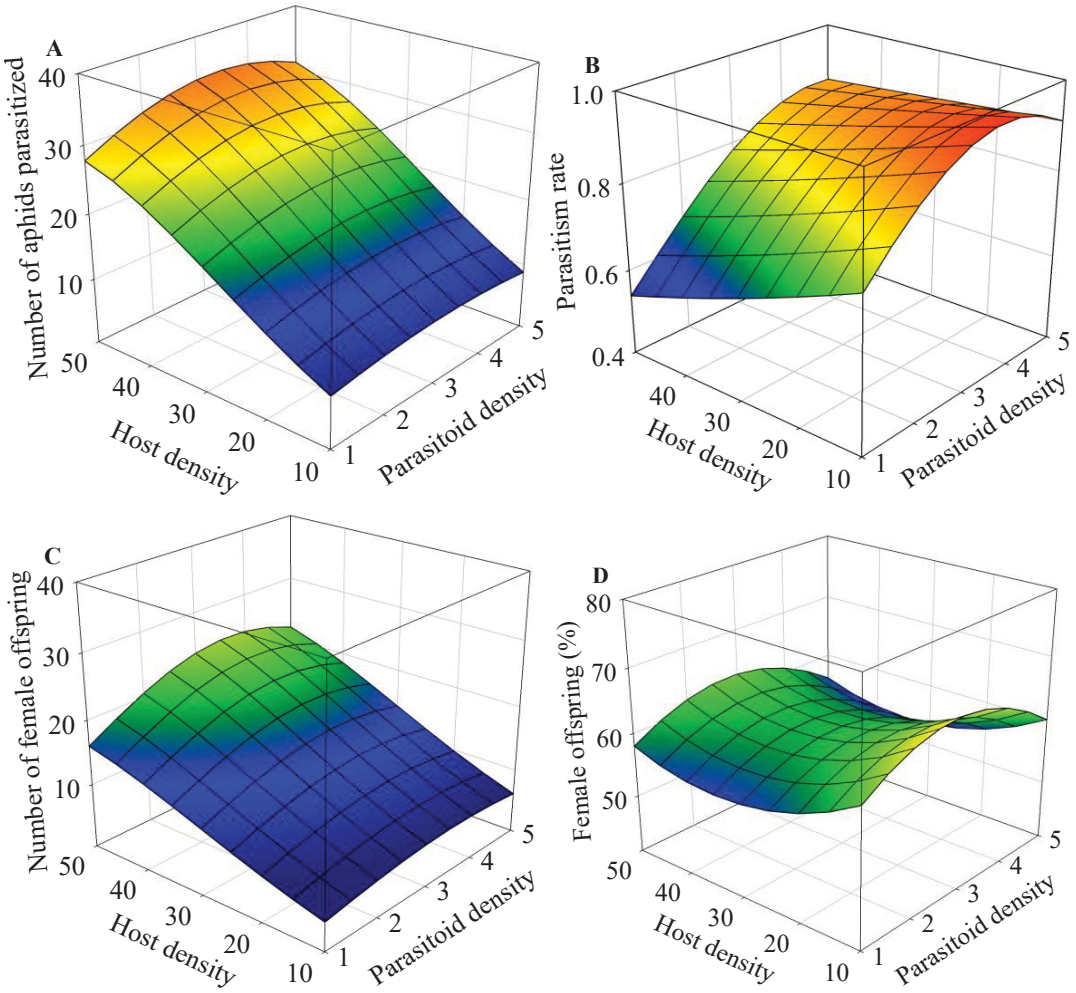

Figure 7.2 Reproductive fitness of *A. colemani* of different density (P_t) in response to host density (N_o). (**A**) Number of hosts parasitised (N_a): $N_a = \exp(1.2399 + 0.2328Pt - 0.0340Pt^2 + 0.0667N_o - 0.0006N_o^2 + 0.0018PtN_o)$ ($F_{5,234} = 224.66$, $P < 0.0001$, $R^2 = 0.8276$); (**B**) Parasitism rate (N_a/N_o): $N_a/N_o = \exp(-0.3950 + 0.2304Pt - 0.0335Pt^2 - 0.0102N_o + 0.0018PtN_o)$ ($F_{4,225} = 28.24$, $P < 0.0001$, $R^2 = 0.3248$); (**C**) Number of female offspring (N_f): $N_f = \exp(0.5612 + 0.4291Pa - 0.0585Pa^2 + 0.0618N_o - 0.0005N_o^2)$ ($F_{4,223} = 94.65$, $P < 0.0001$, $R^2 = 0.6294$); (**D**) Proportion of female offspring [N_f(%)]: $N_f(\%) = \exp(4.1370 + 0.1381Pa - 0.0245Pt^2 - 0.0138N_o + 0.0002N_o^2)$ ($F_{4,233} = 29.10$, $P < 0.0001$, $R^2 = 0.3331$).

With increasing parasitoid age, superparasitism rate significantly increased ($F_{1,175} = 4.39$, $P = 0.0361$) and then significantly decreased ($F_{1,175} = 4.05$, $P = 0.0457$), and with increasing host density, it significantly decreased ($F_{1,175} = 38.42$, $P < 0.0001$) (Figure

7.3A). Superparasitism rate significantly increased with increasing parasitoid density ($F_{1,220}$ = 137.12, P < 0.0001) and then significantly decreased ($F_{1,220}$ = 97.79, P < 0.0001); it significantly decreased with increasing host density ($F_{1,220}$ = 71.04, P < 0.0001) and then significantly increased at higher host densities ($F_{1,220}$ = 20.31, P < 0.0001) (Figure 7.3B).

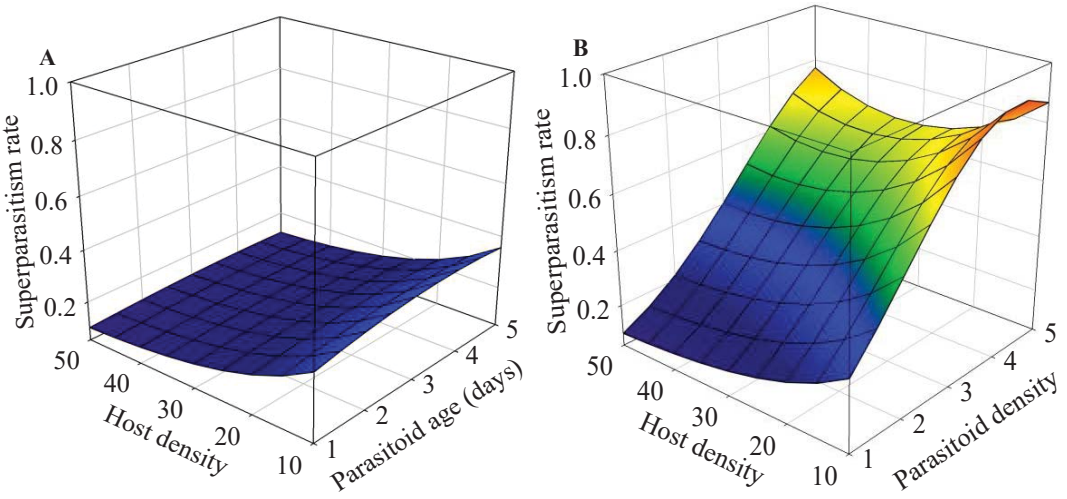

Figure 7.3 Superparasitism rate of *A. colemani* of different age (P_a) and different density (P_t) in response to host density (N_o). (**A**) Effect of different age (P_a): Superparasitism rate = exp(– 0.8005 + 0.1684Pa – 0.0216Pa^2 – 0.0582N_o + 0.0005N_o^2) ($F_{4,175}$ = 63.56, P < 0.0001, R^2 = 0.5924); (**B**) Effect of different density (P_t): Superparasitism rate = exp(– 1.3337 + 0.7965Pa – 0.1004Pa^2 – 0.0660N_o + 0.0005N_o^2 + 0.0066PtN_o) ($F_{5,220}$ = 154.76, P < 0.0001, R^2 = 0.7786).

7.3.3 Demographic response and mutual interference

Although the response patterns generated by the demographic response model (Figure 7.4) and the generalized logistic linear model (Figure 7.2) were similar, the former model fitted my data better than the latter ($F_{1,229}$ = 152.14 and 20.57 for number and proportion of female offspring produced, respectively; P < 0.0001). Furthermore, Figure 7.4 provides more detailed information, for example, the proportion of female progeny produced decreased with increasing parasitoid density at the density from 10 to 20 aphids but increased at the density from 30 to 50 aphids (Figure 7.4B).

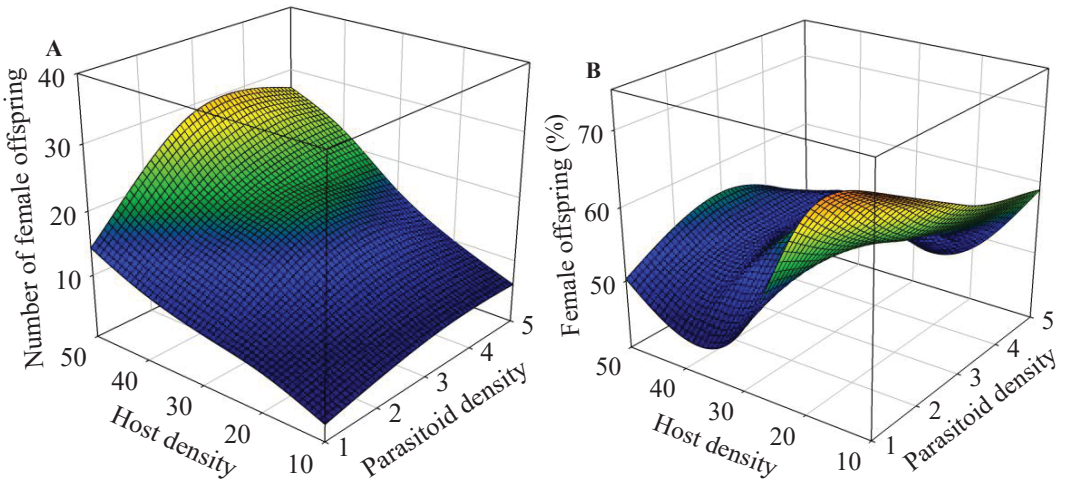

Fig. 7.4 Demographic response of *A. colemani* to parasitoid (P_t) and host densities (N_o). (**A**) The number of female progeny: $P_{t+1} = SN_o[1\text{-}\exp(\text{-}aP_t)]$ ($F_{1,229} = 5656.33$, $P < 0.0001$, $R^2 = 0.8412$), where the constant $a = 0.6405 \pm 0.0252$; (**B**) proportion of female progeny: $S = P_{t+1}/\{N_o[1\text{-}\exp(\text{-}aP_t)]\}$ ($F_{1,229} = 4268.90$, $P < 0.0001$, $R^2 = 0.4350$), where the constant $a = 1.0941 \pm 0.0562$.

In the present study, $m = 0.4983 \pm 0.0495$ and $1\text{-}(F\text{-}1)/(F\ln F) = 0.6245$. The result of $m < 0.6245$ indicates that the interaction between *A. colemani* and *M. persicae* was unstable. Figure 7.5 shows a significant negative relationship between the searching efficiency (a) and parasitoid density (P_t) ($F_{1,238} = 101.35$, $P < 0.0001$).

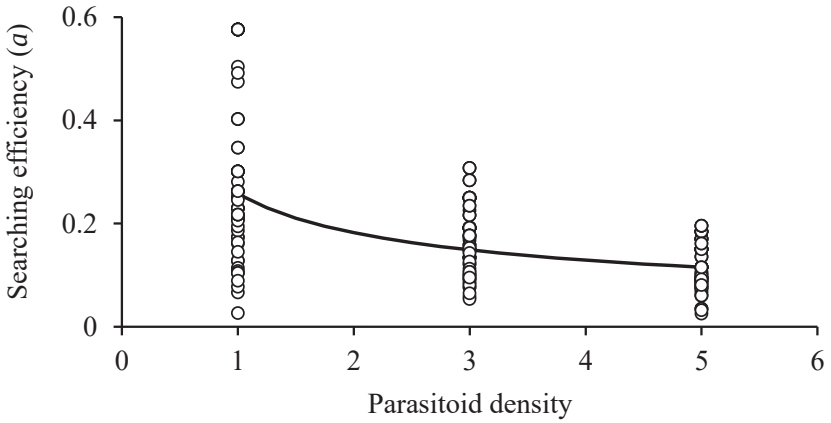

Figure 7.5 The mutual interference in *A. colemani*: $a = QP_t^{-m}$ ($F_{1,238} = 101.35$, $P < 0.0001$, $R^2 = 0.2987$), where the constant $Q = 0.2576$ and mutual interference constant $m = 0.4983$.

7.4 Discussion

My results indicate that the response of *A. colemani* of different age and density to host density fitted Type II functional response with both Holling's (1959) and Rogers' (1972) models. However, Holling's model assumes that the density of available hosts decreases over time which may be more suitable for predator-prey systems while Rogers' model considers that the host density is constant (parasitised hosts are re-encountered) which could be more appropriate for parasitoid-host systems (Hassell 1978). For example, *A. colemani* females encounter the parasitised and unparasitised *A. gossypii* with similar frequency (van Steenis & El-Khawass 1995b). The occurrence of superparasitism (Figure 7.3) suggests that *A. colemani* females do re-encounter and re-attack the hosts. For overall parasitoid density the coefficient of determination (R^2) from Rogers' model was more than twice as high as that from Holling's model (Table 7.4). Therefore, Rogers' model is more appropriate than Holling's model to fit the data of my study. Furthermore, the searching efficiency (a) estimated by Rogers' model was significantly higher than that by Holling's model for *A. colemani* of any age (Table 7.5) and density (Table 7.6). I therefore suggest that Holling's model could underestimate the biological control potential of *A. colemani*.

Theories predict that natural enemies with a Type III functional response are more successful in biological control due to their density dependent parasitism rates (Khan et al. 2016). However, biological control success using parasitoids with a Type II functional response has been widely reported (Hughes et al. 1992; van Roermund et al. 1996; Sagarra et al. 2000; Hanan et al. 2017) although the mechanisms behind the success are not well known. Therefore, Type II functional response found in the present study does not imply a potential failure of *A. colemani* to control *M. persicae*. After reviewing more than 90 papers dealing with functional response, Fernández-arhex and Corley (2003) have found no relationship between the type of functional response and success in biological control. Furthermore, van Lenteren et al. (2016) suggest that parasitoids with a Type II functional response are more suitable for inundative biological control programmes because they intend to bring immediate pest suppression. For example, *A. colemani* has been produced commercially for biological control of *M. persicae* and *A. gossypii* around the world (Fernandez & Nentwig 1997; Starý 2002; van Lenteren 2003; Vásquez et al. 2006; Teulon et al. 2008; van Driesche et al. 2008) for augmentation of aphid biological control programmes (Burgio et al. 1997; Jacobson & Croft 1998; Vásquez et al. 2006; van Driesche et al. 2008; Bioforce 2017).

Previous studies show that age of parasitoids can affect their host searching and oviposition behaviour (Völkl & Mackauer 1990; Asadi et al. 2012; Nikbin et al. 2014; Pasandideh et al. 2015). In the present study, 3-d-old *A. colemani* females had higher searching efficiency than did 1-d-old females and shorter host handling time than did 1- and 5-d-old females (Table 7.5). These may explain why 3-d-old females parasitise more aphids (Figure 7.1A), produce more female offspring (Figure 7.1C), and have higher parasitism rate (Figure 7.1B) and superparasitism rate (Figure 7.3A).

In the field, several foraging parasitoids may visit a host patch simultaneously (Chong & Oetting 2006; He & Wang 2014), which may cause competition and interference among parasitoids and reduce their per capita host search and attack efficiency (Hassell & Varley 1969). My findings (Table 7.6 and Figure 7.5) appear to agree with Hassell & Varley (1969). However, reproductive fitness of *A. colemani* was actually the highest when the parasitoid density was between intermediate and high

(Figure 7.2). This could be attributed to the significantly positive interactions between parasitoid and host densities. Furthermore, the *m* value (0.4983) from my study was lower than the critical *m* value (0.6245) calculated for the stable parasitoid-aphid system, indicating unstable *A. colemani*-*M. persicae* interactions. This property should encourage aggregation of parasitoids on host patches of high density, leading to the collapse of host patches (Hassell & May 1973; Kidd & Jervis 2005; Hanan et al. 2017) and preventing pest outbreaks from these patches (Rosenheim 1990; Umbanhowar et al. 2003; He & Wang 2014). Moreover, parasitoid age and density had significantly more effect than host density on parasitism, parasitism rate, and female offspring produced (Figures 7.1 and 7.2), suggesting that mass release of *A. colemani* can be a good option for effective control of aphids when the pest density is high.

In conclusion, my studies show that although *A. colemani* has a Type II functional response, it can still successfully control *M. persicae* regardless of pest density. I propose two mechanisms behind the success: (1) parasitoid density has significantly more effect than host density on parasitoid reproductive fitness, making mass release more effective, and (2) the low mutual interference (*m*) among the searching parasitoids encourages aggregation of the parasitoids on host patches of high density, leading to the collapse of host population these patches. The present study provides some insights into the success of aphid biological control programmes using the parasitoid augmentation approach.

Chapter Eight
General Conclusions

8.1 Introduction

During my PhD studies, I carried out a large number of experiments to investigate many aspects of the biological control ecology in the *A. colemani*-*M. persicae* system from general biology of the parasitoid, life history strategies of both aphid and parasitoid, to parasitoid-host interactions. I statistically analysed my data and attempted to interpret my findings. In this chapter, I summarise my main findings and discuss their relevance to biological control.

8.2 Adult activity patterns

In the present study I demonstrate that *A. colemani* is a diurnal species with emergence, mating and oviposition mainly occurring during the day, particularly in the morning. These findings are in accordance with reports in several other parasitoid species, such as *E. formosa* Gahan (van Lenteren et al. 1992), *Trichogramma* spp. (Corrigan et al. 1995; Pompanon et al. 1995; Reznik et al. 2008), *T. busseolae* (Fantinou et al. 1998) and *A. ervi* (He et al. 2004). Synchronised with this activity pattern may be maximal sex pheromone by females in the morning (McNeil & Brodeur 1995; Marchand & McNeil 2000; McClure et al. 2007; Benelli et al. 2013) during which time, the environmental conditions are favourable for males to locate females. Furthermore, my results show that females take longer time to complete development than males, and thus males emerge earlier than do females. The observed emergence pattern in *A. colemani* may be a life history strategy for maximal mating success in both sexes (Morbey & Ydenberg 2001).

Although *A. colemani* females undertake most oviposition during the day, they can still lay some eggs during the night, generally in agreement with previous findings in *C. bartetti* (Walter 1988), *M. pseudoplatani* (Collins & Dixon 1986), and *A. ervi* (He et al. 2004). Contradictory to previous findings that parasitoids may alter sex allocation

patterns of their offspring in response to photoperiod, for example, *U. lariophaga* females produce more female offspring in the photophase than in the scotophase (van Huis & Appiah 1995; Sagarra et al. 2000), the sex ratio of *A. colemani* offspring is consistently female-biased throughout the 24-h cycle. It is thus suggested that increasing light period cannot raise proportion of female offspring produced for this parasitoid.

My findings show that both sexes *A. colemani* require about 2 hours for sex maturation and females need to feed before mating. Moreover, like other parasitoid wasps (Adams & Morse 2014), mating success is much higher in *A. colemani* when space for mating is smaller. Therefore, to improve the efficiency of augmentative programmes, newly emerged parasitoids should be provided with food and stored in small containers for at least two hours before release. It is probably a good idea if adult food is also provided in the release areas.

8.3 Life history strategies of both aphid and parasitoid

Theories and empirical studies suggest that life history strategy parameters of both pests and their natural enemies should be measured before we understand whether a parasitoid can be an effective biological control agent (Bellows et al. 1992; van Driesche & Bellows 1998; Bellows & van Driesche 1999; van Lenteren 2009; Lins et al. 2011). Therefore, I have measured these parameters of both *A. colemani* and *M. persicae* simultaneously. My findings indicate that young and mid-aged *M. persicae* nymphs parasitised by *A. colemani* die before reaching adulthood and parasitised late instar nymphs and adults produce only very few offspring. I also show that compared to healthy aphids, parasitised aphids have lower intrinsic rate of increase and net population growth rate, longer doubling time and shorter survival time. These results indicate that *M. persicae* parasitised by *A. colemani* can contribute little to their population growth and make limited damage to plants regardless of age when parasitised.

Although *A. colemani* has lower intrinsic rate of increase and longer lifecycle than *M. persicae*, the former produces twice as many female offspring and has twice as great net population growth rate as the latter does. Therefore, the parasitoid's overwhelmingly

higher reproductive output and faster population growth may overcome the constraints of its longer lifecycle and lower intrinsic rate of increase and potentially make it an effective biological control agent of *M. persicae*. My results may explain why the relatively lower intrinsic rate of increase in *A. colemani* than in *A. gossypii* (Torres et al. 2007) does not compromise the biological control efficiency for *A. gossypii* using *A. colemani* (van Steenis & El-Khawass 1995b; Vásquez et al. 2006; Prado et al. 2015).

So far, none of the recommended parasitoid release rates for aphid control is based on aphid density at the time of release. My current study demonstrates that *A. colemani* reaches 50% and 90% of their lifetime reproductive potential five and ten days earlier than healthy *M. persicae*, respectively. This suggests that *A. colemani* has high potential to suppress the aphid population quickly if it is released at the right time. Based on the fact that one *A. colemani* female can parasitise ≈ 220 *M. persicae* in a week without any preference for aphid age and parasitised aphids contribute little to population growth, it is estimated that a small release rate of ≈1:220 (*A. colemani* female:*M. persicae*) at a weekly interval during the first three weeks after the onset of *M. persicae* population could quickly suppress the aphid population growth and effectively control the pest. However, to achieve successful and cost-effective control of *M. persicae* by release of *A. colemani*, a close monitoring of the onset of the aphid population early in the season is required.

8.4 Host age preference

In theory, larger hosts carry more resources for parasitoids (Liu 1985; Opp & Luck 1986), and as a result, parasitising larger hosts is expected to be more profitable than smaller ones in terms of parasitoid fitness (He et al. 2005a; Wyckhuys et al. 2008; Henry et al. 2009). However, larger hosts at the parasitisation may have better defence ability (Wyckhuys et al. 2008; He et al. 2011) and the hosts of koinobiont parasitoids continue to change in resources after being parasitised (Brough et al. 1990; Lin & Ives 2003).

Like previous findings in other parasitoid-aphid systems such as *M. paulensis* (Chau & Mackauer 2000) and *A. ervi* (He et al. 2011) on *A. pisum*, *A. colemani* females are significantly more likely to encounter their hosts with the increase of host size.

However, encounter and attack attempt do not proportionally translate into an ovipositor probing, and the ovipositor probing does not proportionally turn to an oviposition. These results suggest that *A. colemani* females prefer to attack larger aphids but the counterbalance between the parasitoid preference and host defence makes any host age preference pattern undetectable.

Contradictory to previous reports, my study shows that the host age at parasitisation has no effect on progeny sex ratio and emergence rates, suggesting that in the *A. colemani-M. persicae* system pupae have similar survival rate regardless of host size at parasitisation. The similar sex ratio of progeny that emerge from hosts of different ages at parasitisation may result from the fact that (1) the parasitoid females do not adjust sex allocations depending on host age, (2) the progeny of both sexes have similar survival rate during the larval stage regardless of host size, or both (1) and (2).

Several authors reveal that the larger progeny produced from larger hosts at parasitisation carry more eggs at emergence (Chau & Mackauer 2001; He et al. 2005a). However, they have not tested whether these larger progenies have higher lifetime parasitism. My study indicates that larger parasitoids produced from 3-d-old aphids at parasitisation parasitise more aphids than smaller ones that emerge from 1-d-old hosts at parasitisation. These results demonstrate that larger progenies produced from larger hosts at parasitisation are more fecund and probably more effective in the control of aphids.

8.5 Functional response in relation to parasitoid age and host and parasitoid density

My current study shows that the response of *A. colemani* of different age and density to host density fits a Type II functional response. Theories envisage that parasitoids with a Type III functional response are more successful in biological control due to their density dependent parasitism rates (Khan et al. 2016). However, biological control success using parasitoids with a Type II functional response has been widely reported (e.g., Hughes et al. 1992; van Roermund et al. 1996; Sagarra et al. 2000; Hanan et al. 2017) although the mechanisms behind the success are not well known. Therefore, Type II functional response found in the present study does not imply a potential failure

of *A. colemani* to control *M. persicae.* More recently, van Lenteren et al. (2016) suggest that parasitoids with a Type II functional response are more suitable for inundative biological control programmes because they intend to bring immediate pest suppression.

Similar to previous studies (Völkl & Mackauer 1990; Asadi et al. 2012; Nikbin et al. 2014; Pasandideh et al. 2015), 3-d-old *A. colemani* females have higher searching efficiency than 1-d-old females and shorter host handling time than 1- and 5-d-old females. These findings may explain why 3-d-old females parasitise more aphids, produce more female offspring, and have higher parasitism rate.

Contrary to general predictions that parasitoids foraging on the same host patch may compete and interfere with each other, reducing parasitisation efficiency, my results indicate that *A. colemani* achieve the maximal reproductive fitness when the parasitoid density is relatively high. This could be attributed to the significantly positive interactions between parasitoid and host densities.

I show unstable *A. colemani-M. persicae* interactions from my study. This property should encourage aggregation of parasitoids on host patches of high density, leading to the collapse of host patches (Hassell & May 1973; Kidd & Jervis 2005; Hanan et al. 2017) and preventing pest outbreaks from these patches (Rosenheim 1990; Umbanhowar et al. 2003; He & Wang 2014). Furthermore, parasitoid age and density have significantly more effect than host density on parasitism, parasitism rate, and female offspring produced. It is thus suggested that mass release of *A. colemani* can be a good option for effective control of aphids when the pest density is high.

8.6. Conclusion

My PhD studies have provided insight into biological control ecology in the *A. colemani- M. persicae*, which is useful for the development of effective biological control programmes for this and probably other parasitoid-aphid systems, for example, laboratory handling, mass rearing, and release time and rate. My results indicate that *A. colemani* can successfully suppress *M. persicae* population via augmentative release

regardless of pest density. However, my study has limitations. Future research on this topic should focus on parasitoid-aphid interactions in glasshouses.

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Appendix: Publications from My PhD Studies

In the next 13 pages I attach two publications in an international journal, which are from my PhD work.