Biological control ecology of *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) on *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae)

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Abstract

Tamarixia triozae (Burks) (Hymenoptera: Eulophidae) is an important primary parasitoid of tomato-potato psyllid *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), a serious invasive pest of solanaceous crops. It kills its hosts by both feeding and parasitisation. However, its biological control ecology is still not well known, making it difficult to develop an effective biological control programme using this parasitoid. In this thesis, I investigated adult circadian rhythms, diets, life history strategies, and host selection behaviour in *T. triozae.* My results show that most emergence occurred in the morning and most mating took place early the next morning. Oviposition only occurred during the daytime, peaking between mid-morning and mid-afternoon while host feeding had three peaks in the early morning, late afternoon, and dawn. Adults fed with honey for four days with no access to hosts or with water or yeast for one day followed by host feeding for three days had similar longevity and lifetime pest killing ability. Adults fed with only water for one day immediately before release had significantly greater intrinsic rate of increase, shorter doubling time, and higher daily fecundity peak. Adults fed with honey or yeast for one day followed by host feeding for three days significantly flattened their daily oviposition curves. *T. triozae* females could feed on nymphs of all instars but preferred mid-aged ones for feeding. Most parasitisation occurred on older nymphs. Host feeding and parasitism peaked during the first week of female life and declined markedly after two weeks. Parasitoids allocated more fertilised eggs to older and larger nymphs. The oviposition of fertilised eggs peaked when females were four to five days old, with > 90 % of daughters produced during the first half of their life. There was a positive relationship between the host size at parasitisation and parasitoid offspring fitness. The parasitoid behaviours consisted of encounter, evaluation, piercing for feeding, feeding, oviposition probing, and oviposition, and host defence behaviours included body swaying and escaping. Female wasps were more likely to encounter and evaluate older and larger hosts. However, encounter and evaluation did not necessarily translate into feeding and oviposition success. Older nymphs had more resources, thicker integument and stronger defence ability and the opposite was the case for younger ones. The present study provides novel knowledge for development of effective *T. triozae* mass rearing, shipment, and release programmes for the control of *B. cockerelli*.

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General introduction

1.1 Introduction

Many psyllid species (Hemiptera: Psylloidea: Triozidae) are important pests in agriculture and horticulture, damaging crops by direct feeding and transmitting plant diseases (Munyaneza et al. 2010; Butler and Trumble 2012a; Espinosa-Zaragoza et al. 2021). They camouflage under the leaves of their host plants and are usually unnoticed, making it easy for them to be accidentally transported through agricultural activities. They can also disperse over long distance through wind (de Queiroz et al. 2012). Near 100 psyllid species have been found in New Zealand (Martoni et al. 2016), many of which are economic pests of vegetables and fruit crops (Teulon et al. 2009).

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), native to the United States and Mexico, is a serious pest of solanaceous crops such as potatoes, tomatoes, eggplants, and peppers (Cranshaw 1994; Crosslin et al. 2010; Butler and Trumble 2012a; Rojas et al. 2015). It has invaded New Zealand, Australia, Canada, and Ecuador, where its economic impact is under evaluation (Teulon et al. 2009; Ogden 2011; FAO 2017; WADPIRD 2018; Castillo Carrillo et al. 2019; Olaniyan et al. 2020; Sarkar et al. 2023). It damages host plants by direct feeding, promoting sooty mould growth (Rojas et al. 2015), and transmission of zebra chip disease (*Candidatus* Liberibacter solanacearum) (Liefting et al. 2009; Munyaneza 2012). In the United States, particularly in Texas, the potato production is severely affected by zebra chip disease (Goolsby et al. 2007). Only a few years after its first detection in New Zealand in 2006, TPP has cost New Zealand potato industry more than $120 million (Ogden 2011).

Management of TPP is challenging due to its high reproductive rate and cryptic feeding sites under the leaf surface (Yang and Liu 2009; Butler and Trumble 2012b). Currently, insecticide applications remain the main option to control TPP (Munyaneza 2012; Martinez et al. 2015; Wright et al. 2017). For example, in New Zealand potato growers regularly apply mainly broad-spectrum insecticides to control this pest (Wright et al. 2017) with a trend of increasing applications (Vereijssen 2020). However, repeated use of insecticides causes the development of insecticide resistance (Martin 2005). For example, TPP has already developed resistance to some insecticides in United States and Mexico (Dávila et al. 2012; Cerna et al.

2013; Chávez et al. 2015; Szczepaniec et al. 2019). In addition, insecticide applications may cause pest resurgence (Prager et al. 2016) because of the destruction of natural enemies (Martinez et al. 2015; Morales et al. 2018). Therefore, there is an urgent need for development of an integrated pest management (IPM) programme including natural enemies to reduce the reliance on insecticides and to maintain or increase levels of control (Luna-Cruz et al. 2011; Liu et al. 2012; Cerón-González et al. 2014; Rojas et al. 2015).

Biological control is an important tactic in pest management (Waage et al. 1988; Naranjo et al. 2015) and can avoid the negative impacts of chemical insecticides (van Lenteren et al. 2018). The parasitoid *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) and predatory bug *Dicyphus hesperus* Knight (Hemiptera: Miridae) are two important biological control agents of TPP (Calvo et al. 2016). In addition, *Engytatus nicotianae* (Koningsberger) (Hemiptera: Miridae) may also have potential to control TPP (Veronesi et al. 2022a). *T. triozae* is a primary ectoparasitoid of TPP (Rojas et al. 2015; Yang et al. 2015). It has great potential to control TPP, particularly in greenhouse crops because of its high fecundity, short life cycle, and ability to kill hosts by both parasitisation and host feeding (Cerón-González et al. 2014; Rojas et al. 2015; Yang et al. 2015). *T. triozae* was introduced to New Zealand in 2016 for the control of TPP (EPA 2016). Preliminary studies show that released parasitoid can overwinter and establish self-sustaining populations in New Zealand where the climate is warming up (Anderson 2020; StatsNZ 2020).

1.2 Relevance of research

The success of pest biological control using parasitoids heavily relies on our knowledge about the biological control agents. To develop effective *T. triozae* mass rearing, shipment, and release programmes for the control of TPP, we need to understand (1) their circadian activity patterns, (2) the effect of adult diets with or without temporary host deprivation on their survival, host feeding and oviposition, (3) their life history strategies in response to host stage and their own age, and (4) parasitoid-host interaction behaviours.

1.2.1 Circadian rhythms

In parasitoids, circadian emergence, mating, host feeding, and oviposition are usually rhythmic (Walter 1988; Vogt and Nechols 1991; Hanan et al. 2009; Bertossa et al. 2010). Understanding circadian activity patterns of parasitoids may help develop effective field release programmes for biological control and make recommendations for the timing of irrigation or insecticide applications that could reduce negative impacts on parasitoids (Vogt and Nechols 1991; van Lenteren et al. 1992). For example, morning release of *Gryon pennsylvanicum* (Ashmead) (Hymenoptera: Scelionidae) may be more effective because adults find mates easier in the morning (Vogt and Nechols 1991). Irrigation and insecticide sprays should be avoided when the adult emergence peaks (van Lenteren et al. 1992). However, circadian rhythms of emergence, host feeding and reproduction are still unknown in *T. triozae*.

1.2.2 Adult diets for population manipulation

Augmentative release of natural enemies, particularly parasitoids, is one of the most widely used approaches in biological control in annual crops and greenhouses (van Lenteren and Bueno 2003; Hoy 2008; Amadou et al. 2019; Bueno et al. 2020; Kazak et al. 2020). Feeding on hosts or non-host foods can increase parasitoids' fecundity and longevity (Bartlett 1964; Heimpel and Rosenheim 1995; Liu et al. 2015; Benelli et al. 2017; Picciau et al. 2019). Therefore, providing adult parasitoid diets before release can help parasitoids establish their population at release sites (Tena et al. 2017; Stahl et al. 2019) and improve their biological control effectiveness after release (Hougardy et al. 2005; Hougardy and Mills 2006, 2007; Benelli et al. 2017). Because adult diets can also change parasitoids' lifetime oviposition patterns (Bai and Smith 1993; Wade et al. 2008; Hill et al. 2020), the pre-release holding period may provide opportunities for us to manipulate parasitoid life history traits according to our needs. Yet, it is not clear how adult diets with or without temporary host deprivation affect survival, lifetime fecundity, host feeding, oviposition patterns and life table parameters in *T. triozae*.

1.2.3 Life history strategies in response to host stage and parasitoid age

Host-feeding parasitoids usually prefer hosts of certain stages for feeding and oviposition (e.g., Kidd and Jervis 1991; Choi et al. 2001; Ebrahimifar and Jamshidnia 2022). It is still unknown how *T. triozae* females partition their hosts of different instars for feeding and parasitisation when TPP nymphs of all instars are present simultaneously, information of which may help understand the host selection for feeding and oviposition and evaluate the biological control effectiveness of *T. triozae*. Parasitoid wasps usually adjust their offspring sex ratio depending on host body size (e.g., Charnov et al. 1981; Favaro et al. 2018; Pérez-Rodríguez et al. 2019). To date, sex allocation strategies and their consequences are still poorly understood for *T. triozae*, making it difficult to develop an effective mass rearing programme. Furthermore, the age of parasitoids may affect their feeding (Zhang et al. 2014), oviposition (Wade et al. 2008; Bodino et al. 2019), and offspring sex ratio (Latham and Mills 2010; Mawela et al. 2021), knowledge of which is important for evaluation of biological control programmes and timing of parasitoid releases. This information is currently lacking for *T. triozae.*

1.2.4 Parasitoid-host interaction behaviours

Female parasitoids including *T. triozae* usually prefer larger hosts for parasitism and smaller ones for feeding (Kidd and Jervis 1991; Choi et al. 2001; Ebrahimifar and Jamshidnia 2022; Chen et al. 2023). Although host defence generally increases with host size and age (He et al. 2011; Khatri et al. 2016), older hosts provide more and better resources for offspring development (Liu et al. 2011; Hanan et al. 2015; Luo et al. 2022; Chen et al. 2023). Younger hosts have thinner integument that is easier to penetrate by the parasitoids before feeding (Kidd and Jervis 1991; Veronesi et al. 2022a). Therefore, such host stage preference may help maximise parasitoids' own and their offspring fitness. However, the host feeding and oviposition behaviour of *T. triozae* in relation to host stage and the defence behaviour of *B. cockerelli* nymphs in response to parasitoid attack are still unclear, making it difficult to unfold the underlying behavioural mechanisms of host preference and evaluate the host-parasitoid interactions.

1.3 Aim and objectives of this study

To provide information for development of effective biological control programmes using *T. triozae*, I aim to investigate aspects of biological control ecology of this parasitoid on *B. cockerelli*, with four objectives:

(1) to determine the circadian patterns of emergence, host feeding and reproduction in *T. triozae*;

(2) to investigate the effects of adult diets with or without temporary host deprivation on survival, lifetime fecundity, host feeding, oviposition patterns and life table parameters in *T. triozae*;

(3) to determine parasitoid age- and host stage-dependent host feeding, parasitisation and sex allocation and their fitness consequences in *T. triozae*, and

(4) to study the host selection behaviour of *T. triozae* and defence behaviour of *B. cockerelli*.

1.4 Literature review

This section reviews the current knowledge relevant to my studies on *T. triozae*.

1.4.1 Origin and distribution of *Bactericera cockerelli* and *Tamarixia triozae*

1.4.1.1 *Bactericera cockerelli*

Bactericera cockerelli is native to the United States and Mexico and widely distributed in the United States, Mexico, and New Zealand (EPPO 2013; CABI 2018). It was also found in Australia (WADPIRD 2018; Sarkar et al. 2023). In New Zealand, *B. cockerelli* was first reported in greenhouse crops and volunteer potatoes in 2006 (Gill 2006).

The taxonomy of *B. cockerelli* is:

Order: Hemiptera
  Family: Triozidae
    Genus: *Bactericera*
      Species: *cockerelli* (Šulc)

Previously described as *Trioza cockerelli* by Šulc (1909), *B. cockerelli* is the current accepted name. *B. cockerelli* was moved from Psyllidae to Triozidae by Burckhardt and Lauterer (1997).

1.4.1.2 *Tamarixia triozae*

Parasitism of TPP nymphs by *T. triozae* was first noted in southern Arizona (Romney 1939). The parasitoid is widely distributed in the United States and Mexico (Pletsch 1947; Jensen 1957; Lomeli-Flores and Bueno 2002). New Zealand introduced and released it in 2016 to control TPP (EPA 2016). It has successfully established in Hawke’s Bay and Canterbury and is expected to establish in other regions in New Zealand (Davidson et al. 2023).

The taxonomy of *T. triozae* is:

Order: Hymenoptera
  Family: Eulophidae
    Genus: *Tamarixia*
      Species: *triozae* (Burks)

Previously described as *Tetrastichus triozae* Burks (1943), *T. triozae* is the current accepted name.

1.4.2 Host range of *Tamarixia triozae*

Tamarixia triozae is a generalist parasitoid that attacks *Calophya californica* Schwarz (Hemiptera: Calophyidae), *C. nigrella* Jensen (Hemiptera: Calophyidae), *C. nigripennis* Riley (Hemiptera: Calophyidae), *C. triozomima* Schwarz (Hemiptera: Calophyidae), *Ceanothia ceanothi* (Crawford) (Hemiptera: Psyllidae), *Euglyptoneura minuta* (Crawford) (Hemiptera: Psyllidae), *Euphalerus vermiculosus* Crawford (Hemiptera: Psyllidae), *Pexopsylla cercocarpi* Jensen (Hemiptera: Psyllidae), *B. cockerelli* (Hemiptera: Triozidae), *B. minuta* (Crawford) (Hemiptera: Triozidae), *B. nigricornis* (Förster) (Hemiptera: Triozidae), *Trioza albifrons* Crawford (Hemiptera: Triozidae), and *T. beameri* Tuthill (Hemiptera: Triozidae) (Zuparko et al. 2011). These host species are all members of the former family Psyllidae.

1.4.3 General biology of *Bactericera cockerelli* and *Tamarixia triozae*

1.4.3.1 *Bactericera cockerelli*

Bactericera cockerelli deposits eggs on both sides of the leaves and usually along the margins but occasionally on the petioles and stems (Knowlton and Janes 1931). The eggs are transparently white or pale greenish-yellow, oblong, and attached to the leaves with short stalks (Essig 1917; Fig. 1.1a). *B. cockerelli* nymphs pass through five instars (Knowlton and Janes 1931), prefer the abaxial leaf surface and seldom move (Lehman 1930). First-instar nymphs are pale yellow in colour with an orange head and abdomen (Essig 1917; Fig. 1.1b). During the second instar, divisions between head, thorax and abdomen are evident (Vega-Chávez 2010; Fig. 1.1c). During the third instar, the wing pads are evident (Fig. 1.1d) and become more pronounced with each subsequent moult (Munyaneza 2012; Fig. 1.1e and Fig. 1.1f). Newly emerged adults are pale green or light amber, but soon become darker amber (Knowlton and Janes 1931; Fig. 1.1g). Adult females have rounded and robust abdomen ending with a short ovipositor while males have sharper abdomen than females (Abdullah 2008).

Bactericera cockerelli requires 385 and 368 degree-days to complete a life cycle on potato and tomato, respectively, and the lower developmental thresholds reared on potato and tomato are 7.1°C and 7.5°C, respectively (Tran et al. 2012). At 26.7 ± 2°C, 75 ± 5% RH and a photoperiod of 14:10 hours (L:D), the developmental time of this species reared on bell pepper is 5.9, 20.2, and 26.2 days for eggs, nymphs, and adults, respectively. Adult females can live for 55 days, have a pre-oviposition period of 8 days, and produce 227.3 offspring on bell pepper (Yang and Liu 2009).

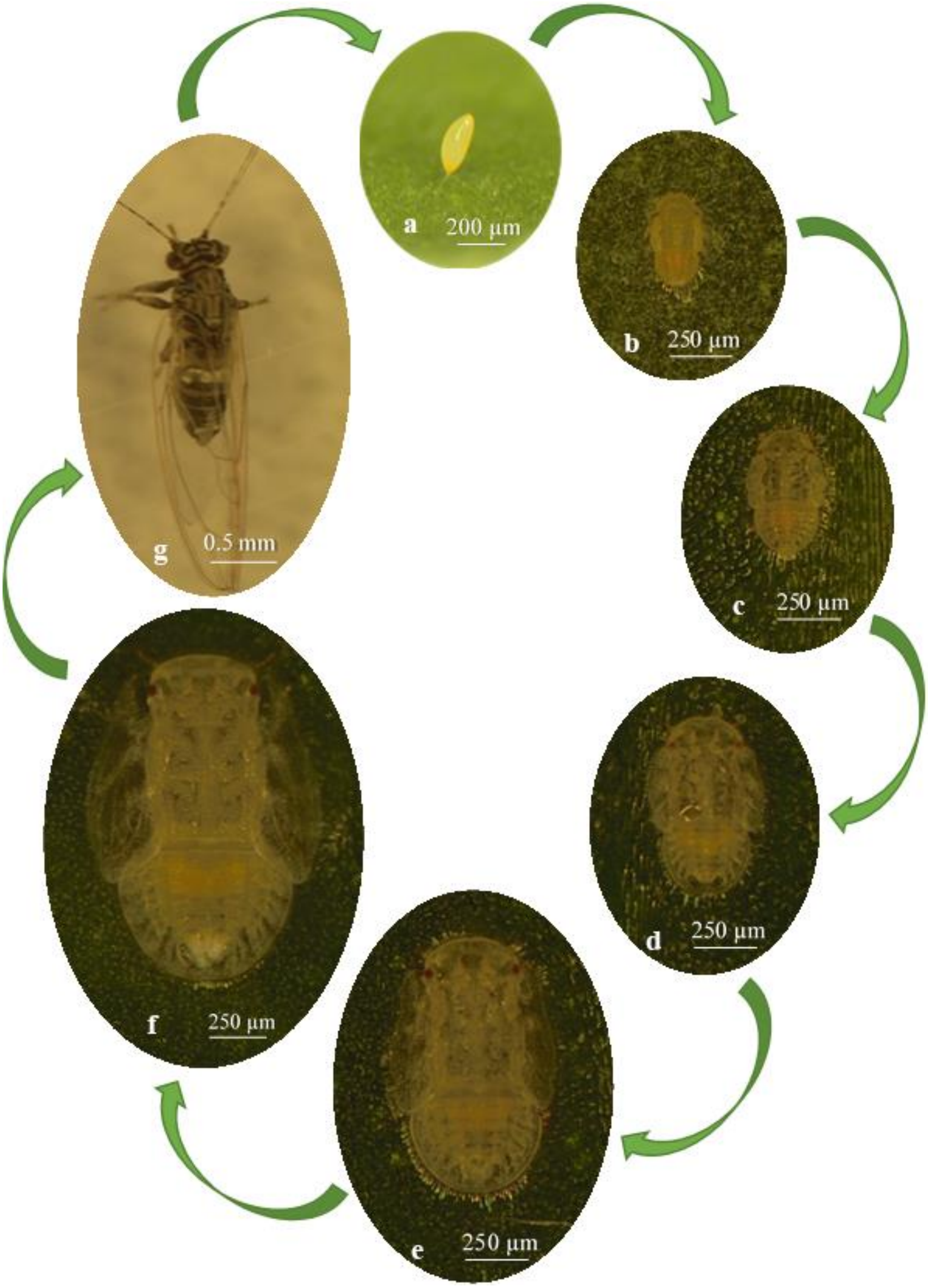

Fig. 1.1 Life cycle of *Bactericera cockerelli*: (**a**) egg, (**b**) first instar nymph, (**c**) second instar nymph, (**d**) third instar nymph, (**e**) fourth instar nymph, (**f**) fifth instar nymph, and (**g**) adult (female)

1.4.3.2 *Tamarixia triozae*

Tamarixia triozae females probe psyllid nymphs with the ovipositor between the hosts and leaflet surface several times, and then deposit one egg either between the prothoracic and mesothoracic coxes or between the mesothoracic and metathoracic coxes of a host and secrete adhesive substance to attach the egg to the cuticle during oviposition (Martinez et al. 2015; Rojas et al. 2015). Females occasionally deposit two or more eggs on a host under laboratory conditions but only one can complete development (Vega-Chávez 2010; Rojas et al. 2015). The eggs are long, gelatinous and translucent yellow (Martinez et al. 2015; Fig. 1.2a). After hatching, the parasitoid larva (Fig. 1.2b) starts to feed on host hemolymph and develop under the host (Vega-Chávez 2010). The exoskeleton colour of the parasitised nymph changes from green to brown (Pletsch 1947; Johnson 1971) when the mature parasitoid larva (Fig. 1.2c) develops to pupa (Fig. 1.2d), and then the nymph becomes a mummy (Fig. 1.2e). The adult parasitoid (Fig. 1.2f) emerges by making a hole in the upper part of the nymph's dorsal surface with its jaws (Pletsch 1947; Rojas et al. 2009).

Tamarixia triozae can complete a life cycle in a temperature range of 4.8°C to 35°C (Vega-Chávez et al. 2016). The low temperature threshold for *T. triozae* is lower than that (7.1 ~ 7.5°C, see above) for TPP nymphs (Tran et al. 2012; Vega-Chávez et al. 2016). *T. triozae* requires 243.9 degree-days to complete a life cycle (Vega-Chávez et al. 2016). At 26 ± 2°C, 60 ± 10% RH and a photoperiod of 14:10 hours (L:D), the developmental time of *T. triozae* is 1.5, 3.5, 5.7, 11.6, and 12.0 days for eggs, larvae, pupae, and male and female adults, respectively. *T. triozae* is a synovigenic species (Rojas et al. 2015). To obtain energy for egg maturation females pierce the soft integument area of their hosts using the ovipositor to make wound and then feed on the haemolymph exuding from the wound (Martinez et al. 2015). Males do not feed on hosts (Martinez et al. 2015). Host feeding and oviposition in this species are non-concurrent, and females neither feed on parasitised hosts nor parasitise those already fed upon (Cerón-González et al. 2014; CC pers. observ.). Adult females can live for about 21 days (Chen et al. 2022) with a pre-oviposition period of 1.9 ± 0.8 days (Rojas et al. 2015) when exposed to hosts and honey solution. A female kills an average of 312 TPP nymphs throughout lifetime, of which 56% by parasitism and 44% by feeding (Cerón-González et al. 2014).

The body size of *T. triozae* offspring increases with the host size or stage at parasitisation, and female offspring are larger than male offspring (Chen et al. 2023). This parasitoid allocates more fertilised eggs to older and larger nymphs (Chen et al. 2023). Females have geniculate antennae with an enlarged end, while males do not have such end and have many setae along their antennae (Vega-Chávez 2010; Fig. 1.3).

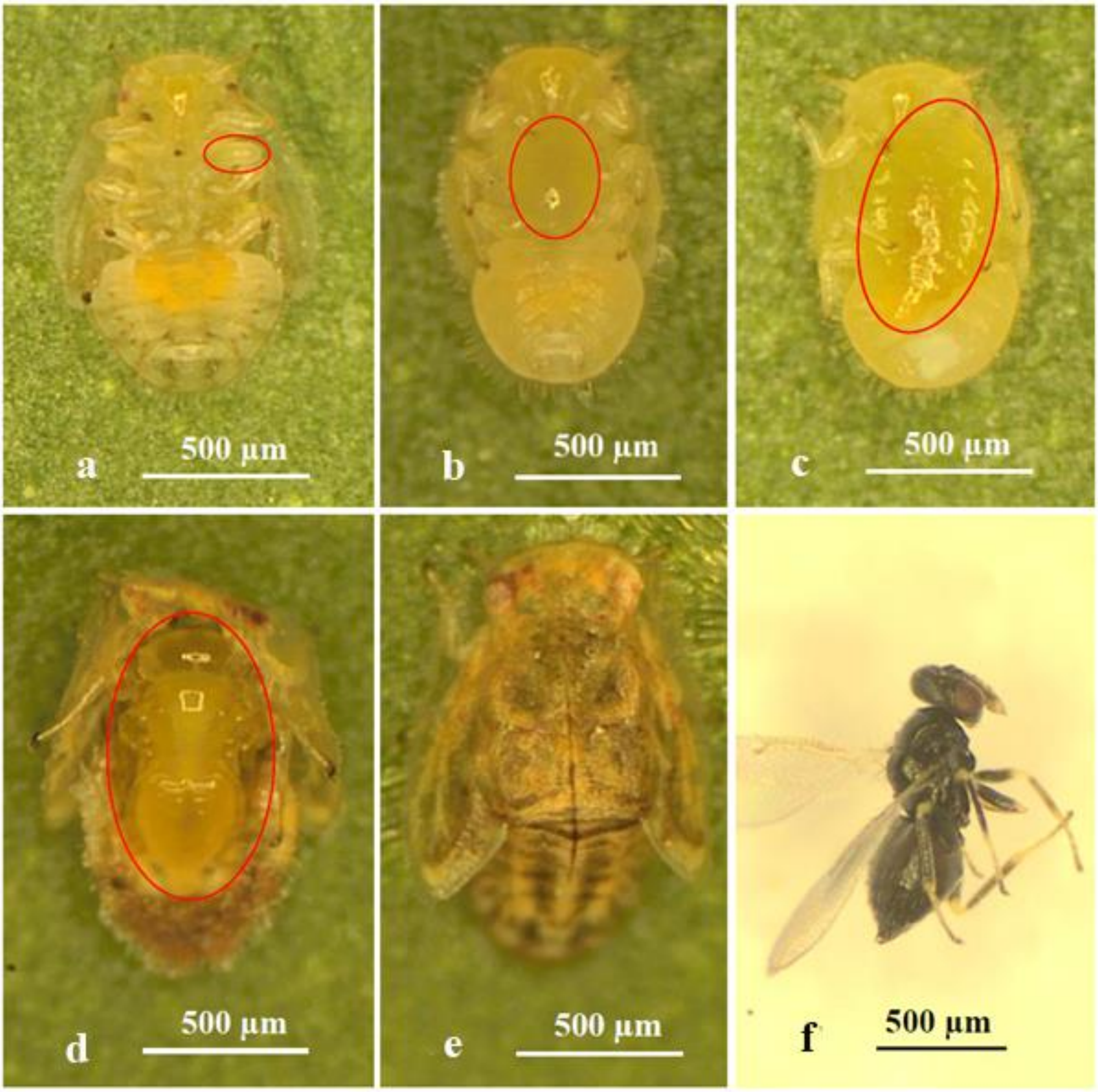

Fig. 1.2 Life cycle of *Tamarixia triozae*: (**a**) egg, (**b**) larva (1 day old), (**c**) larva (3 day old), (**d**) pupa, (**e**) psyllid mummy, and (**f**) adult (female)

Fig. 1.3 Adults of *Tamarixia triozae*: female (left) and male (right)

1.4.4 Application of *Tamarixia* species in biological control of psyllids

Some *Tamarixia* species have been used to control psyllids. For example, an augmentative biological control programme using *T. radiata* (Waterston) (Hymenoptera: Eulophidae) to combat the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) has been implemented in Mexico since 2010 (Gonzalez-Cabrera et al. 2018). *T. triozae* has been found from TPP nymphs in the field and greenhouses in Mexico and USA (Pletsch 1947; Jensen 1957; Lomeli-Flores and Bueno 2002) and up to 80% parasitism rate has been reported in the fields of Oaxaca where insecticides have not been applied extensively (Bravo and López 2007). It has a Type III functional response to its host density, i.e., the parasitism rate initially increases and then decreases with the increase of the host density, suggesting that this parasitoid is very likely to be an efficient biological control agent (Bernal et al. 1994; Yang et al. 2015). Furthermore, *T. triozae* has a higher population growth potential than its host (Rojas et al. 2015). It has been used in greenhouse crops to control TPP in North America (Workman and Whiteman 2009). This parasitoid has been imported and released since 2016 as a classical biological control agent of TPP in New Zealand (EPA 2016). Horticulture New Zealand processed the request for the import and release of *T. triozae*, and host testing was carried out on seven native and one introduced species of psyllids before its release (Gardner-Gee 2012).

1.4.5 Circadian activity patterns of parasitic wasps

Organisms have evolved circadian clocks or biological clocks to synchronize their activities with diel rhythms and achieve a survival advantage (Panda et al. 2002). Circadian activity patterns are species-specific (Brown and Schmitt 2001), which may help species consuming the same resources reduce competition and facilitate the genetic isolation between closely related species (Saunders 2002; Bertossa et al. 2013; Krittika and Yadav 2019).

1.4.5.1 Adult emergence, sexual maturation, and mating rhythms

In many parasitic hymenopterans, adult emergence mainly occurs in the first few hours after the onset of the photophase (e.g., Kainoh 1986; Pompanon et al. 1995; He et al. 2004; Karpova 2006; Hanan et al. 2009; Bourdais and Hance 2019; Wang et al. 2019). Such emergence rhythms are adaptative in diurnal insects probably because the higher air humidity and lower temperature in the early morning reduce water loss through the cuticle of a newly emerged adult and facilitate wing expansion, initial dispersal, and search for mates (Lankinen 1986; Fantinou et al. 1998; Marchand and McNeil 2000; Karpova 2006).

Adult males have shorter development period but need longer time to become sexually mature than females in some hymenopteran parasitoids (e.g., Kainoh 1986; He et al. 2004; Khatri et al. 2009; Rojas et al. 2015). Consequently, males emerge earlier than females, indicating a protandrous nature (Pompanon et al. 1995; Quicke 1997; Morbey and Ydenberg 2001; Bourdais and Hance 2019; Li et al. 2022). Protandry allows males to synchronize with sexually mature mates and increase mating success and the female to reduce the risk of death before reproduction (Waage and Ming 1984; Quicke 1997; Morbey and Ydenberg 2001; Khatri et al. 2009). In addition, in many parasitoid species (Benelli et al. 2012; Khatri 2017), mating predominantly occurs in the morning because cool temperatures and high humidity can reduce the risk of desiccation (Benelli et al. 2012), and the production of sex pheromones is maximal in the morning (McClure et al. 2007).

1.4.5.2 Oviposition and feeding rhythms

Knowledge on circadian oviposition patterns of parasitoids is important for the development of field release programmes for biological control. For example, simultaneous release of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Doryctobracon crawfordi* (Viereck) (Hymenoptera: Braconidae) may result in substantial competition for hosts and thereby reduce their control efficacy due to overlaps in their circadian oviposition patterns (Miranda et al. 2015). In addition, the understanding of daily oviposition and host feeding patterns facilitates the exploration of the role of host feeding on egg production in parasitoids. For example, *Eretmocerus warrae* Naumann and Schmidt (Hymenoptera: Aphelinidae) females mainly feed on hosts before the oviposition peak during the photophase, indicating that this parasitoid acquires nutrients for egg production by host feeding (Hanan et al. 2009).

1.4.6 Effects of adult diets on parasitoid performance

Knowledge on effects of adult diets on parasitoid performance is important in mass rearing and augmentative release programmes because diets often influence parasitoid reproductive traits (Benelli et al. 2017). For example, adults feeding on hosts (Liu et al. 2015) or honey (Hossain and Haque 2015; Liu et al. 2015; Rossetti et al. 2022) have higher fecundity and live longer. Furthermore, because parasitoids may adjust fecundity schedule in response to adult diets (e.g., Bai and Smith 1993; Wade et al. 2008; Hill et al. 2020), the timing and type of adult diets provided for parasitoids before release may change their reproductive schedule, affecting the effectiveness of fit-for-purpose biological control. For instance, when the pest population density is high, we may want to manipulate diet supply before release and allow the agent to achieve immediate pest suppression (Karacaoğlu et al. 2018). If we aim to reduce the risk of massive removal of hosts and increase establishment success of wasps in the field when the pest population density is low (Eggenkamp-Rotteveel Mansveld et al. 1982), we may want to delay parasitoids' oviposition peaks and flatten their oviposition curves by adjusting diet supply (Stahl et al. 2019).

1.4.7 Factors affecting parasitism, host feeding and sex allocation of parasitoids

1.4.7.1 Parasitism and host feeding in relation to host stage

According to the optimal foraging theory, a female parasitoid should adopt the host selection strategies that maximise her lifetime fitness gain (Goubault et al. 2003; Danchin et al. 2008). For idiobiont parasitoids, the host size at the time of parasitism determines the resources for progeny development (Strand 1986; Gauld and Bolton 1988). To maximise offspring fitness, they usually prefer to lay eggs on larger hosts that provide more resources for their offspring (Choi et al. 2001; Liu et al. 2011). Host-feeding parasitoids often prefer larger hosts for parasitisation and smaller hosts for feeding (Kidd and Jervis 1991; Choi et al. 2001; Ebrahimifar and Jamshidnia 2022; Zhang et al. 2022).

1.4.7.2 Superparasitism in relation to host stage and density

Most parasitoids can recognize and reject parasitised hosts (Montoya et al. 2012) because parasitoid offspring developed on these hosts may have poor fitness (Vet et al. 1994; Potting et al. 1997; Jones et al. 1999). However, parasitoids may still lay eggs in or on parasitised hosts (van Alphen and Visser 1990), resulting in superparasitism. Therefore, superparasitism could be an adaptive strategy under certain conditions (van Alphen and Visser 1990; Visser et al. 1992). For example, laying more than one egg in or on a host may ensure the survival of her offspring from that host (van Alphen and Visser 1990; Hanan et al. 2016). Superparasitism rate is often higher on older/larger hosts (Ho and Ueno 2008; Chen et al. 2023) because the fitness of parasitoid offspring may be greater on these hosts (Choi et al. 2001; Liu et al. 2011; Luo et al. 2022). In addition, superparasitism strategy may stabilize the host-parasitoid dynamics (Sirot and Krivan 1997). For example, parasitoids may increase superparasitism rate when host density is low to avoid collapse of the host population and reduce it when the host density is high to maximise the use of available resources (Hanan et al. 2016; Chen et al. 2023).

1.4.7.3 Sex allocation in relation to host stage

Hymenopteran parasitoids have the haplodiploid sex determination system where fertilised eggs develop into females and unfertilised eggs develop into males, allowing them to control offspring sex ratio by adjusting the proportion of fertilised eggs at oviposition (King 1988). The host size-dependent sex ratio model predicts that female parasitoids lay fertilised eggs on larger hosts and unfertilised eggs on smaller ones (Charnov et al. 1981) because the fitness of parasitoid offspring generally increases with host size at parasitisation (Hanan et al. 2015; Khatri et al. 2016; Tian et al. 2021; Luo et al. 2022), and daughter fitness generally increases more steeply with host size than son fitness (Charnov 1982). Overproducing males is common in hymenopteran parasitoid rearing and can lead to high costs in mass rearing programmes (Ode and Heinz 2002). Therefore, understanding sex allocation based on host size and the effect of host stage at parasitisation on offspring fitness may help develop effective procedures for parasitoid production.

1.4.8 Host stage selection and host defence behaviours

Foraging parasitoids often encounter their hosts of various stages and sizes with different nutritional values and defensive abilities (Gross 1993; Cadée and van Alphen 1997; He et al. 2011; Khatri et al. 2016). Host-feeding parasitoids thus need to rationalise their host selection for fitness of both their offspring and themselves (Kidd and Jervis 1991; Heimpel and Collier 1996; Lauzière et al. 2001; Hanan et al. 2015). For example, larger or older hosts provide more resources for parasitoid offspring (e.g., Liu et al. 2011; Hanan et al. 2015; Wang and Aparicio 2020; Luo et al. 2022), and smaller or younger hosts are easier to make a wound for feeding (Kidd and Jervis 1991; Veronesi et al. 2022a). Therefore, female parasitoids are more likely to accept the former for parasitism and the later for feeding. However, the expected parasitism and host feeding patterns may be altered by handling time associated with host defence (Gross 1993; Cadée and van Alphen 1997; He et al. 2011; Khatri et al. 2016). Therefore, behavioural observation and analysis of parasitoid host selection and host defence are essential to understanding behavioural mechanisms behind the host stage selection.

Chapter 2

Tamarixia triozae, an important parasitoid of *Bactericera cockerelli*: circadian rhythms and their implications in pest management

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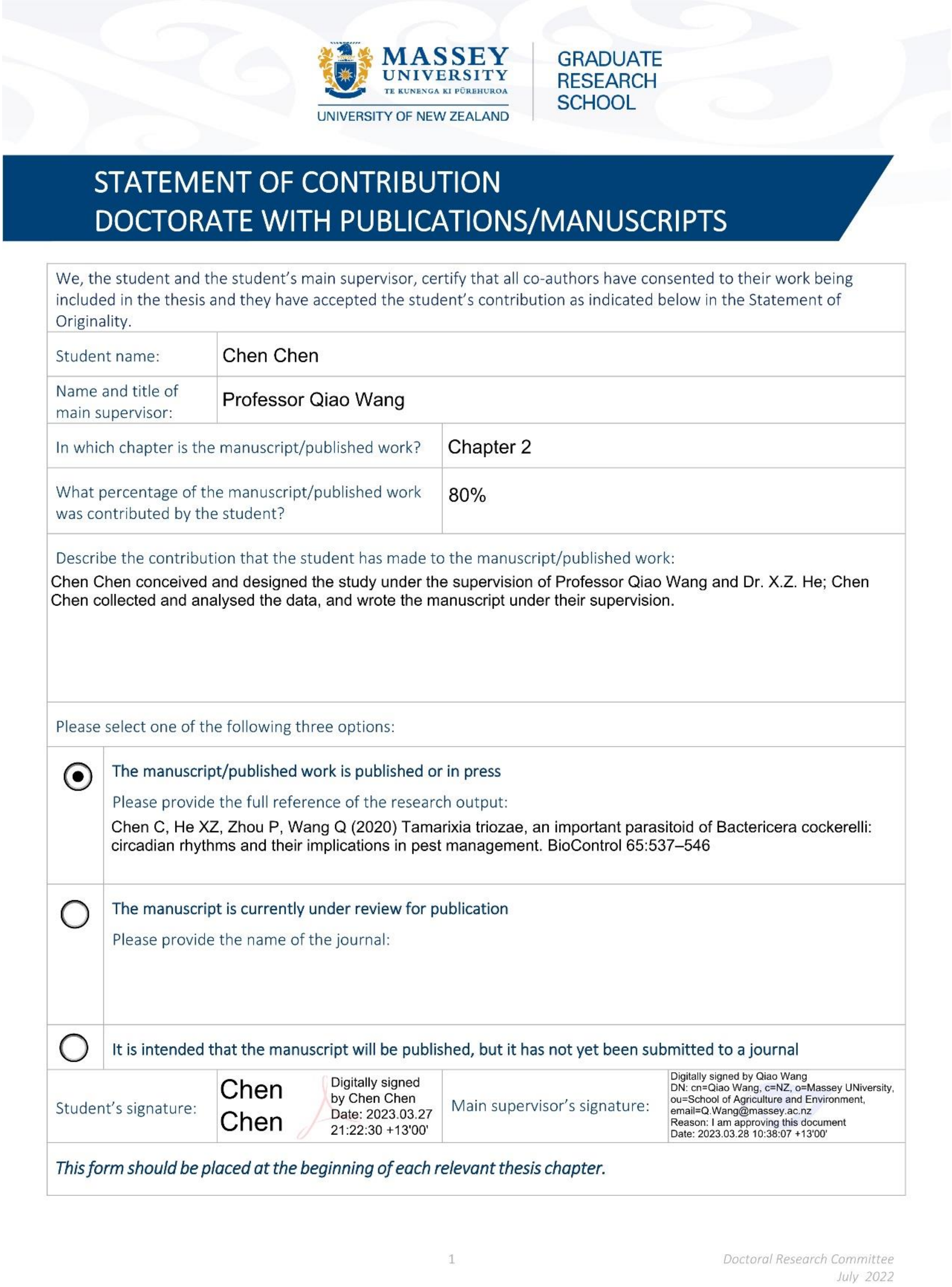

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Chen Chen
Name and title of main supervisor:	Professor Qiao Wang
In which chapter is the manuscript/published work?	Chapter 2
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:
Chen Chen conceived and designed the study under the supervision of Professor Qiao Wang and Dr. X.Z. He; Chen Chen collected and analysed the data, and wrote the manuscript under their supervision.

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1 Doctoral Research Committee
July 2022

Abstract *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is an important primary parasitoid of *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). This study aimed to unveil the parasitoid's circadian rhythms and provide information for enhancement of its biological control. Most emergence occurred in the morning and most mating took place early next morning. Oviposition only occurred during the daytime, peaking between mid-morning and mid-afternoon while host feeding had three peaks in the early morning, late afternoon and dawn, respectively. I demonstrate that circadian activity peaks for different life functions did not overlap, suggesting that *T. triozae* have developed strategies for maximal fitness gain. These findings have implications in pest management, which can be tested under field conditions. For example, release of sexually mature and host-fed adults in the early morning may help achieve better control. Moreover, insecticide sprays, when necessary, may be applied as late in the evening as possible to reduce side effects on this parasitoid.

2.1 Introduction

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an economically important pest of cultivated solanaceous crops, particularly potatoes, tomatoes, eggplants and peppers, in the USA, Mexico (Cranshaw 1994; Crosslin et al. 2010; Butler and Trumble 2012a; Rojas et al. 2015) and New Zealand (Teulon et al. 2009; Yang and Liu 2009). It has also established in Western Australia since 2017 (FAO 2017) and its economic impact there is under evaluation. Due to its invasive nature, the pest is expected to enter other parts of the world in the future (CABI 2018). TPP damages host plants by direct feeding and transmission of *Candidatus* Liberibacter solanacearum, the causal agent of zebra chip, and other diseases (Munyaneza et al. 2007, 2008; Butler and Trumble 2012a; Rojas et al. 2015). Soon after its first detection in New Zealand in 2006, the pest cost the potato industry of this country more than NZ$120 million (Ogden 2011). So far, the application of insecticides is the main method for the control of this pest (Munyaneza 2012; Martinez et al. 2015; Wright et al. 2017). However, the psyllid has already developed resistance against commonly used insecticides (Dávila et al. 2012; Cerna et al. 2013; Chávez et al. 2015; Szczepaniec et al. 2019), reducing their effectiveness. Furthermore, the application of insecticides may cause pest resurgence (Prager et al. 2016) due to destruction of natural enemies (Martinez et al. 2015; Morales et al. 2018). Therefore, there is an urgent need to develop more sustainable control methods for this pest including the use of natural enemies (Luna-Cruz et al. 2011; Liu et al.

2012; Cerón-González et al. 2014; Rojas et al. 2015) compatible with integrated pest management programs.

Tamarixia triozae (Burks) (Hymenoptera: Eulophidae) is an important primary ectoparasitoid of TPP (Rojas et al. 2015; Yang et al. 2015) with potential for higher population increase than its host (Rojas et al. 2015) and up to 80% parasitism rate (Bravo and López 2007). It is an arrhenotokous synovigenic wasp, killing its hosts by both parasitisation and host feeding (Martinez et al. 2015; Rojas et al. 2015). A female consumes an average of 1.4 psyllid nymphs for each egg laid (Cerón-González et al. 2014). Females feed on all instars of host nymphs (Vega-Chávez 2010) to gain nutrients necessary for egg maturation (Jervis and Kidd 1986; Burger et al. 2005) and prefer to parasitise fourth instar nymphs (Caudillo Ruiz 2010). Each female lays an average of 165 eggs during her lifespan, and the pre-oviposition period and longevity of adult females are about two and 20 days, respectively (Rojas et al. 2015). These features suggest that *T. triozae* can be an effective biological control agent of TPP. Consequently, New Zealand has recently introduced and released the parasitoid for TPP control (Barnes 2017). However, the parasitoid is very sensitive to insecticides which may render it ineffective (Luna-Cruz et al. 2011; Liu et al. 2012), and recommendation for the timing of insecticide sprays to minimize side effects on this natural enemy is yet to be developed.

Most organisms adjust their physiology and behaviour through their circadian clock in response to daily photoperiod (Panda et al. 2002; Bertossa et al. 2010; Vaze and Sharma 2013; Saunders 2020). For instance, adults of different parasitoid species have their own unique circadian activity patterns (Brown and Schmitt 2001). Species-specific circadian rhythms may be of adaptive significance, for example different rhythmicity can reduce competition between species consuming the same resources and improve the efficiency of genetic isolation between more closely related species (Saunders 2002; Bertossa et al. 2013). Therefore, understanding circadian activity patterns of pest parasitoid adults may help develop effective laboratory handling and field release programs for biological control. For example, the morning release of the squash bug egg parasitoid *Gryon pennsylvanicum* (Ashmead) appears to be more effective because it is easier for adults to find mates in the morning (Vogt and Nechols 1991). Due to significant overlaps in circadian oviposition patterns of *Diachasmimorpha longicaudata* (Ashmead) and *Doryctobracon crawfordi* (Viereck), two parasitoids of tephritid fruit flies, simultaneous release of both species may result in substantial competition, reducing control efficacy (Miranda et al. 2015).

Knowledge on circadian rhythms of adult parasitoids may also contribute to decisions on the timing of insecticide applications that could reduce impacts on natural enemies. For instance, *G. pennsylvanicum* is most vulnerable to insecticide sprays when adult activity peaks, during which time no insecticide should be applied (Vogt and Nechols 1991). To spare *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), van Lenteren et al. (1992) recommend that irrigation and insecticide sprays be avoided during the peak of adult emergence. However, circadian rhythms of emergence, host feeding and reproduction of *T. triozae* are still unknown, hampering the development of its effective laboratory handling and field releases. Moreover, it may help understand potential competition with other parasitoid species in the field. Without this knowledge, it would be impossible to make recommendations for the timing of insecticide applications or irrigation.

In this study, I simulated New Zealand summer conditions of 25ºC and a photoperiod of L:D 14:10 h and carried out a series of experiments to determine the circadian patterns of emergence, host feeding and reproduction in *T. triozae*. Knowledge generated is essential for development of an effective TPP biological control program using this parasitoid and strategies for its integration with other control practices (e.g., the release of other natural enemies, pesticide applications, and watering).

2.2 Materials and methods

2.2.1 Breeding colonies and experimental conditions

I established *B. cockerelli* and *T. triozae* colonies with adult insects from BioForce Ltd., Auckland, New Zealand. I maintained the psyllid colony on five two-month-old bell pepper (*Capsicum annuum* L.) plants, each grown in a pot (9.5 cm in height × 10.5 cm in top diameter × 8.5 cm in bottom diameter) with potting mix. The potted plants were randomly placed in an aluminium-framed cage (43 × 42 × 40 cm) with a fine metal mesh (aperture size = 0.25 mm) on the back and both sides and Perspex on the top and front and aluminium alloy on the bottom. I reared *T. triozae* on 3rd–5th instar psyllid nymphs feeding on five infested potted plants in a cage as before. These two insect colonies were kept in two separate climate rooms to ensure the psyllid colony was parasitoid-free for experiments. Environmental conditions for colony maintenance were 25 ± 1°C, 40–60% RH and a photoperiod of L:D 14:10, which normally occur in New Zealand summer. The photophase was set from 08h00 to 22h00 for both colonies.

I carried out experiments in two climate rooms under the above environmental conditions. I set the photophase from 08h00 to 22h00 for one room (a normal light regime) and the scotophase from 10h00 to 20h00 for the other (a reverse light regime). I recorded the data in the normal light room during the period of 08h00 to 22h00 and in the reverse light room during the period of 10h00 to 20h00. Therefore, all data for the photophase were from the normal light room and those for the scotophase were from the reverse light room. I collected parasitoid adults from normal light regime and introduced them into reversed light regime.

2.2.2 Sex-dependent emergence rhythm and developmental period

I recorded sex-dependent emergence patterns and developmental period of *T. triozae* in both climate rooms. In each room, I set up 15 plastic cylinders made of two identical transparent containers (6.5 cm in diameter × 8.5 cm in height). The bottom container was filled with tap water and covered by a lid bearing a hole (1.0 cm in diameter) at the centre through which I inserted the petiole of a bell pepper leaf infested with 50 3rd–5th instar psyllid nymphs. The top container had three holes, two (3 cm in diameter) covered by a metal mesh (aperture size = 0.25 mm) at the opposite sides for ventilation and one (1 cm in diameter) with a cotton wool plug between the ventilation holes for transferring wasps. A cotton wool ball (0.5 cm in diameter) saturated with 10% honey solution was placed on the lid of the bottom container to provide food for wasps. The two containers were held together with a piece Parafilm®.

At the beginning of the photophase in each room, I released four *T. triozae* females randomly collected from the colony into each plastic cylinder through the hole in the top container using an aspirator, and then plugged the hole with a cotton wool plug. I allowed the wasps to lay eggs during the first three hours of the photophase and then removed them from the cylinder and transferred them back to the colony. I collected the parasitoid pupae seven days after oviposition from each cylinder and individually placed them in glass vials (5 cm in height × 1.5 cm in diameter) in their rooms of origin. Eight days after oviposition, I observed and recorded hourly emergence rhythm of 181 and 195 pupae for three successive days in the normal light and reverse light rooms, respectively. I sexed newly emerged adults according to Vega-Chávez (2010) and calculated the developmental time of each sex (101 males and 88 females) as the time from the egg deposition to adult emergence.

2.2.3 Sexual maturation and circadian mating rhythm

To determine sexual maturation, I individually paired newly emerged virgin females or males with a one-day-old virgin mate in the above-mentioned glass vials immediately after lights on in the normal light room where these experimental insects originated. I observed the mating events of each pair until mating ended or lights off. I placed a cotton wool ball saturated with 10% honey solution in each vial to provide food for wasps. For each pair, I recorded the pre-mating period from pairing to mating using a stopwatch. I tested 55 males and 52 females, respectively, of which 28 males and 16 females did not mate.

To record circadian mating rhythm, I made observations in both normal light and reverse light rooms with insects from their original rooms. I individually paired one-day-old virgin males with one-day-old virgin females in the above-mentioned glass vials at the beginning of the photophase and scotophase in the normal light and reverse light rooms, respectively, and observed mating events continuously throughout the photophase and scotophase, respectively. I observed 84 and 40 pairs in the normal light and reverse light rooms, respectively, and provided 10% honey solution for each pair as described above. I used red light for observations during the scotophase.

2.2.4 Host feeding and oviposition rhythms

Because most *T. triozae* females begin to lay eggs when they are three day old under conditions similar to those in the present study (Rojas et al. 2015), I used four-day-old mated females for host feeding and oviposition experiments. To obtain four-day-old mated females, I individually paired one-day-old virgin wasps that fed on honey solution before pairing in the above-mentioned glass vials and observed their mating events in each room. In each light regime, I set up five cylinders, each of which held a bell pepper leaf infested with about 80 3rd–5th instar psyllid nymphs and a cotton wool ball saturated with 10% honey solution as described above. I introduced four one-day-old mated females into each cylinder and allowed them to stay for 24 h. I then transferred these females into a new cylinder with psyllids and honey solution as above using an aspirator and allowed them to stay for additional 24 h. I repeated this process until I obtained 20 females at normal and reverse light regimes, respectively, for the following experiment.

I placed a bell pepper leaf upside down with its petiole wrapped with water-saturated cotton wool in a Petri dish (8.5 cm in diameter × 2.4 cm in height) and then gently transferred 20 4th-instar psyllid nymphs onto the leaf and covered the dish with a lid. The lid had three holes, one (1 cm in diameter) covered with metal mesh for ventilation, one (1 cm in diameter) with a cotton wool plug for introducing wasps and one (0.5 cm in diameter) inserted with a cotton wool ball saturated with 10% honey solution as food for wasps. I released one 4-day-old mated female wasp obtained as above into the Petri dish through the introduction hole in the lid, plugged the hole with cotton wool and allowed her to stay for two hours (first feeding and oviposition bouts). I then transferred her to a new Petri dish with 20 fourth-instar psyllid nymphs using an aspirator and allowed her to stay for two more hours. I repeated the process until I achieved seven 2-h bouts in the photophase and five 2-h bouts in the scotophase. I tested 20 female wasps in each light regime and treated each female as a replicate. Since exuded body fluid is the evidence of host feeding and *T. triozae* females deposit their eggs under the host nymphs (Martinez et al. 2015), I counted the number of nymphs fed and then turned over all nymphs to count the number of eggs laid under a stereomicroscope (Leica MZ12, Germany) for each 2-h bout.

2.2.5 Statistical analysis

I carried out all analyses using SAS 9.4 (SAS Institute Inc 2019) with a rejection level set at $\alpha < 0.05$. I used a χ^2 test (FREQ procedure) to compare differences in proportion of adult emergence between photophase and scotophase and in mating success between males and females. I used non-linear regression models (NLIN procedure) to fit the data of adult emergence, mating, host feeding and oviposition rhythms, and estimated the parameters by a standard least squares method. The estimated parameters in non-linear regression models were significantly different from 0 if both low and up 95% confidence limits (CLs) > 0 or < 0. The coefficients of determination (R^2) for regressions were calculated as the sum of square due to the model divided by the total sum of square.

An exponential decline model (Archontoulis and Miguez 2015) was applied to fit the hourly emergence (y) and mating success (y): $y = ae^{(-bx)}$, where x (≥ 1) is the hour, a is the maximum y value and b is a decrease rate of y. An asymptotic exponential model (Archontoulis and Miguez 2015) was used to fit the hourly cumulative emergence (y): $y = a\,(1 – e^{(-bx)})$, where x (≥ 1) is the hour, a is the maximum y value and b is an increase rate of y. The difference in each estimated parameter (a or b) in a model was compared between sexes according to Julious (2004): if the 95% CLs overlap, then there is no significant difference.

A Gaussian functional model (Archontoulis and Miguez 2015) was modified to fit the data on oviposition, which occurred exclusively during the photophase: $y = ae^{\{-b[\ln{(x/x_0)}/c]^2\}}$, where x is the hour, a is the oviposition peak at time x_0 (h) and b (default = 0.5 for the Gaussian function) and c are coefficients controlling the width of the peak. Because host feeding took place throughout 24 h cycle, I improved the above Gaussian functional model to fit the data on host feeding in the photophase: y = 1/($ae^{\{-b[\ln(x/x_0)/c]^2\}}$), where 1/$a$ is the minimum of hosts fed at time x_0. I then used an exponential increase model to estimate the number of hosts fed in the scotophase: $y = e^{[a(x-x_0)]}$, where a is the maximum of hosts fed at time x_0.

I used a Shapiro-Wilk test (UNIVARIATE procedure) to check whether data on the pre-mating duration and developmental time followed a normal distribution. I analysed those on pre-mating duration using an ANOVA due to their normal distribution. Because data on the developmental time of both sexes were not normally distributed even after transformation, they were analysed using the non-parametric Kruskal-Wallis test (NPAR1WAY procedure).

2.3 Results

2.3.1 Sex-dependent emergence rhythm and developmental period

I show that the vast majority of emergence (91% of males and 79% of females) occurred during the photophase (x_1^2 = 58.91 and 34.47 for males and females, respectively; $P < 0.0001$) (Fig. 2.1a). Emergence of both sexes was the highest during the early photophase and then significantly decreased (95% CLs of coefficient = 0.43 – 0.91 for males, and 0.13 – 0.22 for females) (Fig. 2.1a). Male emergence declined significantly faster (coefficient = 0.67) than female emergence (coefficient = 0.17) (non-overlapped 95% CLs of coefficients). Cumulative emergence reveals that 50% of males and females emerged two hours and four hours after lights on, respectively (95% CLs of coefficient = 0.33 – 0.60 for males, and 0.17 – 0.18 for females) (Fig. 2.1b). The cumulative emergence increased significantly faster in males (coefficient = 0.47) than in females (coefficient = 0.17) (non-overlapped 95% CLs of coefficients). My results indicate that the developmental period from egg to adult in males (mean ± SE, 9.90 ± 0.03 days) was significantly shorter than that in females (10.12 ± 0.04 days) (x_1^2 = 49.73, $P < 0.0001$).

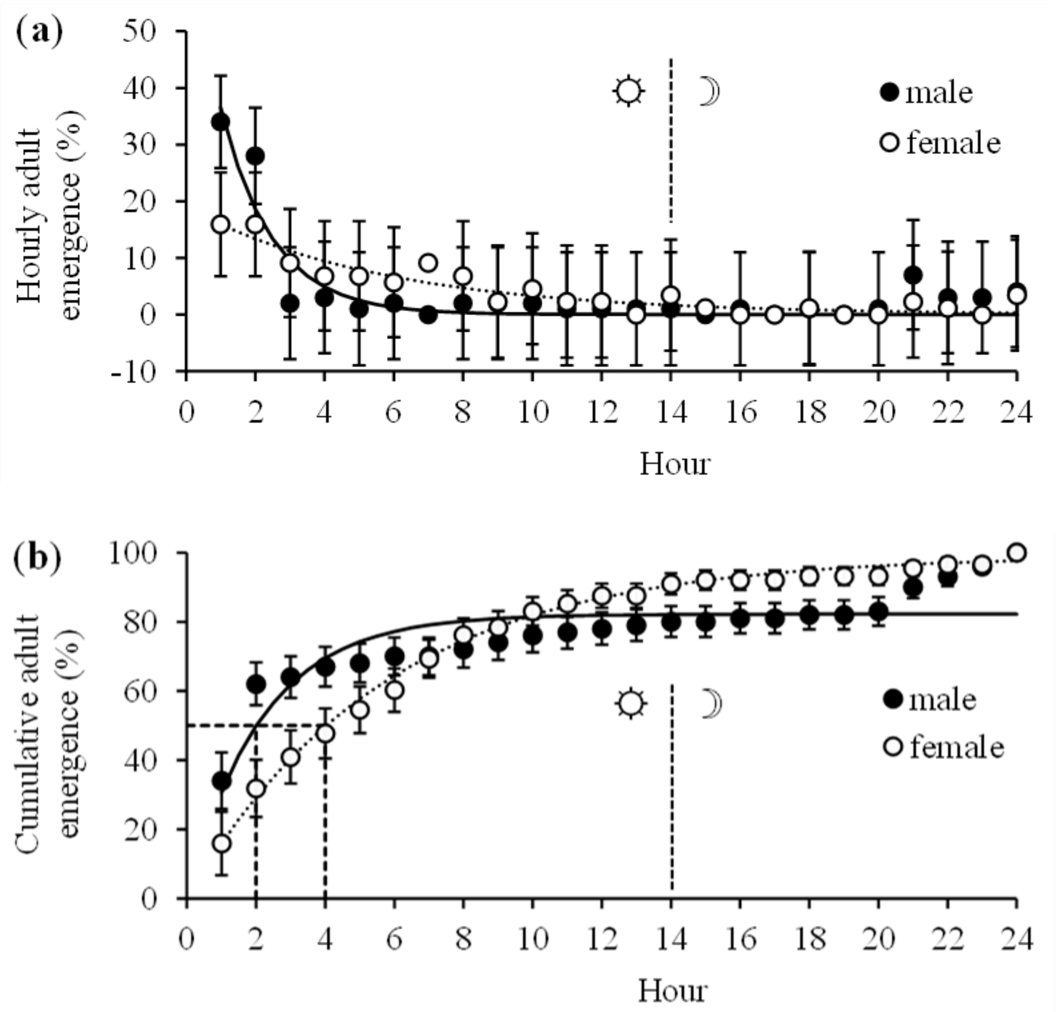


Fig. 2.1 Hourly (**a**) and cumulative (**b**) emergence patterns in *Tamarixia triozae* (photophase = 0–14 h and scotophase = 14–24 h). (**a**) Hourly emergence of males (%): y = $71.48e^{(-0.67x)}$, $R^2 = 0.8754$; hourly emergence of female (%): y = $18.97e^{(-0.17x)}$, $R^2 = 0.9285$. (**b**) Cumulative emergence of males (%): y = $82.16(1-e^{(-0.47x)})$, $R^2 = 0.9915$; cumulative emergence of females (%): y = $99.13(1-e^{(-0.17x)})$, $R^2 = 0.9996$; the points where the dotted horizontal and vertical lines intersect indicate 50% of cumulative emergence for males and females, respectively. Lines on dots are SE.

2.3.2 Sexual maturation and circadian mating rhythm

Significantly more newly emerged females (69.2 ± 6.4%) mated successfully than newly emerged males (49.1 ± 6.7%) when they were offered one-day-old mates (x_1^2 = 4.48, P = 0.0343). Similarly, females became sexually receptive (1.3 ± 0.1 h) significantly earlier than males (2.3 ± 0.6 h) ($F_{1,61}$ = 4.18, P = 0.0456).

About 70% of one-day-old adults mated during the first hour of the photophase and then mating success significantly declined within four hours into the photophase (95% CLs of coefficient = 1.18 – 1.55) (Fig. 2.2). Very few matings occurred after four hours into the photophase and during the scotophase (Fig. 2.2).

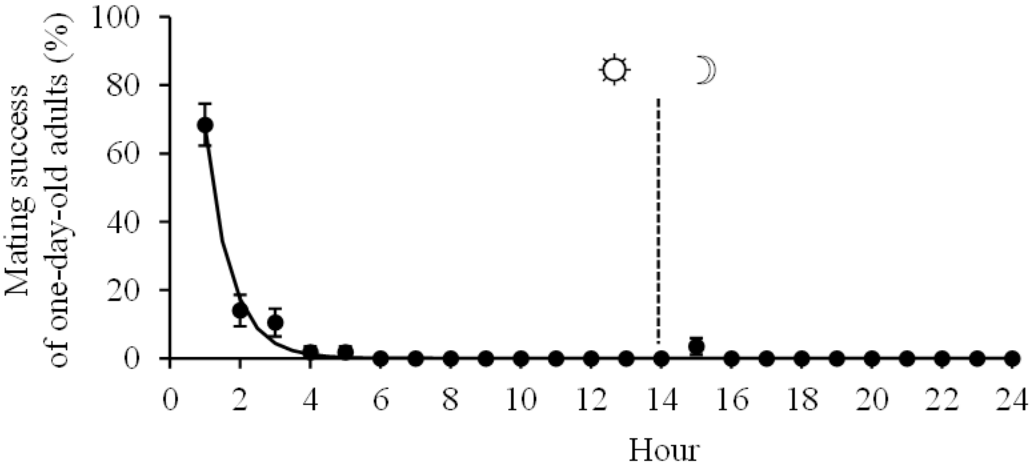


Fig 2.2 Mean hourly mating success of one-day-old adults (photophase = 0–14 h and scotophase = 14–24 h): y = $266.20e^{(-1.36x)}$, R^2 = 0.9874. Lines on bars are SE

2.3.3 Host feeding and oviposition rhythms

Adult females fed on hosts throughout the day but laid eggs only in the photophase (Fig. 2.3). In the photophase the number of hosts fed on significantly decreased in the first three 2 h feeding bouts and then significantly increased (95% CLs of coefficient = 0.45 – 1.95) but in the scotophase this significantly increased over time (95% CLs of coefficient = 0.03 – 0.42). The number of eggs laid significantly increased in the first three 2 h bouts and then significantly declined (95% CLs of coefficient = 0.45 – 0.91).

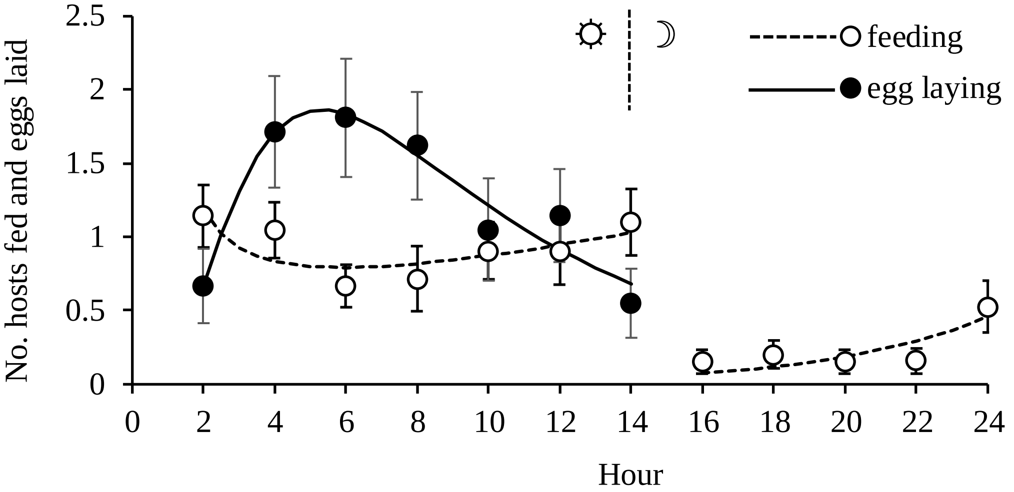


Fig. 2.3 Mean number of hosts fed and eggs laid by *Tamarixia triozae* females (photophase = 0–14 h and scotophase = 14–24 h): number of hosts fed in the photophase: y = $1/(1.26e^{\{-0.5[\ln(x/5.86)/1.20]^2\}})$, $R^2 = 0.5117$; number of hosts fed in the scotophase: y = $e^{[0.22(x-27.46)]}$, $R^2 = 0.2323$; number of eggs laid in the photophase: y = $1.86e^{\{-0.5[\ln(x/5.31)/0.68]^2\}}$, $R^2 = 0.4336$. Lines on bars are SE

2.4 Discussion

The present study shows that *T. triozae* adults emerged throughout the day with the vast majority of emergence occurring in the photophase, particularly during the first few hours after the onset of the photophase (Fig. 2.1). Such emergence patterns may be beneficial to this primarily diurnal insect because the lower temperature and higher humidity in the early morning are ideal for wing expansion, initial dispersal and search for mates with less water loss (Lankinen 1986; Fantinou et al. 1998; Marchand and McNeil 2000; Karpova 2006; McClure et al. 2007). Similar circadian emergence patterns take place in some other parasitoid wasps such as *Ascogaster reticulatus* Watanabe (Hymenoptera: Braconidae) (Kainoh 1986), *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) (England 1995), *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) (He et al. 2004) and *Eretmocerus warrae* Nauman and Schmidt (Hymenoptera: Aphelinidae) (Hanan et al. 2009).

Similar to some earlier studies on hymenopteran parasitoids (e.g., Kainoh 1986; He et al. 2004; Rojas et al. 2015), *T. triozae* males had significantly shorter developmental periods than did females. Consequently, most males emerged significantly earlier than females (Fig. 2.1), indicating a protandrous nature, which is a strategy to increase male mating success and reduce the risk of female pre-reproductive death (Waage and Ming 1984; Quicke 1997; Morbey and Ydenberg 2001). Furthermore, *T. triozae* males became sexually mature significantly later than females, and when offered sexually mature one-day-old mates, females were more likely than males to mate during the photophase of their emergence. However, when I paired one-day-old wasps during the entire photophase, 95% of pairs mated during the first four hours of the photophase with > 65% of mating occurring in the first hour of the photophase (Fig. 2.2). These findings suggest that, unlike *A. reticulatus* where emergence occurs early in the morning and mating in the afternoon of the same day (Kainoh 1986), most *T. triozae* adults may not mate on the day of emergence, rather they are more likely to mate in the early morning of the following day when both sexes are sexually mature. This strategy may reduce mate-searching time, mating failure, and risk of predation as reported in other insects (Rymer et al. 2007) and allow time for *T. triozae* females to mature their eggs because most females begin to lay eggs in the third photophase following emergence (Rojas et al. 2015).

Adults of many parasitoid species including a congeneric species *T. radiata* (Waterston) (Gebiola et al. 2018) feed on their hosts to gain nutrients necessary for egg maturation and production (Jervis and Kidd 1986; Burger et al. 2005; Hanan et al. 2009). I show that *T. triozae* females laid eggs only in the photophase but fed on hosts throughout the day with most host feeding happening during the photophase (Fig. 2.3). In some parasitoids such as *Ooencyrtus kuwanai* (Howard) (Hymenoptera: Encyrtidae) (Weseloh 1972) and *A. ervi* (He et al. 2004), oviposition occurs throughout the 24 h cycle while in others such as *E. warrae* (Hanan et al. 2009), host feeding and oviposition take place in both photophase and scotophase. In the photophase, host feeding and oviposition in *T. triozae* had reverse patterns where maximum oviposition and minimum host feeding happened four to eight hours into the photophase (Fig. 2.3). Although host feeding activity was generally low during the scotophase, it reached a peak just before its end (Fig. 2.3). These findings reveal a clear strategy that female *T. triozae* take to maximise their fitness, i.e., they have the highest host feeding rate before and after the oviposition peak to acquire nutrients for egg production and somatic maintenance. *E. warrae* females probably use the same strategy to achieve the best fitness because their circadian oviposition and host feeding activity peaks do not overlap (Hanan et al. 2009).

In general, male harassment can reduce female fitness in insects (e.g., Sakurai and Kasuya 2008; Li et al. 2014). In the present study, I found that circadian mating (Fig. 2.2) and oviposition (Fig. 2.3) peaks mostly did not overlap in *T. triozae*, suggesting that males are not able to harass females after oviposition starts because males are active in the early morning. This clearly defined circadian activity pattern for host feeding and reproduction in *T. triozae* indicates that this parasitoid species has developed a highly adaptive strategy for maximum fitness, which may make it an effective biological control agent.

My findings provide important knowledge for development of laboratory handling and field release programs for biological control of TPP. For example, to achieve the highest reproductive output and best control of TPP, we may allow sexually mature wasps to mate in the early morning of the next day following their emergence. Because most *T. triozae* females start ovipositing when they are three day old (Rojas et al. 2015) and host feeding is essential for egg production and maturation (Cerón-González et al. 2014), we may achieve better results if we carry out field or greenhouse release of mated and host-fed females in the early morning of the third day following emergence. Alternatively, if growers decide to release newly emerged wasps in the field or greenhouses, they may do so in the morning when adults are more active and environmental conditions are more favorable. This approach has proved to be effective in the application of another diurnal parasitoid, *G. pennsylvanicum* (Vogt and Nechols 1991). When we introduce new natural enemies to control TPP in the future, we need to ensure that their circadian oviposition and host feeding peaks do not completely overlap with those of *T. triozae* to minimize competition that may reduce biological control efficacy (Miranda et al. 2015; Ramírez-Ahuja et al. 2017). Furthermore, a recent report shows that circadian rhythms of predatory mites may vary in different seasons (Pérez-Sayas et al. 2018). It is thus worth exploring whether those of *T. triozae* differ seasonally.

Non-selective pest control measures including application of insecticides and crop production procedures such as overhead irrigation can make natural enemies ineffective. Therefore, to reduce impact on biological control agents, particularly parasitoids, the timing of these operations is important (Vogt and Nechols 1991; van Lenteren et al. 1992; Martinez et al. 2015). In the present study, I demonstrate that most emergence and mating occurred in the morning (Figs. 2.1 and 2.2), and oviposition concentrated from mid-morning to mid-afternoon and host feeding peaked in the early morning, late afternoon and dawn (Fig. 2.3). Therefore, I suggest that field trials be conducted to test whether insecticide sprays and overhead irrigations outside the activity peaks of *T. triozae* would reduce their impact on the parasitoid. Because *T. triozae* pupae are less susceptible to insecticides than other immature stages (Morales et al. 2018) and the population peak of *T. triozae* is 2–3 weeks behind that of TPP in the field (Butler and Trumble 2012c), the difference in the phenology of these two organisms should be taken into consideration when integrating biological control and other cultural practices. In addition, biopesticides with short residual activity and low toxicity to natural enemies (Liu and Stansly

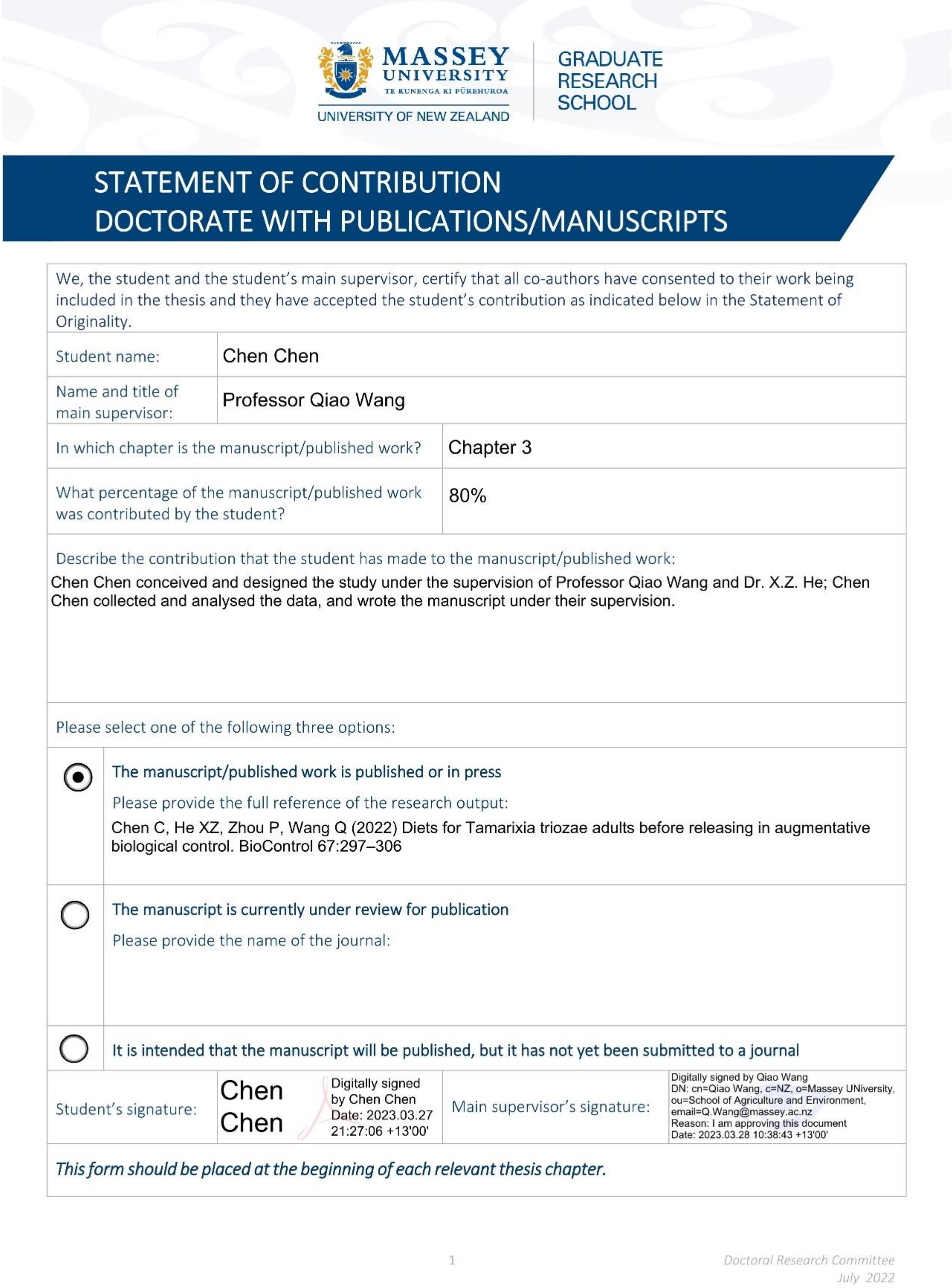
1995; Gharalari et al. 2009; Buteler and Stadler 2011) should be considered in the integrated management program involving *T. triozae*.

In summary, most adult *T. triozae* emerge in the morning with males emerging earlier than females and females becoming sexually mature earlier than males. Most individuals mate at the very beginning of the morning of the second photophase following emergence. Oviposition only occurs during the daytime, peaking between mid-morning and mid-afternoon. Host feeding has three peaks taking place in the early morning, late afternoon and dawn, respectively. My findings demonstrate that most mating, host feeding and oviposition activities do not overlap over the 24 h cycle, suggesting that *T. triozae* have developed strategies to perform different life functions at the particular times for maximal fitness gain.

Chapter 3

Diets for *Tamarixia triozae* adults before releasing in augmentative biological control

This chapter was published in *BioControl* 67:297–306 (2022).



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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Chen Chen
Name and title of main supervisor:	Professor Qiao Wang
In which chapter is the manuscript/published work?	Chapter 3
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:

Chen Chen conceived and designed the study under the supervision of Professor Qiao Wang and Dr. X.Z. He; Chen Chen collected and analysed the data, and wrote the manuscript under their supervision.

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Student's signature:	Chen Chen Digitally signed by Chen Chen Date: 2023.03.27 21:27:06 +13'00'	Main supervisor's signature:	Digitally signed by Qiao Wang DN: cn=Qiao Wang, c=NZ, o=Massey UNIversity, ou=School of Agriculture and Environment, email=Q.Wang@massey.ac.nz Reason: I am approving this document Date: 2023.03.28 10:38:43 +13'00'

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1

Doctoral Research Committee
July 2022

Abstract The effectiveness of augmentative biological control using parasitoids often depends on their physiological state and the pest population density at the time of release. *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is a primary host-feeding parasitoid of a serious invasive pest *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). Here I investigated the effects of adult diets (honey, water, yeast, and hosts) and timing of their provision on *T. triozae* fitness and oviposition patterns, providing knowledge for enhancement of its biological control potential. Adults fed with honey for four days with no access to hosts or with water or yeast for one day followed by host feeding for three days had similar longevity and lifetime pest killing ability. Adults fed with only water for one day before release had significantly greater intrinsic rate of increase, shorter doubling time, and higher daily fecundity peak. Adults fed with honey or yeast for one day followed by host feeding for three days significantly flattened their daily oviposition curves. These findings have several implications for augmentative biological control using *T. triozae*. First, honey diet may allow at least four days for successful shipment of host-deprived adults without compromising biological control effectiveness. Second, the release of host-deprived adults with one-day water feeding may achieve rapid pest suppression when the pest population density is high. Finally, releasing host-deprived adults with one-day honey or yeast feeding followed by three-day host feeding can increase their establishment success and reduce the risk of massive removal of hosts when the pest population density is low.

3.1 Introduction

Augmentative release of natural enemies, particularly parasitoids, is one of the most widely used approaches to pest biological control in annual crops and greenhouses (van Lenteren and Bueno 2003; Hoy 2008; Amadou et al. 2019; Bueno et al. 2020; Kazak et al. 2020). It is also commonly applied to control resurgent pests resulting from disruptions of natural enemy populations by seasonal factors and harvesting activities (Hajek and Eilenberg 2018; Stenberg et al. 2021) or insecticide applications (Coppel and Mertins 1977; DeBach and Rosen 1991). Evidence shows that adult host feeding is important for synovigenic parasitoids (Jervis and Kidd 1986; Giron et al. 2004; Burger et al. 2005; Jervis et al. 2008; Kapranas and Luck 2008; Liu et al. 2015; Benelli et al. 2017; Hanan et al. 2017; Gebiola et al. 2018), and supplementary adult diets such as honey and honeydew (Bezemer et al. 2005; Wade et al. 2008; Hossain and Haque 2015; Benelli et al. 2017; Dong et al. 2018; Picciau et al. 2019) and yeast (Bartlett 1964; Heimpel and Rosenheim 1995) can increase parasitoid fecundity and longevity. Therefore, provision of adult parasitoid diets before release can not only improve parasitoids' ability to establish at release sites (Tena et al. 2017; Stahl et al. 2019) but also increase their biological control effectiveness after release (Hougardy et al. 2005; Hougardy and Mills 2006, 2007; Benelli et al. 2017).

Several studies report that adult diets can change parasitoids' lifetime oviposition patterns (Bai and Smith 1993; Wade et al. 2008; Hill et al. 2020). This suggests that we may be able to modify parasitoids' lifetime oviposition trajectory by tailoring their adult diets before release to achieve effective augmentative biological control under different circumstances. For example, when the pest population density is high, we may aim to achieve quick pest suppression (Karacaoğlu et al. 2018) where the parasitoids are expected to perform maximum host killing through host feeding and parasitisation immediately after release. When the pest population density is low, we may want to delay their oviposition peaks and flatten their oviposition curves (Stahl et al. 2019) where the released parasitoids are expected to spread their oviposition and host feeding more evenly or move these activities toward their later life. Nevertheless, it is not clear how alteration of timing and type of diets provided for adult parasitoids before release can change their life history traits towards our advantage. It is also unclear whether such manipulation would compromise their overall pest killing ability in terms of fecundity and host feeding. This knowledge is crucial for the development of successful augmentative biological control programs using parasitoids.

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a severe pest of solanaceous crops such as potatoes, tomatoes, eggplants and peppers in the USA, Mexico and New Zealand (Cranshaw 1994; Teulon et al. 2009; Crosslin et al. 2010; Butler and Trumble 2012a; Rojas et al. 2015), causing significant economic losses. It is native to the United States and Mexico (EPPO 2013; CABI 2018) and has invaded New Zealand, Australia, Canada, and Ecuador (Teulon et al. 2009; Ogden 2011; FAO 2017; WADPIRD 2018; Castillo Carrillo et al. 2019; Olaniyan et al. 2020). *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is an important synovigenic parasitoid of TPP (Bravo and López 2007; Rojas et al. 2015; Yang et al. 2015). It kills the pest by both host feeding and parasitisation (Martinez et al. 2015). However, it is still unknown how provision of different adult diets and temporary deprivation of hosts could affect reproductive fitness and lifetime feeding and oviposition patterns in *T. triozae*. This information is of vital importance for development of strategies to optimize augmentative biological control of TPP using *T. triozae*.

In the present study, I tested the effects of adult diets with or without temporary host deprivation on survival, lifetime fecundity, host feeding, oviposition patterns and life table parameters in *T. triozae*. Knowledge presented in this work could help enhance the success of augmentative biological control using *T. triozae*.

3.2 Materials and methods

3.2.1 Insects

I obtained *B. cockerelli* and *T. triozae* from BioForce Limited, Auckland, New Zealand, and established the colonies in the laboratory. I maintained the psyllid colony on five two-month-old bell pepper plants, each of which was grown in a pot (9.5 cm in height × 10.5 cm in top diameter × 8.5 cm in bottom diameter) with potting mix. The potted plants were placed in an aluminium-framed cage (43 × 42 × 40 cm) with a fine metal mesh (aperture size = 0.25 × 0.25 mm) on the back and both sides and Perspex on the top and front and aluminium alloy on the bottom. I kept the parasitoid colony on the 3rd–5th instar psyllid nymphs feeding on five potted plants in another aluminum-framed cage of the same size. I provided 10% honey solution in a plastic tube (7.5 cm in length × 1 cm in diameter) fitted with cotton wick (3.5 cm in length × 1 cm in diameter) for parasitoid adults as supplementary food in the cage. To ensure the psyllid colony was parasitoid-free for experiments, I kept psyllid and parasitoid colonies separate in two climate rooms at 25 ± 1°C, 40–60% RH and a L:D 14:10 photoperiod. I carried out all experiments under the above environmental conditions.

I transferred about 400 psyllid adults from the colony onto four two-month-old un-infested pepper plants in an aforementioned cage for oviposition. I transferred the infested plants into another cage and replaced them with the same number of un-infested plants once every two days. I obtained a total of 84 infested plants, observed them daily, and collected the 4th instar nymphs for experiments. I used transparent plastic cylinders consisting of two identical containers for production of experimental parasitoids. Briefly, I transferred about 100 fourth instar nymphs onto a bell pepper leaf, inserted the leaf petiole through a hole (1.0 cm in diameter) in the lid of a container (6.5 cm in diameter × 8.5 cm in height) filled with tap water. I then introduced five female wasps from the colony to the infested leaf and covered the container with another container upside down. I allowed these wasps to oviposit in the cylinder for 24 h before removing them. The top container had two holes (3 cm in diameter) covered by a metal mesh (aperture size = 0.25 mm) at the opposite sides of the wall for ventilation. I collected parasitoid pupae seven days later and placed them individually in glass vials (5 cm in height × 1.5 cm in diameter) plugged with cotton wool until emergence. I set up 20 such cylinders to obtain sufficient wasps for experiments.

3.2.2 Treatments

To determine how adult diets and temporary host deprivation affected parasitoid longevity, lifetime fecundity, daughter production, oviposition and host feeding patterns, and life table parameters, I carried out two phases of treatments. In the first phase, I provided one of the following three diets for wasps: (1) Honey—10% honey solution made of 10 g honey + 90 ml distilled water, (2) Yeast—5% yeast solution consisting of 5 g yeast extract + 95 ml distilled water, and (3) Water—distilled water only. For each diet treatment, I individually introduced 60 newly emerged female and 60 newly emerged male wasps into glass vials (5 cm in height × 1.5 cm in diameter). Each vial was provided with a diet saturated in a cotton ball (0.5 cm in diameter) and plugged with cotton wool. Because one-day-old wasps can successfully copulate within one hour after encountering a mate (Chen et al. 2020), I kept wasps in their vials for 24 h, and then individually paired males and females that fed on the same diet in glass vials and allowed 2 h for mating to occur. I obtained 28, 30, and 28 mated pairs that fed on honey, yeast, and water diets, respectively, for the second phase of treatments.

In the second phase, I divided the above pairs into two groups and maintained them in one of the following two conditions: (1) no host deprivation (+ Host)—each pair was released into a Petri dish (8.5 cm in diameter × 2.4 cm in height) containing the same adult diet saturated in a cotton ball and 24 4th instar psyllid nymphs feeding on a bell pepper leaf (5 cm in diameter, upside down) with its petiole embedded in water-saturated cotton wool and wrapped with parafilm. The lid of the Petri dish had two holes (1 cm in diameter), one plugged with a cotton wool for introducing wasps and one covered with metal mesh for ventilation. I allowed the pair to stay in the dish for 24 h, and then transferred them to another dish with the same diet and fresh hosts once every day until both male and female died. I replaced the adult diet with fresh one once every day. (2) Temporary host deprivation (− Host)—each pair was released into a Petri dish of the same size containing the same adult diet but no hosts. Three days later (live wasps were now four days old), I provided them with the same adult diet and 24 4th instar psyllid nymphs as above and allowed each pair to stay in the Petri dish for 24 h. I then transferred the pair into another Petri dish with the same adult diet and fresh hosts once every day until both male and female died. I replaced the adult diet with fresh one once every day. I tested 15, 13, 13, 13, 17 and 15 pairs for treatments Honey + Host, Yeast + Host, Water + Host, Honey − Host, Yeast − Host and Water − Host, respectively.

3.2.3 Effect of adult diets and temporary host deprivation on survival, reproduction, host feeding and life table parameters

I started recording daily survival of *T. triozae* adults after I transferred one-day-old, mated wasps to Petri dishes. I commenced to count the daily number of hosts fed and parasitised one day after hosts were provided, i.e., when wasps were two days old for + Host treatments and five days old for − Host treatments. I did not record reproduction data for treatments Yeast − Host and Water − Host because all females died before I provided them with hosts. I examined host feeding and parasitism under a stereomicroscope (Leica MZ12, Germany). Host feeding was identified by bleeding that occurred from the nymph (Martinez et al. 2015) or an inverted V-shape mark on the hollowed body (Morales et al. 2013). Because *T. triozae* females deposit eggs under the nymphs (Martinez et al. 2015), I turned all nymphs over and placed them on the surface of 1% agar to determine parasitism. I then transferred all parasitised nymphs from each dish onto a fresh bell pepper leaf in an above-mentioned plastic cylinder. I collected the parasitoid pupae seven days after parasitisation and individually placed them in above-mentioned glass vials plugged with cotton wool until emergence. I recorded the developmental time from egg to adult and sex of each emerged adult. I used the data collected for life table calculations.

3.2.4 Statistical analysis

I conducted all data analyses using SAS software (SAS 9.3, SAS Institute Inc., NC, USA). I analysed the data on adult survival using the Kaplan-Meier method. I compared the survival curves between treatments with a non-parametric Wilcoxon test (LIFETEST procedure). Data on lifetime host feeding were normally distributed (Shapiro-Wilk test, UNIVARIATE Procedure) and analysed using an ANOVA (GLM procedure) followed by a Tukey's Studentized (HSD) range test for multiple comparisons. Data on lifetime fecundity were not normally distributed even after transformation, and thus analysed using a Kruskal-Wallis test.

I developed two Gaussian functional models according to Archontoulis and Miguez (2015): $y = a\ e^{(-0.5\{[(x-c)-(x_0-c)]/b\}^2)}$ to fit the daily host feeding and $y = a\ e^{(-0.5\{\ln[(x-c)/(x_0-c)]/b\}^2)}$ to fit the daily oviposition and daughter production for each treatment, where x is the age of female wasps (days), a is the peak at time x_0 (wasp age), b is the coefficient controlling the width of the peak, and c is the period (days) before the females were exposed to hosts (i.e., $c = 1$ and 4 days for the + Host and − Host treatments, respectively). These models allowed us to compare the host feeding and reproduction patterns between treatments. If the 95% CLs of a given parameter overlap, then there is no significant difference between treatments.

To estimate how adult diets and temporary host deprivation affected population growth, I calculated the life table parameters (Jervis et al. 2005) using the data on daily survival and daughter production of each *T. triozae* female. The intrinsic rate of increase (r, daughters per female per day) was calculated by solving the Lotka-Euler equation: $\sum e^{-rx} \mathrm{l}_x \mathrm{m}_x = 1$, where x is the pivotal age, l_x is the proportion of females surviving to age x, and m_x is the number of daughters produced per female at age x. Other life table parameters included the net reproductive rate ($R_0 = \sum \mathrm{l}_x \mathrm{m}_x$, daughters per female per generation), doubling time [$D_t = \log_e(2)/r$, days] and generation time [$T = \log_e(R_0)/r$, days]. For each treatment, I used the bootstrap method with 100,000 bootstrap samples to calculate the pseudo-values of life table parameters (Huang and Chi 2012; Yu et al. 2013). I then employed a paired-bootstrap test (MULTTEST procedure) for multiple comparisons between treatments (Efron and Tibshirani 1993; Mou et al. 2015; Reddy and Chi 2015). Because multiple comparisons raise the Type I error (Noble 2009), the overall P value was adjusted by the Bonferroni correction (MULTTEST procedure) (Gravandian et al. 2022).

3.3 Results

3.3.1 Effect of adult diets and temporary host deprivation on survival and lifetime reproductive outputs

My results reveal that most wasps in treatments Yeast − Host and Water − Host died within two days after emergence. These wasps lived significantly shorter than those in other treatments ($x_5^2 = 94.56$ and 93.35 for females and males, respectively; $P < 0.0001$) (Fig. 3.1). Wasps in other treatments including either hosts or honey lived for about 21 days (Fig. 3.1), where females had similar survival curves and males in treatment Honey + Host lived significantly longer. As shown in Fig. 3.2, female wasps in treatments Honey + Host, Yeast + Host, Water + Host and Honey − Host had similar lifetime host feeding and fecundity ($F_{3,50} = 0.36$, $P = 0.7802$ for host feeding; $x_3^2 = 2.97$, $P = 0.3961$ for fecundity).

Treatments significantly affected all life table parameters measured in this study (Table 3.1). Wasps in treatment Honey − Host had greatest net reproductive rate (R_0) and longest generation time (T) while those in treatment Water + Host had the highest intrinsic rate of increase (r) and shortest doubling time (D_t).

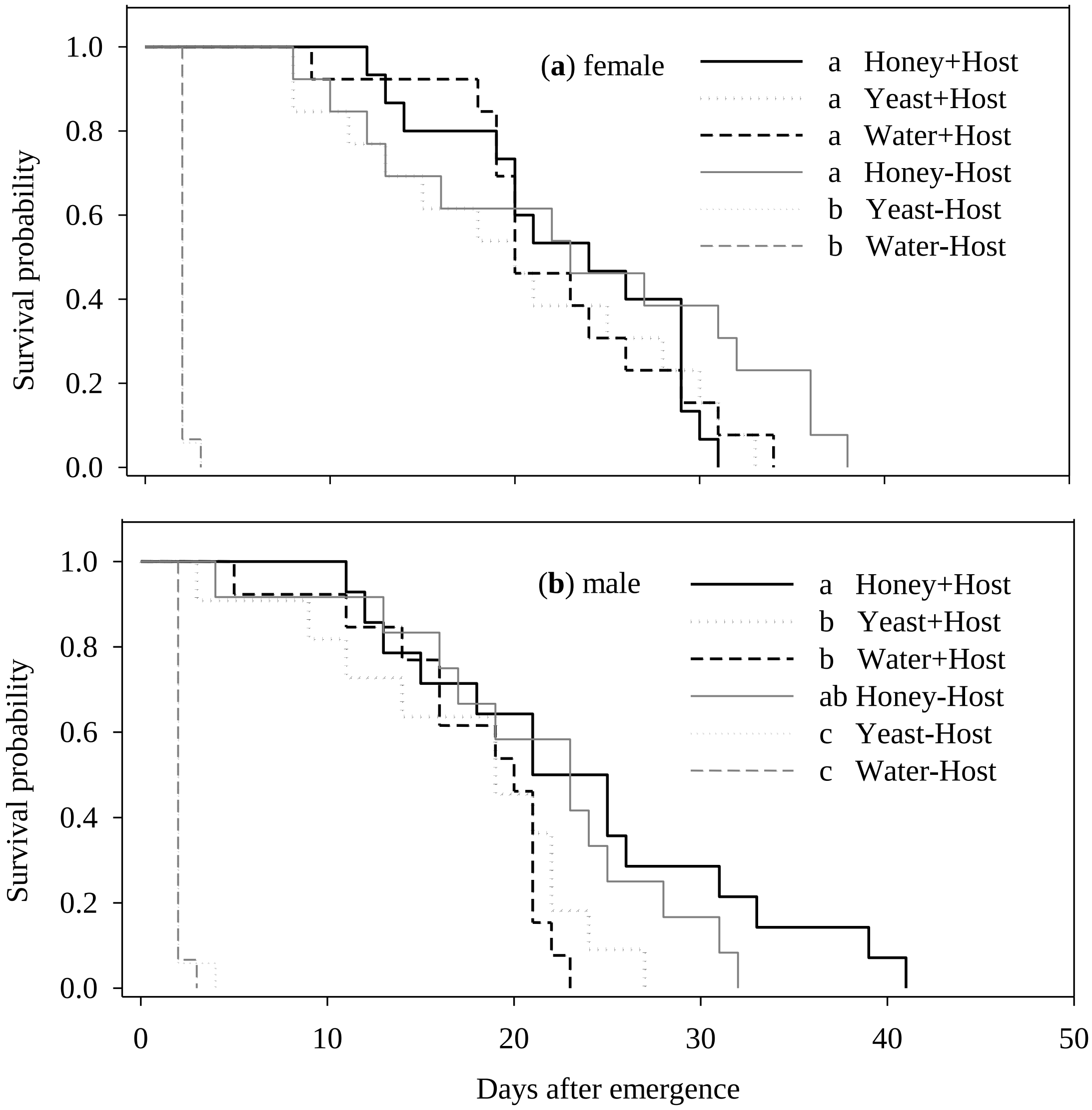


Fig. 3.1 Survival probability of *Tamarixia triozae* females (**a**) and males (**b**) feeding on different diets with (− Host) and without temporary host deprivation (+ Host). Treatments followed by different letters are significantly different (P < 0.05)

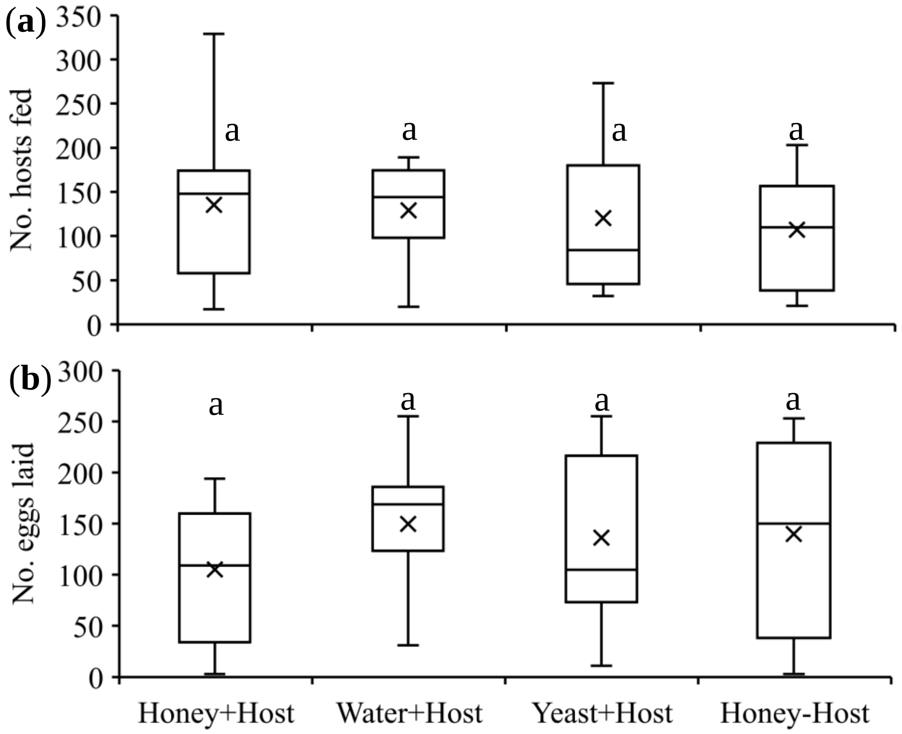


Fig. 3.2 Number of hosts fed (**a**) and eggs laid (**b**) by *Tamarixia triozae* females in different treatments. Each box plot shows the mean (×), median line, the upper and lower quartiles (i.e., the range where 25% of scores fall above and 25% fall below the median), and scores outside the 50% middle (upper ‘T’ and lower ‘⊥ ’). Columns followed by the same letters are not significantly different (P > 0.05)

Table 3.1 Effects of different diets with (− Host) or without (+ Host) temporary host deprivation on life table parameters (mean ± SE)

Diet	R_0 (daughters/female/generation)	r (daughters/female/day)	T (days)	D_t (days)
Honey + Host (H)	16.85±0.19d	0.1596±0.0008d	17.70±0.08b	4.35±0.02a
Yeast + Host (Y)	19.91±0.30c	0.1797±0.0007b	16.64±0.04d	3.86±0.02c
Water + Host (W)	26.43±0.34b	0.1909±0.0001a	17.14±0.06c	3.63±0.00d
Honey − Host (H1)	30.91±0.56a	0.1705±0.0007c	20.11±0.06a	4.07±0.02b
$P_{(Bon.)}$	< 0.0378	< 0.0001	< 0.0001	< 0.0001

Estimated values in columns followed by different letters are significantly different based on the paired-bootstrap test with Bonferroni correction;

$P_{(Bon.)}$ Bonferroni corrected P value

3.3.2 Effect of adult diets and temporary host deprivation on lifetime host feeding and reproductive patterns

When I provided one-day-old wasps with hosts immediately after their mating, daily host feeding (Fig. 3.3a) peaked between eight and 13 days after emergence, and daily fecundity (Fig. 3.3b) and daughter production (Fig. 3.3c) peaked between five and eight days after emergence. Host deprivation for three days after mating delayed the peaks of daily host feeding, fecundity and daughter production for three to four days (Fig. 3.3). There was no significant difference in the width of daily feeding (Fig. 3.3a) and daughter production (Fig. 3.3c) peaks between treatments. However, daily fecundity peak in Yeast + Host was significantly wider than in Honey + Host and Water + Host (Fig. 3.3b). Treatment had no significant effect on the height of daily host feeding peaks (Fig. 3.3a). Daily fecundity peak in Water + Host was significantly higher than in Honey + Host and Yeast + Host, and that in Honey − Host significantly higher than in Honey + Host (Fig. 3.3b). Daily daughter production peak was the highest in Honey − Host (Fig. 3.3c). Detailed statistical data are provided in Supplementary Table S3.1.

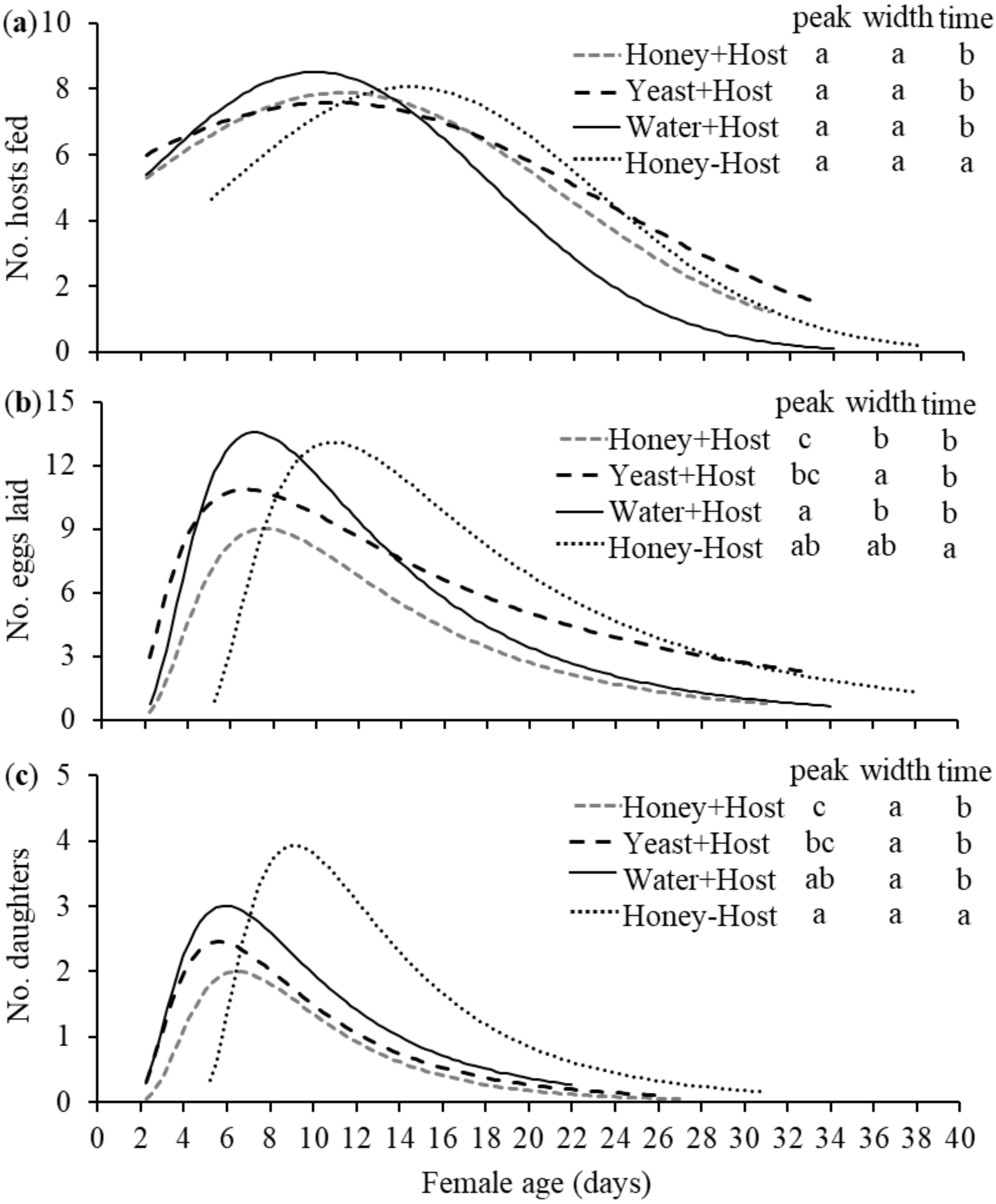


Fig. 3.3 Daily number of hosts fed (**a**), eggs laid (**b**) and daughters produced (**c**) in *Tamarixia triozae* females that fed on different diets with or without host deprivation. Diet + Host = both adult diets and hosts were provided after mating, and Diet − Host = adult diets were provided but hosts were unavailable for three days after mating. For each parameter, treatments followed by the same letters in a column are not significantly different (overlapped 95% CLs of coefficients)

3.4 Discussion

In augmentative release programs, entomologists sometimes ship parasitoid pupae or adults (e.g., van Lenteren and Tommasini 1999; Yokoyama et al. 2010; Cancino and López-Arriaga

2016) from their production sites to the release sites in the absence of hosts, which can last days, depending on distance and transport methods. Like other synovigenic parasitoids (e.g., Giron et al. 2004; Liu et al. 2015; Gebiola et al. 2018), adult feeding on honey and/or hosts was essential for the survival of *T. triozae* because carbohydrates in these diets provided energy to prolong their longevity (e.g., Jervis et al. 2008; Picciau et al. 2019). However, my results do not support previous findings about the beneficial effect of yeast feeding on parasitoid fitness (Bartlett 1964; Heimpel and Rosenheim 1995) because *T. triozae* adults died in about two days if they only fed on yeast solution (Yeast − Host) or water (Water − Host) after emergence. My findings suggest that honey diet should be provided for *T. triozae* adults immediately after emergence regardless of whether hosts are present, or for pupae during shipment to ensure newly emerged adults can get access to honey food in the absence of hosts.

I demonstrate that adult diets and temporary host deprivation altered daily feeding and oviposition patterns of *T. triozae* females. This information can be used to develop strategies for TPP biological control. For example, we could have four days to successfully ship honey-fed and host-deprived females from production to release sites without compromising their longevity and lifetime host-killing ability. Wasps in treatments Water + Host and Honey − Host had the highest fecundity peaks, suggesting that when the pest density is high, we can quickly suppress the pest population by releasing wasps that have fed on water for 24 h or on honey for four days in the absence of hosts. The current study shows that wasps that fed on honey or yeast without temporary host deprivation (Honey + Host and Yeast + Host) flattened their daily oviposition curves, which may reduce the risk of massive removal of hosts and increase establishment success of wasps in the field when the pest population density is low (Eggenkamp-Rotteveel Mansveld et al. 1982). Furthermore, wasps that fed on honey for four days without access to hosts (Honey − Host) had the highest peak of daughter production, suggesting that soon after release to the field (hosts become available), these wasps are not only able to quickly suppress the pest population but also increase their own.

In the absence of hosts for four days after emergence, honey-fed wasps had longest generation time (T) and relatively low intrinsic rate of increase (r) and long doubling time (D_t) due to delayed oviposition. However, these host-deprived and honey-fed adults achieved greatest net reproductive rate (R_0), i.e., produced the highest number of daughters, promoting future population growth (Sabbatini Peverieri et al. 2012). These findings suggest that treatment Honey − Host can lead to better and more sustainable control when the pest density is low at the time of release. On the other hand, wasps in treatment Water + Host had the highest *r* and shortest D_t, the second highest R_0 and relatively short *T*. Therefore, if we aim to achieve immediate control of the pest when the pest population density is high, we should feed newly emerged wasps with water for 24 h and then immediately release them into the infested crops. The released wasps can kill more pest individuals within a shorter time period and quickly build up their own population. The life table parameters also provide references for producers to adjust their mass-rearing programs depending on whether they want to yield more parasitoids quickly or to slow production down.

In conclusion, adult diets can affect survival and reproductive patterns of *T. triozae*. In the absence of hosts at emergence, wasps feeding on water or yeast diet cannot live for more than two days, which is not long enough for successful shipment. However, adult *T. triozae* feeding on honey solutions for four days or other diets for one day followed by provision of hosts can survive for about 21 days with similar lifetime pest killing ability. Furthermore, wasps feeding on water for one day before access to hosts have greater intrinsic rate of increase, shorter doubling time, and higher daily fecundity peak while those fed with honey or yeast for one day followed by host feeding for three days flatten their daily oviposition curves. These findings have three implications: (1) honey diet can allow at least four days for successful shipment of host-deprived adults without compromising host-killing ability; (2) releasing host-deprived wasps with one-day water feeding can achieve rapid pest suppression when the pest population density is high, and (3) releasing host-deprived adults with one-day honey or yeast feeding followed by three-day host feeding can increase the establishment success and reduce the risk of massive removal of hosts when the pest population density is low. These implications can be tested under field conditions.

Table S3.1 Effects of different diets with (– Host) or without (+ Host) temporary host deprivation on the number of eggs laid, hosts fed and daughters produced in *Tamarixia triozae* females in relation to female age

Treatment	*a*		*b*		x_0	
	Estimate	95% CLs	Estimate	95% CLs	Estimate	95% CLs
No. hosts fed						
Honey + Host	7.88a	7.04~8.73	10.18a	7.83~12.53	11.18b	9.48~12.88
Yeast + Host	7.59a	6.81~8.37	12.47a	8.91~16.03	10.70b	8.28~13.11
Water + Host	8.52a	7.74~9.30	8.10a	6.68~9.52	9.89b	8.82~10.96
Honey – Host	8.07a	7.40~8.75	8.69a	7.45~9.92	14.25a	13.30~15.19
No. eggs laid						
Honey + Host	9.03c	8.10~9.96	0.69b	0.60~0.79	7.45b	6.83~8.06
Yeast + Host	10.87bc	9.71~12.03	0.99a	0.82~1.15	6.55b	5.71~7.39
Water + Host	13.55a	12.39~14.72	0.69b	0.61~0.77	7.01b	6.53~7.48
Honey – Host	13.10ab	11.85~14.35	0.76ab	0.67~0.86	10.65a	10.01~11.28
No. daughters						
Honey + Host	2.00c	1.68~2.32	0.57a	0.46~0.68	6.34 b	5.70~6.98
Yeast + Host	2.46bc	2.01~2.90	0.68a	0.53~0.84	5.49 b	4.74~6.25
Water + Host	3.01ab	2.61~3.41	0.67a	0.56~0.78	5.79 b	5.20~6.37
Honey – Host	3.93a	3.39~4.47	0.66a	0.55~0.78	8.98 a	8.37~9.58

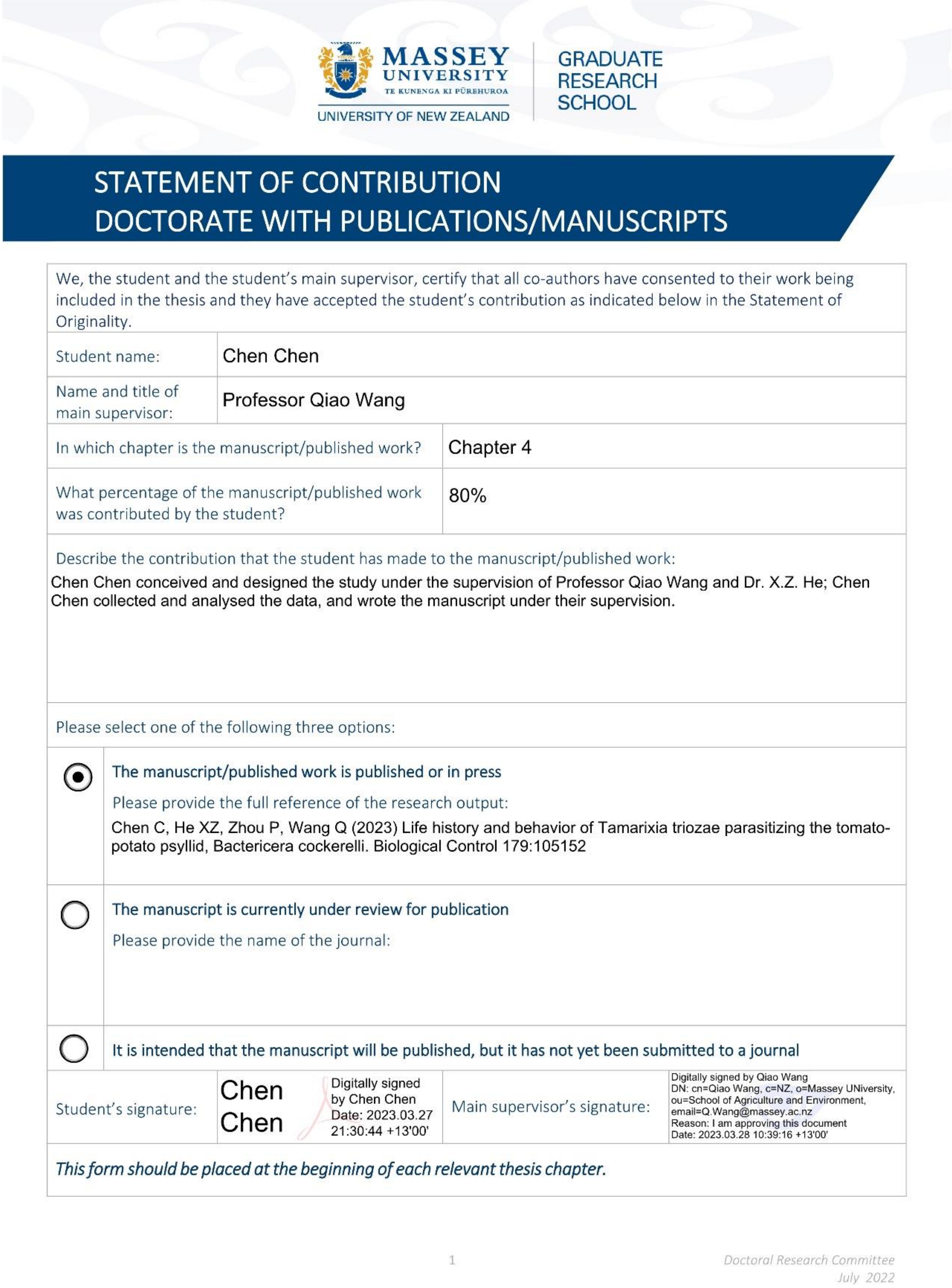
$y = a\, e^{(-0.5\{[(x-c)-(x_0-c)]/b\}^2)}$ for number of hosts fed;

$y = a\, e^{(-0.5\{\ln\,[(x-c)/(x_0-c)]/b\}^2)}$ for number of eggs laid or daughters produced. a is the peak at time x_0, b is the coefficient controlling the width of the peak, and c is the period before the females got access to hosts (i.e., c = 1 and 4 days for the + Host and – Host treatments, respectively). For each parameter, values in a column followed by the same letters do not differ significantly (the 95% CLs overlap)

Chapter 4

Life history and behaviour of *Tamarixia triozae* parasitising the tomato-potato psyllid, *Bactericera cockerelli*

This chapter was published in *Biological Control* 179:105152 (2023).



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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Chen Chen
Name and title of main supervisor:	Professor Qiao Wang
In which chapter is the manuscript/published work?	Chapter 4
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:
Chen Chen conceived and designed the study under the supervision of Professor Qiao Wang and Dr. X.Z. He; Chen Chen collected and analysed the data, and wrote the manuscript under their supervision.

Please select one of the following three options:

● The manuscript/published work is published or in press
Please provide the full reference of the research output:
Chen C, He XZ, Zhou P, Wang Q (2023) Life history and behavior of Tamarixia triozae parasitizing the tomato-potato psyllid, Bactericera cockerelli. Biological Control 179:105152

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Student's signature:	Chen Chen Digitally signed by Chen Chen Date: 2023.03.27 21:30:44 +13'00'	Main supervisor's signature:	Digitally signed by Qiao Wang DN: cn=Qiao Wang, c=NZ, o=Massey UNiversity, ou=School of Agriculture and Environment, email=Q.Wang@massey.ac.nz Reason: I am approving this document Date: 2023.03.28 10:39:16 +13'00'

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1 Doctoral Research Committee
July 2022

Abstract *Tamarixia triozae* is an important primary parasitoid of the tomato-potato psyllid, *Bactericera cockerelli,* a serious cosmopolitan pest of solanaceous crops. However, without better information about its life history and behaviour, it will be difficult to use this parasitoid in effective biological control programs. I carried out a series of experiments to characterize its parasitism, adult feeding, and oviposition behaviours and its sex allocation in response to different life stages of its host, and their fitness consequences. I show that *T. triozae* females fed on all host instars with a preference for mid-aged ones, and preferred to parasitise later instars, thus inflicting mortality on all instars simultaneously. Host feeding and parasitism peaked during the first week of female life and declined markedly after two weeks. Parasitoids allocated more fertilised eggs to older and larger nymphs, and superparasitism declined with increasing host density. The oviposition rate of fertilised eggs peaked when females were four to five days old, with > 90% of daughters produced during the first half of adult life. The body size and egg loads of progeny increased with increasing host instar at parasitism, demonstrating a positive relationship between the host size and offspring fitness.

4.1 Introduction

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a serious pest of solanaceous crops native to the United States and Mexico, causing significant losses (Cranshaw 1994; Crosslin et al. 2010; Butler and Trumble 2012a; Rojas et al. 2015). It has invaded New Zealand, Australia, Canada, and Ecuador, where the assessment of its economic impact is under way (Teulon et al. 2009; Ogden 2011; FAO 2017; WADPIRD 2018; Castillo Carrillo et al. 2019; Olaniyan et al. 2020). The psyllid damages plants by feeding and transmission of zebra chip and other diseases (Munyaneza et al. 2008; Butler and Trumble 2012a; Rojas et al. 2015; Chen et al. 2020). To date, insecticides are still the main tactic for the control of this pest in the field (Munyaneza 2012; Martinez et al. 2015; Wright et al. 2017). *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae), which has recently been introduced from Mexico to New Zealand for TPP control (Barnes 2017), is an important primary ectoparasitoid of the pest (Rojas et al. 2015; Yang et al. 2015). It has a higher population growth potential than its host (Rojas et al. 2015) and about 80% parasitism rate (Bravo and López 2007). Furthermore, it is a synovigenic wasp, killing its hosts by both parasitisation and host feeding (Martinez et al. 2015; Rojas et al. 2015; Chen et al. 2020, 2022). It can overwinter successfully and establish self-sustaining populations in New Zealand (Anderson 2020). This parasitoid can thus be highly effective in TPP control, particularly in greenhouse crops.

Parasitoids foraging on host plants normally encounter hosts of different stages and sizes. The optimal foraging theory predicts that parasitoid females should adopt the host selection strategies that maximise their lifetime fitness gain (Goubault et al. 2003; Danchin et al. 2008). Many studies show that parasitoids gain better reward in offspring fitness by parasitising larger hosts because these hosts provide more resources for their offspring (e.g., Liu et al. 2011; Hanan et al. 2015; Luo et al. 2022). However, *T. triozae* neither feed on parasitised hosts nor parasitise those already fed upon (Cerón-González et al. 2014; CC pers. observ.), suggesting that they must have developed a host partitioning strategy for feeding and parasitisation to maximise their offspring's and their own fitness. For example, like other host-feeding parasitoid species (e.g., Kidd and Jervis 1991; Choi et al. 2001; Ebrahimifar and Jamshidnia 2022), *T. triozae* females may select smaller hosts for feeding and larger ones for oviposition. Although several authors have examined host stage selections in this parasitoid (Morales et al. 2013; Yang et al. 2015; Ramírez-Ahuja et al. 2017), it is still not clear how *T. triozae* females partition their hosts of different stages for feeding and parasitisation when *B. cockerelli* nymphs of all instars are present simultaneously, which is the most likely scenario in the field. This knowledge may contribute to better understanding of host selection for feeding and oviposition and evaluation of biological control effectiveness of the parasitoid.

Due to the nature of haplodiploidy, female parasitic wasps can control offspring sex ratio by adjusting the proportion of fertilised eggs at oviposition (King 1988). Previous studies show that they usually allocate more fertilised eggs to larger hosts (e.g., Charnov et al. 1981; Favaro et al. 2018; Pérez-Rodríguez et al. 2019) because their offspring developing from larger hosts have larger body size (e.g., Hanan et al. 2015; Luo et al. 2022) and higher reproductive fitness (e.g., Hanan et al. 2015; Khatri et al. 2016). However, it is not clear how *T. triozae* females allocate the sex of their offspring based on the host stage they attack and whether host stage at parasitisation has any effect on their offspring fitness. This knowledge can help design mass rearing programs for effective parasitoid production. Furthermore, although only one offspring survives on each parasitised host, *T. triozae* females often deposit more than one egg under a host (Rojas et al. 2015). To date, it is unknown whether their egg and sex allocation to each host varies with the host stage and density, making it difficult to understand the mechanisms behind their superparasitism.

Parasitoids of different ages may adjust their daily feeding, oviposition and sex allocation patterns in response to host stage, knowledge of which is currently lacking for *T. triozae* but important for the development of effective pest control programs and decision on the timing of releases. In some parasitoids, younger females may have maximal feeding (Zhang et al. 2014) and oviposition (Wade et al. 2008; Bodino et al. 2019), achieving greatest pest suppression soon after release to the field but these activities decline when they are older, reducing biocontrol effectiveness. Mated females may maximise the use of sperm and produce more daughters in their early life (Latham and Mills 2010; Mawela et al. 2021) to ensure the survival and persistence of their next generation in the field while this reproductive feature may lead to sperm depletion over age, reducing daughter production in their later life (e.g., Pérez-Lachaud and Hardy 1999; Santolamazza Carbone et al. 2007; Hu et al. 2012).

The aim of this study was to determine parasitoid age- and host stage-dependent host feeding, parasitisation and sex allocation and their fitness consequences in *T. triozae*. I exposed mated females of *T. triozae* to *B. cockerelli* nymphs of all instars daily, recorded their lifetime feeding, parasitisation, superparasitism and sex allocation on each instar, and established their offspring fitness. Knowledge generated here provides essential information for the development of effective rearing and releasing programs for the control of TPP.

4.2 Materials and methods

4.2.1 Breeding colony and environmental conditions

I established the colonies of *B. cockerelli* and *T. triozae* using adults from BioForce Limited, Auckland, New Zealand. I utilized the bell pepper (*Capsicum annuum* L.) as host plants for colony maintenance and experiments. To ensure the psyllid colony was parasitoid-free, I kept psyllid and parasitoid colonies separate in two climate rooms. I reared psyllids on five two-month-old plants, each in a pot (9.5 cm in height × 10.5 cm in top diameter × 8.5 cm in bottom diameter) with potting mix, in an aluminium-framed cage (43 × 42 × 40 cm) with a metal mesh (aperture size = 0.25 mm × 0.25 mm) on the back and both sides and Perspex on the top and front and aluminium alloy on the bottom. I maintained the parasitoid colony on the 3rd–5th instar psyllid nymphs feeding on five potted, two-month-old plants in another aluminium-framed cage of the same size. Colonies were maintained and experiments carried out at 25 ± 1°C, 40–60% RH and a photoperiod of 14L:10D hours.

4.2.2 Preparation of insects for experiments

To obtain psyllids of different instars (1st–5th instars) for experiments, I randomly collected about 200 male and female adults from the colony, released them onto two potted, clean, two-month-old pepper plants in an aluminium-framed cage mentioned above. I allowed adults to lay eggs for 24 h, and then removed all adults from the infested plants using an aspirator and transferred those plants into a nylon mesh-framed cage (65 × 50 × 50 cm, aperture size: 0.075 mm × 0.075 mm). I placed two clean plants of similar conditions into the aluminium-framed cage and repeated the above process daily until I obtained sufficient nymphs of desirable instars for experiments. During this period, I examined the aluminium-framed cage daily and added new adults from the colony to assure there were about 200 adults in the cage. When the nymphs reached the 5th instar on the first two infested plants in the nylon mesh-framed cage, I harvested all nymphs from infested plants in all cages to obtain nymphs of different instars for experiments. I determined nymphal instars under a stereomicroscope (Leica MZ12, Germany) according to Vega-Chávez (2010).

To acquire parasitoids for experiments, I randomly collected five females of *T. triozae* from the colony, released them into a plastic cylinder with a bell pepper leaf infested by 100 4th-instar psyllid nymphs and allowed them to stay in the cylinder for 24 h. The cylinder consisted of two identical transparent containers (6.5 cm in diameter × 8.5 cm in height). The bottom container was filled with tap water and covered by a lid bearing a hole (1.0 cm in diameter) at the centre through which I inserted the petiole of the infested leaf. The top container had two holes (3 cm in diameter) covered by a metal mesh at the opposite sides of the wall for ventilation. I set up a total of 10 such cylinders. I collected parasitoid pupae seven days after oviposition and individually placed them in glass vials (5 cm in height × 1.5 cm in diameter) plugged with cotton wools until adult emergence. I individually introduced the newly emerged wasps to the glass vials with a cotton ball saturated with 10% honey solution as food and maintained them there for 24 h. Because both sexes become sexually mature on the day they emerge (Chen et al. 2020), I individually paired one-day-old virgin females and males in the glass vials with honey food until mating ended. Mating usually occurs within 90 mins after pairing and lasts about 15 mins (CC pers. observ.).

4.2.3 Effect of host stage on lifetime host feeding and parasitisation

To determine lifetime parasitism and host feeding in relation to host stage, I transferred 30 psyllid nymphs of five different instars (6 individuals × 5 instars) onto a pepper leaf with its petiole embedded in water-saturated cotton wool wrapped with parafilm in a Petri dish (8.5 cm in diameter × 2.4 cm in height). I covered the dish with the lid bearing two holes (1 cm in diameter), one covered with metal mesh for ventilation and one with a cotton wool plug for introducing wasps. I introduced a one-day-old mated female parasitoid into the dish and allowed it to stay for 24 h. I then transferred the wasp to another dish with a leaf and 30 fresh psyllid nymphs as above and allowed it to stay for 24 h. I repeated this process until the wasp died, which lasted about 20 days. During the experiment, psyllid nymphs were the only food for the wasp and each wasp was exposed to more than 600 nymphs in its lifetime. A cotton ball saturated with water was provided to the wasp and refreshed every day. I tested 18 female wasps, each of which was considered a replicate.

For each replicate, I examined all dishes after removing the wasp and recorded the number and instar of hosts fed and parasitised, and number of eggs laid under the stereomicroscope. Host feeding was determined by the presence of body fluid (Martinez et al. 2015) or an inverted V-shape mark on the hollowed body (Morales et al. 2013). During oviposition female wasps paralyse psyllid nymphs and then deposit eggs on the ventral side of the hosts and secrete adhesive substance to attach the eggs to the host cuticle (Martinez et al. 2015). Therefore, I turned over all host nymphs and put them on the surface of 1% agar to determine oviposition and counted the number of eggs laid under each parasitised nymph.

4.2.4 Effect of host stage at parasitisation on parasitoid offspring fitness

I separated the parasitised nymphs according to their instars in each dish. I then transferred those of the same instar onto a randomly selected leaf on a potted, two-month-old pepper plant in a nylon mesh-framed cage. Immediately after the transfer of psyllid nymphs, I enclosed the leaf with a transparent resealable plastic bag (17.5 cm in length × 10 cm in width) bearing small holes made by insect pins for ventilation and filled the space between the leaf petiole and the zip seal of the bag with cotton wool. Parasitoid pupae were collected seven days after parasitism and individually placed in the glass vials plugged with cotton wool until emergence. For a given host instar, the emergence rate was calculated as the number of emerged offspring divided by the number of hosts parasitised. The developmental time from egg to adult emergence and sex of emerged wasps were recorded.

To measure the effect of host instar at parasitisation on the body size of parasitoid offspring at emergence, I randomly sampled 19 and 30 newly emerged females from the 4th and 5th instar nymphs (no females emerged from the 3rd instar – see Results), and 12, 30 and 28 newly emerged males from the 3rd, 4th and 5th instar nymphs (no 1st and 2nd nymphs were parasitised – see Results), respectively. I measured the hind tibial length of these adults as an index of body size (Khatri et al. 2016) under the stereomicroscope. To determine the egg load of newly emerged females, after tibial measurement I dissected these females in 70% alcohol on a slide under the stereomicroscope. Because immature eggs absorb the stain while mature eggs prevent the stain (Edwards 1954), I added one droplet of stain (1% acetocarmine) into the alcohol. The ovaries were covered with a slide cover, spread by gently pressing 3–5 mins after staining and eggs counted under the stereomicroscope. The number of mature eggs in the ovaries was recorded as egg load at emergence.

4.2.5 Statistical analysis

I performed all analyses using SAS 9.4 (SAS Institute Inc., NC, USA). Because my data were not normally distributed (Shapiro-Wilk test, UNIVARIATE Procedure), I used a generalized linear model (GLIMMIX procedure) to analyse them with the instar as a fixed factor and the replicate as a random factor in the model. A Gamma distribution and a *log* function were applied to the number of eggs laid under each parasitised nymph, developmental time and hind tibial length; a Poisson distribution and a *log* function to the lifetime number of hosts fed and parasitised, number of eggs laid, and egg load, and a Binomial distribution and a *logit* function to the emergence rate and proportion of daughters produced. A Tukey-Kramer test was used for multiple comparisons between the instars.

According to Archontoulis and Miguez (2015), I developed two Gaussian functional models, $y = a\,e^{(-0.5\{[(x-c)-(x_0-c)]/b\}^2)}$, to fit the daily host feeding on nymphs of different instars, and $y = a\,e^{(-0.5\{\ln[(x-c)/(x_0-c)]/b\}^2)}$, to fit the daily number of parasitism on and daughter emergence from nymphs of different instars, where x is the age of female wasps (days), a is the peak at age x_0, b is the coefficient controlling the width of the peak, and c (= 1 day) is the time before the females got access to hosts. The difference in each estimated parameter in the Gaussian functional models was compared between treatments: if the 95% confidence limits (CLs) overlap, then there is no significant difference. I did not fit the data on host feeding for the 1st and 5th instar nymphs, and on parasitism and daughter emergence for 3rd instar nymphs because only a few 1st and 5th instar nymphs were fed, few 3rd instar nymphs were parasitised, and no daughter emerged from the 3rd instar nymphs.

4.3 Results

4.3.1 Effect of host stage on lifetime host feeding and parasitisation

The female parasitoids fed on hosts of all instars but significantly preferred the 2nd, 3rd and 4th to the 1st and 5th instar nymphs for feeding with the highest number of the 3rd instar nymphs fed ($F_{4,68} = 241.19$, $P < 0.0001$) (Fig. 4.1a). They significantly preferred the 4th and 5th to the 3rd instar nymphs for oviposition with the highest number of parasitism and eggs laid on the 5th instar nymphs ($F_{2,34}= 213.56$, $P < 0.0001$ for parasitism; $F_{2,34} = 314.12$, $P < 0.0001$ for eggs laid) but did not parasitise the 1st and 2nd instar nymphs (Fig. 4.1b). Furthermore, the parasitoids deposited significantly more eggs under the 4th and 5th instar nymphs (1.34 and 1.47 eggs per nymph, respectively) than under the 3rd instar nymphs (1.11 eggs per nymph) ($F_{2,27} = 6.46$, $P = 0.0051$).

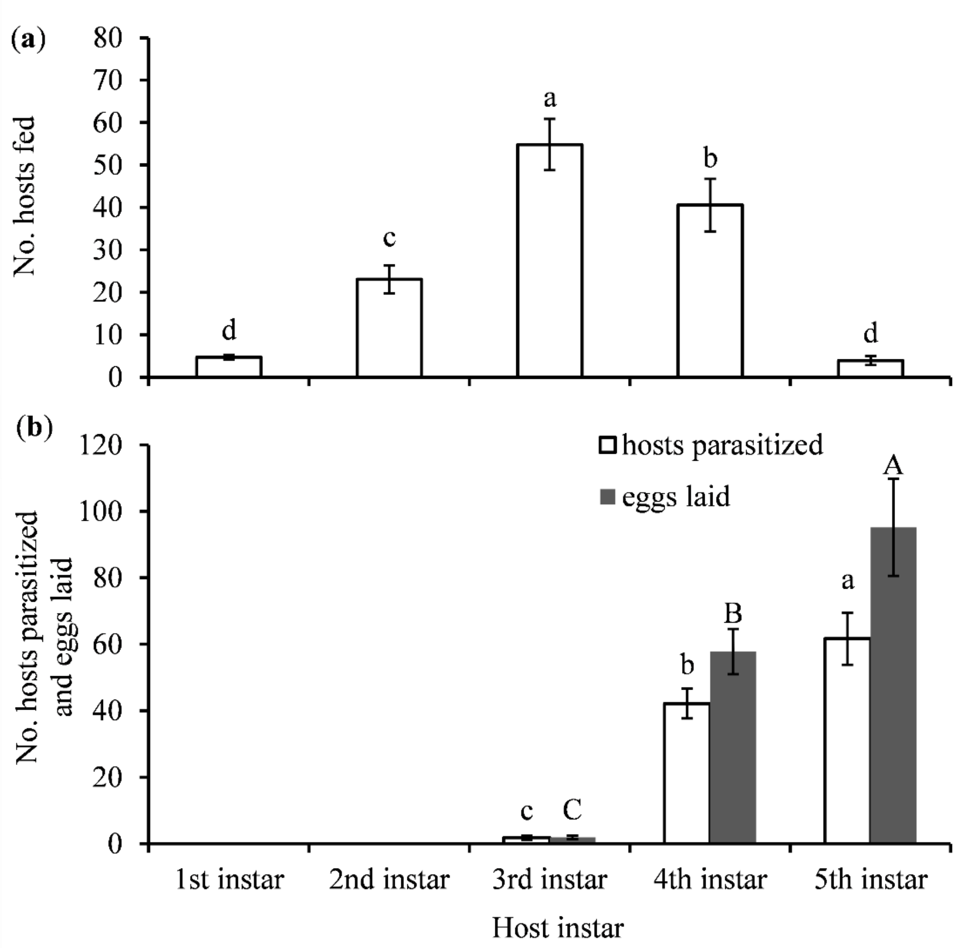


Fig. 4.1 Mean (± SE) lifetime number of hosts fed (**a**), parasitised and eggs laid (**b**) by *Tamarixia triozae* on *Bactericera cockerelli* nymphs of different instars. Columns with different letters are significantly different ($P < 0.05$)

Daily host feeding and parasitisation patterns varied with host stage and parasitoid age (Fig. 4.2, Table S4.1). Daily number of hosts fed by parasitoids on the 3rd and 2nd instar nymphs peaked about six and seven days after emergence, respectively, significantly earlier than that on the 4th instar nymphs which peaked about 12 days after emergence (non-overlapping 95% CLs). The host feeding peak on the 3rd instar nymphs was significantly higher than that on other nymph stages (non-overlapping 95% CLs). Daily parasitism on the 4th instar nymphs peaked about five days after emergence, significantly earlier than that on the 5th instar nymphs which peaked about six days after emergence (non-overlapping 95% CLs). The parasitism peak on the 5th instar nymphs was significantly higher than that on the 4th instar nymphs (non-overlapping 95% CLs). However, there was no significant difference in the width of daily host feeding and parasitism curves between instars (overlapping 95% CLs).

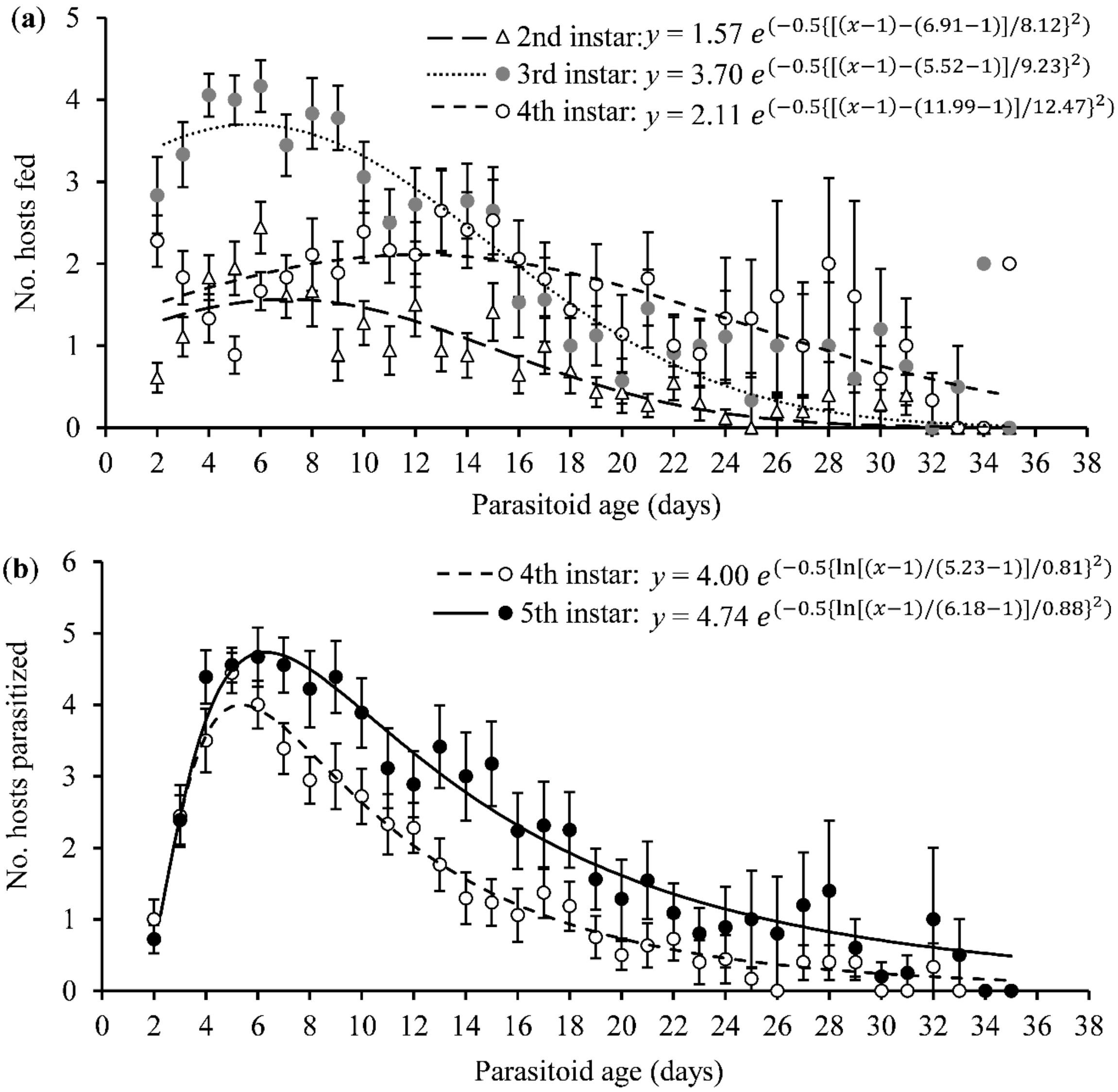


Fig. 4.2 Mean (± SE) daily number of hosts fed (**a**) and parasitised (**b**) by *Tamarixia triozae* females on *Bactericera cockerelli* nymphs of different instars

4.3.2 Effect of host stage at parasitisation on parasitoid offspring fitness

The emergence rate of parasitoid offspring significantly increased with the increase of host instars at parasitisation ($F_{2,27}$ =21.75, P < 0.0001) (Fig. 4.3a). Both sons and daughters emerged from hosts parasitised at the 4th and 5th instars while only sons emerged from those parasitised at the 3rd instar. Emerged offspring were male-biased if the parasitoids parasitised the 4th instar nymphs but female-biased if parasitisation occurred at the 5th instar ($F_{2,23}$ = 71.55, P < 0.0001) (Fig. 4.3b). I show that both host stage and parasitoid age at parasitisation affected parasitoid daughter emergence patterns (Fig. 4.4, Table S4.2). The highest number of daughters emerged from the 4th instar nymphs parasitised by four-day-old parasitoids while that occurred from the 5th instar nymphs parasitised by five-day-old parasitoids (non-overlapping 95% CLs). Daughter emergence peak from the 5th instar nymphs was significantly higher than that from the 4th instar nymphs (non-overlapping 95% CLs). However, there was no significant difference in peak width between the two instars (overlapping 95% CLs). Furthermore, 90% of daughters were produced from the 4th and 5th instar nymphs by the parasitoids of 3-11 days old.

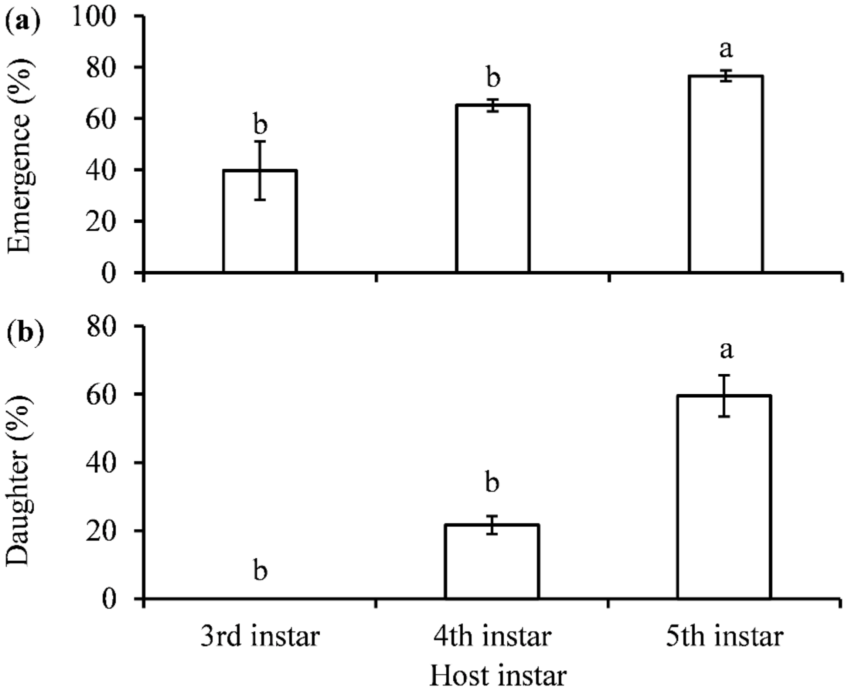


Fig. 4.3 Mean (± SE) emergence rate (**a**) and daughter percentage (**b**) of *Tamarixia triozae* offspring that developed from *Bactericera cockerelli* nymphs parasitised at different instars. Columns with different letters are significantly different (P < 0.05)

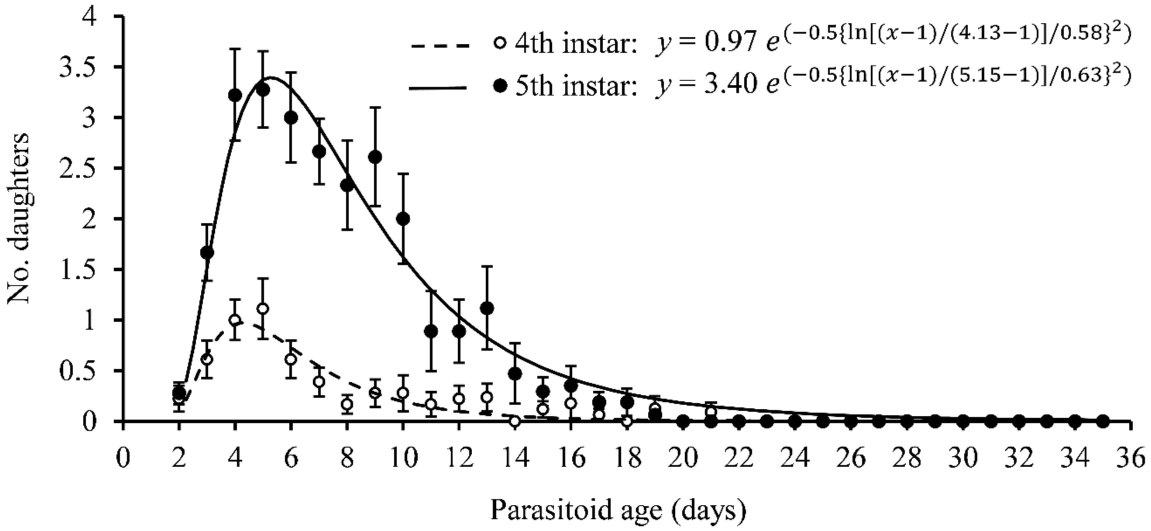


Fig. 4.4 Mean (± SE) number of daughters emerging from *Bactericera cockerelli* nymphs of different instars parasitised by *Tamarixia triozae* over lifetime

Sons required significantly longer time to complete development on the 3rd instar than on the 4th and 5th instar nymphs (Developmental time: mean ± SE = 10.07 ± 0.05, 9.58 ± 0.04 and 9.74 ± 0.07 days for the 3rd, 4th and 5th instars, respectively; $F_{2,23} = 8.13$, $P = 0.0021$) but the host stage at parasitisation had no impact on daughter developmental time (Developmental time: mean ± SE = 10.00 ± 0.05 and 10.06 ± 0.05 days for the 4th and 5th instars, respectively; $F_{1,17} = 0.83$, $P = 0.3749$). Daughters needed significantly longer time to complete development than sons regardless of the host stage parasitised ($F_{1,17} = 53.15$, $P < 0.0001$ for the 4th instar; $F_{1,17} = 11.04$, $P = 0.0040$ for the 5th instar). There was no significant interaction between the host stage parasitised and sex of emerged offspring in their effects on developmental period ($x_1^2 = 1.02$, $P = 0.3115$).

Offspring body size of both sexes significantly increased with the increase of the host stage at parasitisation (Tibial length of sons: mean ± SE = 129.07 ± 3.47, 160.87 ± 1.67 and 190.82 ± 3.00 μm for the 3rd, 4th and 5th instars, respectively; $F_{2,38} = 86.24$, $P < 0.0001$. Tibial length of daughters: mean ± SE =170.06 ± 1.98 and 203.45 ± 1.82 μm for the 4th and 5th instars, respectively; $F_{1,18} = 99.53$, $P < 0.0001$), and regardless of host stage at parasitisation, daughters were significantly larger than sons ($F_{1,18} = 13.52$, $P = 0.0017$ for the 4th instar; $F_{1,27} = 13.38$, $P = 0.0011$ for the 5th instar). There was also no significant interaction between the host stage parasitised and sex of emerged offspring in their effects on offspring body size (x_1^2

= 0.11, P = 0.7420). In addition, newly emerged daughters from hosts parasitised at the 5th instar had significantly higher egg load than those from hosts parasitised at the 4th instar (Egg load: mean ± SE = 0.05 ± 0.05 and 1.67 ± 0.27 for the 4th and 5th instars, respectively; $F_{1,18}$ = 13.19, P = 0.0019).

4.4 Discussion

I demonstrate that *T. triozae* females partitioned hosts of different instars for feeding and parasitisation. When all instars of *B. cockerelli* nymphs were present, which should be the most common scenario in the field, the parasitoids preferred mid-aged nymphs for feeding and older for parasitisation. Because the parasitoids need to pierce the hosts with ovipositors before feeding or parasitisation (Martinez et al. 2015; Rojas et al. 2015) and do not parasitise fed hosts (Cerón-González et al. 2014), they rationalise their effort for optimal fitness gain for both mothers and their offspring. Older hosts are more nutritious for parasitoid offspring development (Li and Mills 2004; Liu et al. 2011) but more difficult to punctuate (Kidd and Jervis 1991; Zhang et al. 2015) due to their thicker integument (Veronesi et al. 2022a) and defensive behaviour (Liu et al. 2011). Therefore, they choose to feed on younger hosts and parasitise older ones, consistent with the optimal foraging theory (Kishani Farahani et al. 2015). As both adult feeding and parasitisation destroy the hosts (Martinez et al. 2015; Rojas et al. 2015), the host partitioning behaviour may allow the parasitoids to control the pest effectively by killing hosts of different life stages simultaneously.

Although only one offspring survives on each parasitised host (Rojas et al. 2015), *T. triozae* females deposited more than one egg under a host with more eggs allocated to older nymphs. This host stage-dependent egg investment may help increase parasitoid offspring survival because the offspring from older nymphs at parasitisation were more likely to develop to adults. Comparing the findings between the current and Rojas et al.'s (2015) studies, I found that the egg allocation was also host density dependent. In the current study, the parasitoids deposited an average of 1.4 eggs per host of the 4th or 5th instar while they laid about 1.15 eggs under each host when the density of the same stage nymphs was twice as that in my study (Rojas et al. 2015). These findings suggest: (1) when the number of nymphs suitable for parasitisation is lower, the parasitoid may increase superparasitism to avoid collapse of the host population for the survival of future generations, but (2) when the number of nymphs suitable for parasitisation is higher, the parasitoid may reduce superparasitism to maximise the use of available resources. This behaviour may make the parasitoids more effective and persistent in the field.

My results indicate that *T. triozae* females adjusted their daily feeding and parasitisation patterns in response to host stage and their own age. They mainly fed on the 3rd nymphs in their early life when they had maximum parasitism on the 4th and 5th instar hosts but they increased their feeding on the 4th instar nymphs after they reached mid-age and their parasitism sharply declined. The increase of feeding on more nutritious 4th instar nymphs at older age is probably due to the combination of declined fecundity and increased demand of nutrition for adult survival. Overall, *T. triozae* completed 90% of their lifetime feeding and parasitism when they were 17 days old. Because my experimental condition (25°C) is optimal for the development of both *B. cockerelli* (Lewis et al. 2015) and *T. triozae* (Vega-Chávez et al. 2016), my findings may provide important knowledge for development of field release programs for biological control of TPP under similar conditions.

The present study reveals that although *T. triozae* preferred to parasitise the 4th and 5th instar nymphs, adults emerged from the 4th instar nymphs at parasitisation were highly male-biased and those from the 5th instar nymphs were highly female-biased. These results strongly imply that the parasitoids allocate significantly more fertilised eggs to the oldest and largest nymphs, which was also reported in other parasitoids (e.g., Charnov et al. 1981; Favaro et al. 2018; Pérez-Rodríguez et al. 2019). Using the 5th instar nymphs as hosts for *T. triozae* during mass rearing may thus increase daughter production and reduce rearing cost. The sex allocation patterns also shifted with the age of parasitoids, i.e., females laid the highest number of fertilised eggs per day when they were four to five days old, with > 90% of daughters produced during their first half of life. I suggest that the females may maximise the use of sperm in their early life to ensure the survival and persistence of their next generation in the field. As a result, they may quickly run out of sperm as they age (Pérez-Lachaud and Hardy 1999; Santolamazza Carbone et al. 2007; Hu et al. 2012) and after they reach mid-age, they produce male-biased offspring.

I show that regardless of the host stage at parasitisation, females had longer developmental time and larger body size than males, suggesting that females need more resources and time to build up larger body size for reproduction (King 1988, 2000; He et al. 2005). Like other parasitoids (e.g., Liu et al. 2011; Hanan et al. 2015; Luo et al. 2022), the host stage affected offspring body size of both sexes in *T. triozae*, which increased with the increase of the host age at parasitisation. Furthermore, newly emerged daughters from hosts parasitised at the 5th instar carried more mature egg than those from hosts parasitised at the 4th instar. Although I have not measured male reproductive potential, other studies show that larger parasitoid males mate more times (Ji et al. 2004; Lacoume et al. 2006) and produce more sperm (He and Wang 2006; Kant et al. 2012). In accordance with other parasitoids (e.g., Hu et al. 2002; Hanan et al. 2015; Li et al. 2018), larger and older hosts increase the fitness of *T. triozae* offspring.

In conclusion, *T. triozae* females adjust their behaviours in response to host stage and their own age. They feed on hosts of all instars with a preference for mid-aged ones and almost always parasitise late instar hosts, thus inflicting mortality on all instars simultaneously. The parasitoids increase and reduce superparasitism at lower and at higher host density, respectively. This may help maintain their own population in the field. Maximum feeding and parasitisation occur during the first week of their life and these activities sharply decline when they are two weeks old. The parasitoids allocate more fertilised eggs to the oldest and largest nymphs. They lay the highest number of fertilised eggs per day when they are four to five days old, with > 90% of daughters produced during their first half of life. Parasitoids' offspring body size and egg load increase with the increase of host instar at parasitisation, demonstrating a positive relationship between host size and offspring fitness.

Table S4.1 Estimated parameters of models fitting the number of nymphs fed and parasitised by *Tamarixia triozae* females on different host instars in relation to female age (Fig. 4.2)

Host instar	a		b		x_0	
	Estimate	95% CLs	Estimate	95% CLs	Estimate	95% CLs
No. hosts fed						
2nd instar	1.57 c	1.38~1.75	8.12 a	5.82~10.41	6.91 b	4.71~9.11
3rd instar	3.70 a	3.43~3.96	9.23 a	7.33~11.13	5.52 b	3.43~7.61
4th instar	2.11 b	1.87~2.35	12.47 a	8.68~16.25	11.99 a	9.57~14.41
No. hosts parasitised						
4th instar	4.00 b	3.70~4.31	0.81 a	0.74~0.89	5.23 b	4.86~5.60
5th instar	4.74 a	4.37~5.11	0.88 a	0.79~0.98	6.18 a	5.68~6.69

$y = a\ e^{(-0.5\{[(x-1)-(x_0-1)]/b\}^2)}$ for host feeding on 2nd, 3rd and 4th instars, respectively;

$y = a\ e^{(-0.5\{\ln[(x-1)/(x_0-1)]/b\}^2)}$ for parasitism on 4th and 5th instars, respectively. a is the peak at time x_0, b is the coefficient controlling the width of the peak. For each parameter, values in a column followed by the same letters do not differ significantly (the 95% CLs overlap)

Table S4.2 Estimated parameters of models fitting the number of daughters emerging from *Bactericera cockerelli* nymphs of different instars parasitised by *Tamarixia triozae* over lifetime (Fig. 4.4)

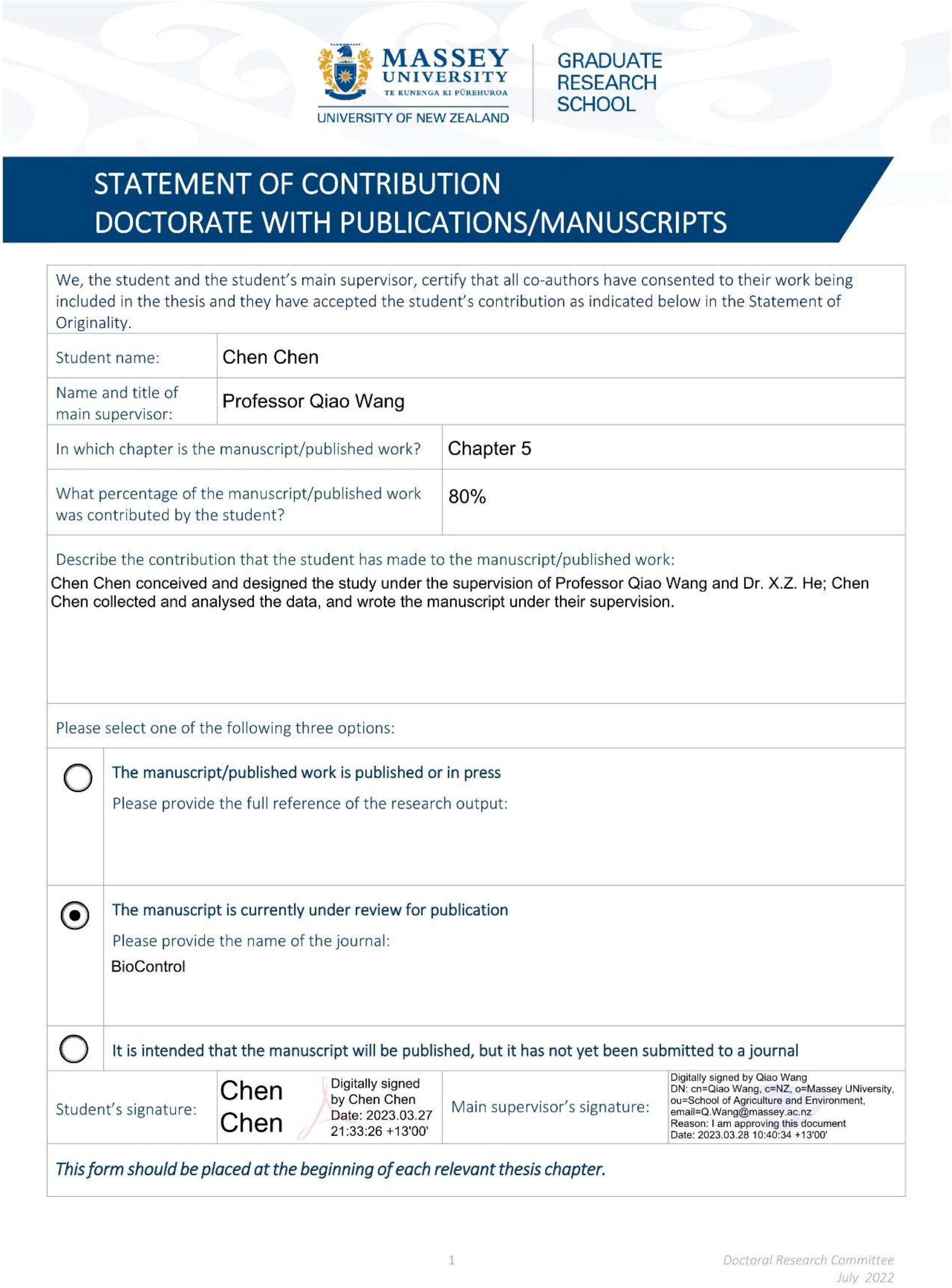
Host instar	a		b		x_0	
	Estimate	95% CLs	Estimate	95% CLs	Estimate	95% CLs
4th instar	0.97 b	0.80~1.14	0.58 a	0.47~0.70	4.13 b	3.70~4.55
5th instar	3.40 a	3.09~3.71	0.63 a	0.56~0.69	5.15 a	4.83~5.47

$y = a\ e^{(-0.5\{\ln[(x-1)/(x_0-1)]/b\}^2)}$ for the number of daughters from 4th and 5th instars, respectively. a is the peak at time x_0, b is the coefficient controlling the width of the peak. For each parameter, values in a column followed by the same letters do not differ significantly (the 95% CLs overlap)

Chapter 5

Parasitoid-host interaction behaviours: a case study in the *Tamarixia triozae-Bactericera cockerelli* system

This chapter was submitted to *BioControl*.



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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Chen Chen
Name and title of main supervisor:	Professor Qiao Wang
In which chapter is the manuscript/published work?	Chapter 5
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:
Chen Chen conceived and designed the study under the supervision of Professor Qiao Wang and Dr. X.Z. He; Chen Chen collected and analysed the data, and wrote the manuscript under their supervision.

Please select one of the following three options:

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Student's signature:	Chen Chen Digitally signed by Chen Chen Date: 2023.03.27 21:33:26 +13'00'	Main supervisor's signature:	Digitally signed by Qiao Wang DN: cn=Qiao Wang, c=NZ, o=Massey UNiversity, ou=School of Agriculture and Environment, email=Q.Wang@massey.ac.nz Reason: I am approving this document Date: 2023.03.28 10:40:34 +13'00'

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Abstract In host-feeding parasitoids, mothers often partition hosts of different stages for feeding and parasitisation but the behavioural mechanisms behind are largely unknown, making it difficult to evaluate parasitoid-host interactions and their effects on biological control success. *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is a key parasitoid of tomato-potato psyllid *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), killing its nymphs by parasitisation and host feeding. The current study presents the first report on behaviours of the parasitoid-host interactions, leading to successful feeding and parasitisation on hosts of different stages by *T. triozae*. The parasitoid behaviours consisted of encounter, evaluation, piercing for feeding, feeding, oviposition probing, and oviposition, and host defence behaviours included body swaying and escaping. Female wasps were more likely to encounter and evaluate older and larger hosts probably due to stronger visual and chemical cues from these hosts. However, encounter and evaluation did not necessarily translate into feeding and oviposition success. Older nymphs had more resources, thicker integument and stronger defence ability and the opposite was the case for younger ones. I show that most feeding occurred on mid-aged nymphs and most parasitisation on late instar ones. These findings suggest that (1) *T. triozae* continues to assess the host using its ovipositor after the evaluation phase, (2) the combination of stage-specific host integument thickness and defence behaviour determines the success of feeding attacks, and (3) the optimal resource for offspring fitness defines host stage selection for oviposition. This study contributes to our understanding of parasitoid-host interactions and mechanisms behind host stage selections.

5.1 Introduction

The optimal foraging theory predicts that parasitoids should adopt the best possible host selection strategies to maximise their lifetime fitness gain (Goubault et al. 2003; Danchin et al. 2008). Parasitoid females often encounter their hosts of various stages with different nutritional values and defensive abilities (Gross 1993; Cadée and van Alphen 1997; Colinet et al. 2005; He et al. 2011; Khatri et al. 2016). Consequently, they should interact with and assess the hosts they encounter before attack occurs (Henry et al. 2005). In host feeding parasitoids, mothers need to rationalise their host selection for fitness of both their offspring and themselves (Kidd and Jervis 1991; Heimpel and Collier 1996; Lauzière et al. 2001; Hanan et al. 2015). This may be achieved through trade-offs between cost and benefit when attacking a host (Rivero 2000). For example, older and larger hosts carry more resources, but parasitoids need more time to handle them (He et al. 2011; Khatri et al. 2016; Uy and Espinoza 2018). Therefore, understanding the host stage selection behaviour could provide information for evaluation of parasitoid-host interactions and their effects on biological control success.

Previous studies report that foraging parasitoids are more likely to encounter larger or older hosts when various stages are present in the same area, but the outcomes of an encounter vary in different parasitoid-host systems (He et al. 2011; Hanan et al. 2015; Khatri et al. 2016). Host feeding parasitoids are expected to prefer larger or older hosts for parasitisation and smaller or younger ones for feeding because the former are of higher nutritional values for their offspring (Liu et al. 2011; Hanan et al. 2015; Luo et al. 2022) and the latter are easier to make a wound for feeding (Kidd and Jervis 1991; Veronesi et al. 2022a). These reported patterns may be altered by handling time associated with host defence (Gross 1993; Cadée and van Alphen 1997; He et al. 2011; Khatri et al. 2016) and integument thickness (Veronesi et al. 2022a). Therefore, observation and analysis of behavioural sequences of host stage-dependent parasitoid-host interactions from encounter to successful feeding and oviposition may provide insight into host stage selection and novel knowledge for effective biological control. To date, behavioural mechanisms behind the host stage selection are unknown for most parasitoid-host systems.

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an important pest of cultivated solanaceous crops in the USA, Mexico and New Zealand, Australia, Canada and Ecuador, causing large economic losses (Cranshaw 1994; Teulon et al. 2009; Crosslin et al. 2010; Butler and Trumble 2012a; Rojas et al. 2015; FAO 2017; WADPIRD 2018; Castillo Carrillo et al. 2019; Olaniyan et al. 2020). *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is an important ectoparasitoid of TPP (Rojas et al. 2015; Yang et al. 2015), killing TPP nymphs by both parasitisation and host feeding (Martinez et al. 2015; Rojas et al. 2015; Chen et al. 2020). It is a diurnally active wasp (Chen et al. 2020), laying egg(s) on the ventral side of the host (Martinez et al. 2015; Rojas et al. 2015) and piercing the soft integument area of the host nymph with its ovipositor and feeding on the haemolymph exuding from the wound (Martinez et al. 2015). The parasitoid is more likely to parasitise larger hosts and feed on smaller ones (Chen et al. 2023) but the behavioural mechanisms behind these patterns are not clear.

Based on the theoretical and empirical frameworks outlined above, the present study aimed to determine the host feeding and oviposition behaviour of *T. triozae* in relation to the stage and defence behaviour of *B. cockerelli* nymphs. I provided mated *T. triozae* females with *B. cockerelli* nymphs of all five instars at the same space and time. I camera-recorded and then quantified interactive behavioural sequences from encountering to successful feeding and oviposition during the entire photophase when the parasitoid wasps were active. Findings from this study contribute to our understanding of parasitoid-host interactions and behavioural mechanisms of host stage selections for feeding and parasitisation.

5.2 Materials and methods

5.2.1 Breeding colonies and environmental conditions

The colonies of *B. cockerelli* and *T. triozae* started from psyllid and wasp adults from BioForce Limited, Auckland, New Zealand. They were maintained in two separate climate rooms to ensure the psyllid colony was not contaminated by parasitoids. Psyllids were reared on five potted two-month-old bell pepper plants (*Capsicum annuum* L.) in an aluminium-framed cage (43 × 42 × 40 cm) with a metal mesh (aperture size = 0.25 × 0.25 mm) on the back and both sides and Perspex on the top and front, and aluminium alloy on the bottom. Wasps were reared in another cage of the same size and made containing five potted two-month-old bell pepper plants infested by 3rd–5th instar psyllid nymphs. The colonies were maintained, and experiments carried out at 25 ± 1°C and RH 40–60 % with a photoperiod of 14:10 hours (L:D).

5.2.2 Insects for experiments

To obtain psyllids of different instars for the experiment, I randomly transferred 70 psyllid adults from the colony onto one potted two-month-old bell pepper plant in an aluminium-framed cage mentioned above. I replaced the infested plant with an un-infested one of the same age once every 24 h for 24 days. Infested plants were individually maintained in a nylon-framed cage (65 × 50 × 50 cm, aperture size = 0.075 × 0.075 mm). When nymphs developed to the 5th instar on the first infested plant, I harvested all nymphs on the plants treated above to obtain insects of all instars at the same time. Nymphal instars were identified under a stereomicroscope (Leica MZ12, Germany) according to Vega-Chávez (2010).

To obtain parasitoids for the experiment, I introduced 10 female parasitoids randomly collected from the colony into an above-mentioned nylon-framed cage maintaining one two-month-old bell pepper plant infested with 200 4th instar psyllid nymphs. After 24 h, I removed all parasitoids using an aspirator. I collected parasitoid pupae seven days later and individually placed them in cotton-plugged glass vials (5 cm in height × 1.5 cm in diameter) until adult emergence. I obtained 30 newly emerged females and 30 newly emerged males and provided them with 10% honey solution as food. Because both sexes become sexually mature within 24 h after emergence (Chen et al. 2020), I individually paired one-day-old virgin males and females in the glass vials until mating ended. Mating usually occurs within 90 mins after pairing and lasts for about 15 mins (CC pers. observ.).

I obtained 14 mated females for the experiments. Using an aspirator, I transferred each mated female into a Petri dish (8.5 cm in diameter × 2.4 cm in height) containing 30 host nymphs (6 individuals × 5 instars) on a bell pepper leaf with its petiole wrapped with water-saturated cotton wool by parafilm. The lid of the Petri dish had two holes (1 cm in diameter), one plugged with a cotton wool ball for transferring wasps and another covered with metal mesh (aperture size = 0.25 × 0.25 mm) for ventilation. I allowed the female to stay in a Petri dish for 24 h and then transferred her to another Petri dish with hosts as above and allowed her to stay for 24 h. I repeated this process until she was 4 days old before experiments because most female wasps start laying eggs at this age (Rojas et al. 2015; Chen et al. 2022). Hosts were the only food for wasps during this period. A cotton ball saturated with water was provided to the wasp and refreshed every day.

5.2.3 Behavioural recording

To determine the host feeding and oviposition behaviour of the parasitoids in relation to the stage and defence behaviour of psyllid nymphs in response to parasitoid attack, I transferred 30 host nymphs (6 individuals × 5 instars) using paintbrush onto a leaf square (5 cm × 2.5 cm) placed upside down on a 1%-agar block (5 cm × 2.5 cm × 0.5 cm) in the centre of a Petri dish mentioned above 30 mins after lights-on. The nymphs of different instars were randomly distributed on the leaf square. Forty minutes after lights-on, I introduced a four-day-old mated and experienced female parasitoid prepared above into the Petri dish and continuously recorded behaviours of both the parasitoid and its hosts of different instars in each dish for 13 h (40 mins after lights-on to 20 mins before lights-off) using a digital video camera (Sony Handycam HDR-CX405, Japan). I repeated the recordings in 14 Petri dishes (14 parasitoids and 420 nymphs). After behavioural recording, I examined all nymphs for evidence of feeding (Morales et al. 2013; Martinez et al. 2015) and turned them over to determine if oviposition had occurred under a stereomicroscope. I correlated each behavioural event with confirmed feeding or oviposition and calculated mean values for each behavioural event.

For each parasitoid I recorded: encounter – the number of times a parasitoid physically contacted a host of a certain instar; evaluation – the number of times a parasitoid examined a host of a certain instar by walking on the host and touching it with its antennae before attack for oviposition or feeding; oviposition probing – the number of times a parasitoid probed a host of a certain instar with its ovipositor between the host body and leaflet surface; oviposition – the number of hosts of a certain instar parasitised; piercing for feeding – the number of times a parasitoid pierced the dorsal soft integument area of a host of a certain instar with its ovipositor; feeding – the number of hosts of different instars fed on by the parasitoid; handling time for feeding – the period a parasitoid spent from the start of encountering a host to the completion of feeding; and handling time for oviposition – the period from start of encountering to the completion of oviposition. For psyllid defence behaviour, I recorded: swaying – the number of times a host of a certain instar swayed its abdomen side to side to prevent attack by a parasitoid; and escaping – the number of a host of a certain instar walked away when attacked by a parasitoid.

5.2.4 Statistical analysis

I conducted all data analyses using SAS software (SAS 9.4, SAS Institute Inc., NC, USA) with a rejection level set at P = 0.05. Data on number of encounters, handling time for feeding were analysed using a linear mixed-effects model (MIXED procedure), with instar stage as a fixed factor and replicate as a random factor. Data on number of evaluations, feeding probing, host feeding, oviposition probing and oviposition, number of escaping and swaying of host nymphs, and handling time for oviposition were analysed using a generalized linear model (GLIMMIX procedure) with a *log* function and Gamma distribution for handling time for oviposition and a Poison distribution for number of evaluations, feeding probing, host feeding, oviposition probing, oviposition, and number of escaping and swaying of host nymphs. Instar stage was a fixed factor and replicate was a random factor in the model. Multiple comparisons between treatment means were performed using the Tukey test.

5.3 Results

5.3.1 Behaviours during parasitoid-host interactions

The parasitoid started foraging after being released to the dish. Upon physical contact with a host, the female evaluated the host by walking on it and frequently touching it with antennae (Fig. 5.1a). Once a host was selected for feeding, the parasitoid pierced the soft integument area on the dorsal side of the host body with its ovipositor to make a wound (Fig. 5.1b) and then fed on the extravasated haemolymph (Fig. 5.1c). When the parasitoid chose to parasitise a host, she probed the host with her ovipositor between the host body and leaf surface and then laid egg(s) (Fig. 5.1d). Eggs were usually deposited on the ventral side of the host between the prothoracic and mesothoracic legs or the mesothoracic and metathoracic legs. Host defence behaviour to avoid attack included swaying its abdomen side to side (Fig. 5.1e) and walking away (Fig. 5.1f).

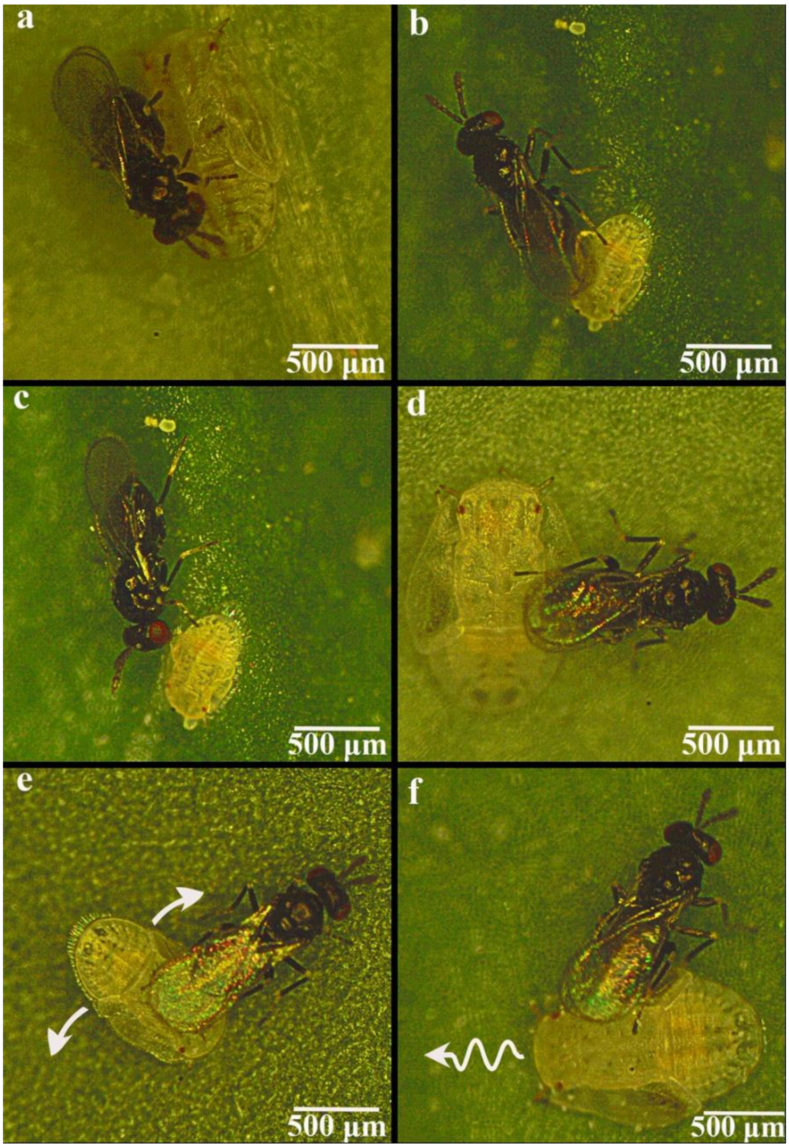

Fig. 5.1 *Tamarixia triozae* attacking and *Bactericera cockerelli* defence behaviours: evaluation (**a**), piercing for feeding (**b**), feeding (**c**), oviposition probing (**d**), swaying to avoid attack (**e**), and escaping to avoid attack (**f**)

5.3.2 Effect of host stage on host feeding, oviposition, and host defence

The parasitoids were more likely to encounter and evaluate the older hosts, i.e., 4th and 5th instar nymphs ($F_{4,52} = 14.84$, $P < 0.0001$ for encounter; $F_{4,52} = 59.69$, $P < 0.0001$ for evaluation) (Fig. 5.2). They pierced all instars but significantly preferred the 3rd instar nymphs for piercing and feeding ($F_{4,52} = 10.52$, $P < 0.0001$ for piercing; $F_{4,52} = 5.01$, $P = 0.0017$ for host feeding) with no feeding on the 1st instar nymphs (Fig. 5.3). The parasitoids performed oviposition probing on the 3rd to 5th instar nymphs but preferred 4th and 5th over 3rd instars for oviposition probing and only laid eggs under 4th and 5th instars ($F_{4,52} = 42.38$, $P < 0.0001$ for oviposition probing; $F_{2,26} = 12.64$, $P = 0.0001$ for parasitism) (Fig. 5.4).

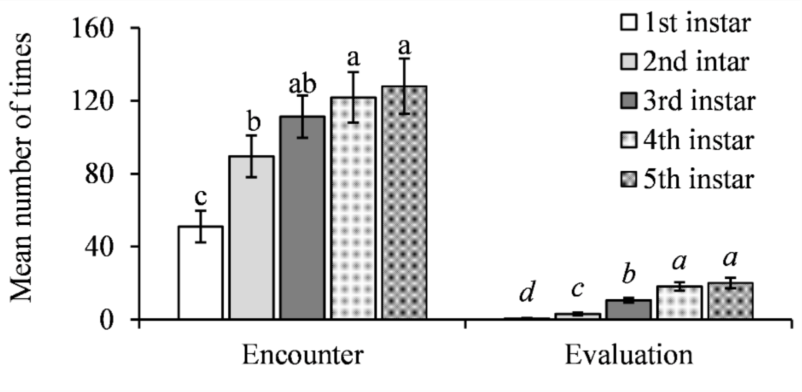

Fig. 5.2 Mean (± SE) number of encounters and evaluations by *Tamarixia triozae* on *Bactericera cockerelli* nymphs of different instars. For each category, columns with different letters are significantly different ($P < 0.05$)

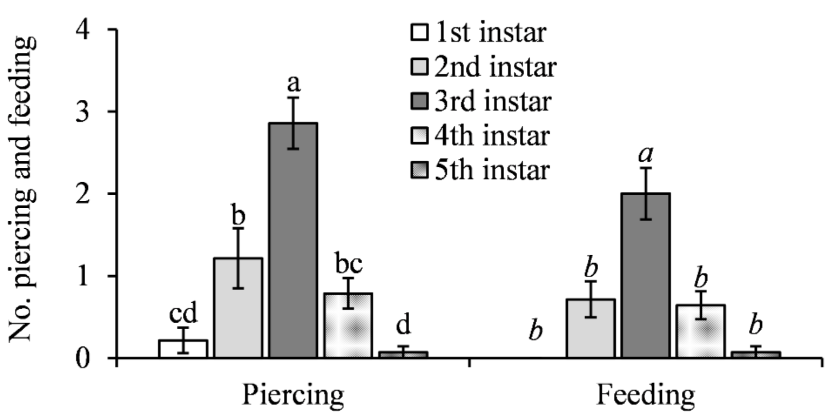

Fig. 5.3 Mean (± SE) number of piercings for feeding and hosts fed by *Tamarixia triozae* on *Bactericera cockerelli* nymphs of different instars. For each category, columns with different letters are significantly different ($P < 0.05$)

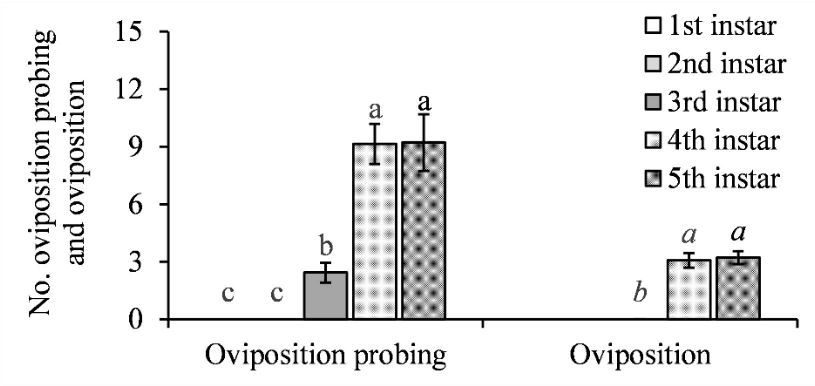

Fig. 5.4 Mean (± SE) number of oviposition probings and hosts parasitised by *Tamarixia triozae* on *Bactericera cockerelli* nymphs of different instars. For each category, columns with different letters are significantly different ($P < 0.05$)

In response to parasitoid attack for feeding, there was no significant difference in frequency of host escaping between instars ($F_{4,16} = 0.58$, $P = 0.6825$) (Fig. 5.5a) but the 4th instar nymphs swayed significantly more frequently than 2nd and 3rd instars ($F_{4,16} = 5.33$, $P = 0.0063$) (Fig. 5.5b). The 5th instar nymphs were significantly more active to defend themselves from oviposition attack than the 3rd and 4th instars ($F_{2,23} = 5.62$, $P = 0.0103$ for escape times; $F_{2,23} = 787.53$, $P < 0.0001$ for sway times) (Fig. 5.5). Wasps spent significantly longer time to handle the 4th and 5th instar nymphs for feeding than the 2nd and 3rd instar nymphs ($F_{3,32} = 10.88$, $P < 0.0001$) (Fig. 5.6a) and to handle the 5th instar nymphs for oviposition than the 4th instars ($F_{1,83} = 19.73$, $P < 0.0001$) (Fig. 5.6b).

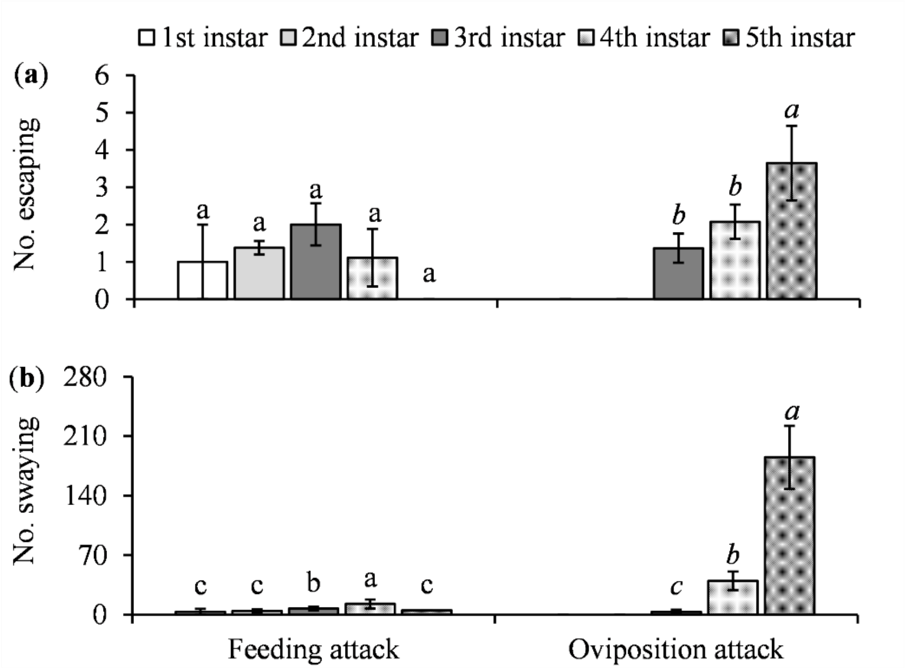

Fig. 5.5 Defence behaviour of *Bactericera cockerelli* nymphs of different instars in response to feeding and oviposition attacks by *Tamarixia triozae*: (**a**) mean (± SE) number of escapes and (**b**) mean (± SE) number of sways. For each category, columns with different letters are significantly different (P < 0.05)

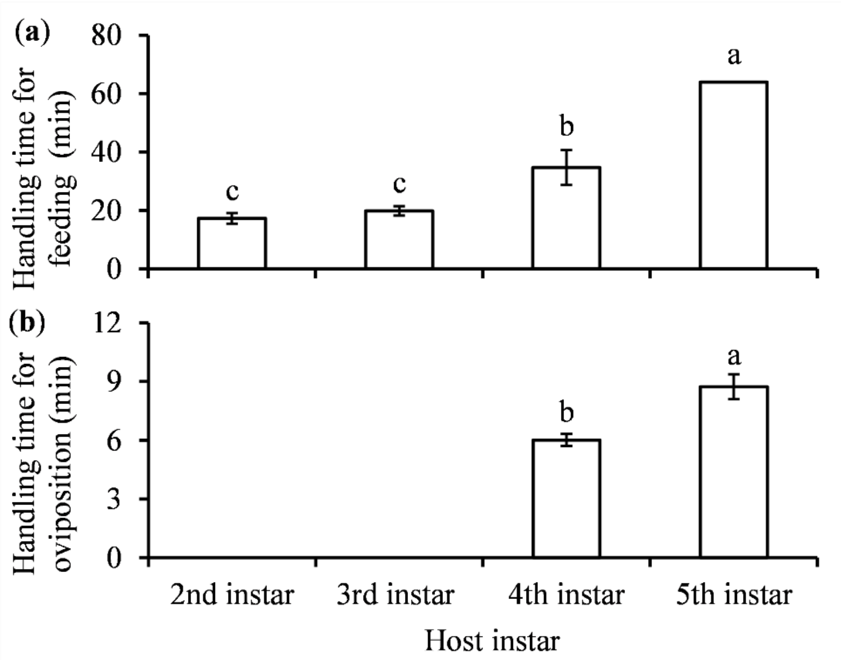

Fig. 5.6 Mean (± SE) handling time for feeding (**a**) and oviposition (**b**) by *Tamarixia triozae* on *Bactericera cockerelli* nymphs of different instars. Columns with different letters are significantly different (P < 0.05)

5.4 Discussion

My results show that *T. triozae* females were significantly more likely to encounter and evaluate older or larger hosts (Fig. 5.2). Following evaluation of the encountered hosts, female wasps might pierce the hosts of all instars with her ovipositor but were significantly more likely to pierce and feed on the 3rd instar hosts (Fig. 5.3). *T. triozae* performed oviposition probing under the 3rd to 5th instar nymphs, significantly preferring the 4th and 5th instars to the 3rd instar and only laying eggs under the 4th and 5th instars (Fig. 5.4). In response to feeding attacks, hosts of all instars might escape at a similar frequency but those of the 3rd and 4th instars swayed more often (Fig. 5.5). Compared to the 3rd and 4th instar nymphs, the 5th instar hosts were significantly more likely to avoid oviposition attacks by escaping and swaying (Fig. 5.5). Similarly, handling older hosts for feeding and oviposition cost the parasitoids significantly more time (Fig. 5.6).

The higher probability of encountering and evaluating older hosts could be attributed to the fact that parasitoids can use visual and chemosensory cues to locate hosts in a short-range (Battaglia et al. 1995, 2000; Mackauer et al. 1996; Powell et al. 1998; Weinbrenner and Volkl 2002; He et al. 2011) and older hosts are more visible and emit stronger semiochemicals (Hanan et al. 2015; Khatri et al. 2016; Bui et al. 2020). Behavioural divergence occurred after the evaluation phase: the females were most likely to pierce and feed on mid-aged nymphs and probe and parasitise late instar ones. Because *T. triozae* females do not feed on parasitised hosts or parasitise those already fed upon (Cerón-González et al. 2014; Chen et al. 2023), my findings suggest that this parasitoid has developed a host partitioning strategy for feeding and parasitisation to minimize the competition for hosts between mothers and their offspring (Lauzière et al. 2001), in accordance with the optimal foraging theory (Kishani Farahani et al. 2015).

I show that piercing the 1st instar nymphs and probing under the 3rd instar ones did not translate into successful feeding and oviposition, respectively. These findings suggest that *T. triozae* continues to assess the host using its ovipositor during piercing or probing because parasitoid ovipositors carry the sensilla that may play key roles in host stage recognition (Wong et al. 2021). The reduced feeding piercing and feeding on younger and older nymphs may result from the cost-benefit assessment by the wasp. Younger hosts have thinner integument which is easier to penetrate (Kidd and Jervis 1991; Veronesi et al. 2022a) and have less nutritional values (Kidd and Jervis 1991; Fellowes et al. 2007; Hanan et al. 2015) while the opposite is the case for older hosts, leading to little or no feeding on younger and older hosts. Although *T. triozae* occasionally parasitises the 3rd instar nymphs, no female offspring emerge from hosts of this instar (Chen et al. 2023), suggesting that hosts younger than the 3rd instar do not have sufficient resources to sustain the parasitoid population.

Significantly more swaying by the 3rd instar nymphs in response to feeding attacks did not increase the handling time by parasitoids, rather, the wasps were significantly more likely to pierce and feed on the hosts of this stage. On the other hand, the parasitoids spent significantly more time to handle the 5th instar hosts but had significantly less success in piercing and feeding. These findings imply that the combination of age-specific host defence behaviour and integument thickness determines the ultimate outcome of feeding attacks. In response to oviposition attacks, host defence activities and parasitoid handling time significantly increased with the increase of host stages. However, the parasitoids were still significantly more likely to perform oviposition probing on and parasitise the 4th and 5th instar hosts. The results suggest that *T. triozae* selects to parasitise older hosts regardless of host defence to maximise the fitness of their offspring. I support the prediction that parasitoid females should adopt the host selection strategies that maximise their lifetime fitness gain (Goubault et al. 2003; Danchin et al. 2008).

Overall, *T. triozae* females are more likely to encounter and evaluate older and larger hosts probably due to stronger visual and chemical cues from these hosts. However, the encounter and evaluation frequencies do not necessarily translate into feeding and oviposition success. They prefer mid-aged hosts for feeding and older ones for oviposition regardless of stage-specific host defence behaviour. Such host partitioning strategies may maximise the fitness of both *T. triozae* adults and their offspring. I suggest that the combination of age-specific host integument thickness and defence behaviour governs the success of feeding attacks while the optimal resource for offspring fitness explains host stage selection for oviposition.

Chapter 6

General discussion and conclusion

6.1 Introduction

During my PhD studies, I have conducted a series of experiments to investigate aspects of the biological control ecology of the parasitoid *T. triozae* on the tomato-potato psyllid *B. cockerelli*. My research has covered circadian rhythms of the parasitoid adult feeding and reproductive activities, effects of supplementary adult diets with or without temporary host deprivation on its female reproductive fitness and lifetime feeding and oviposition patterns, life history strategies in response to host stage and parasitoid's age, and the host selection behaviour in *T. triozae* and defence behaviour in *B. cockerelli*. In this chapter, I summarize and discuss my main findings and their relevance to development of biological control programmes using parasitoids in general, and *T. triozae* in particular. I also recommend further studies.

6.2 Circadian activity patterns of *Tamarixia triozae*

Understanding circadian activity patterns of parasitoids may facilitate development of effective field release programmes for biological control and recommendations for the timing of irrigation or insecticide applications (Vogt and Nechols 1991; van Lenteren et al. 1992). In this study, I demonstrate that *T. triozae* is a primarily diurnal species, with emergence, mating, host feeding and oviposition mainly occurring during the day. These findings are in accordance with reports in some other parasitoid species, such as *A. reticulatus* (Kainoh 1986), *B. curculionis* (England 1995) and *A. ervi* (He et al. 2004). For *T. triozae*, most adults emerge during the first few hours of the photophase. Environmental conditions in the early morning facilitate wing expansion and initial dispersal and search for mates with less water loss (Lankinen 1986; Fantinou et al. 1998; Karpova 2006), which may be beneficial to the primarily diurnal insects.

Similar to previous findings on hymenopteran parasitoids (e.g., Kainoh 1986; He et al. 2004; Rojas et al. 2015), my results show that males have shorter developmental time than females. As a result, most males emerge earlier than females, which may increase male mating success and reduce the risk of female pre-reproductive death (Waage and Ming 1984; Quicke 1997; Morbey and Ydenberg 2001). Furthermore, males become sexually mature later than females, and females are more likely than males to mate with sexually mature one-day-old mates during the day of their emergence. However, most one-day-old adults mate in the first few hours of photophase. These findings suggest that most matings occur in the early morning of the second photophase following emergence in *T. triozae*, which is different from *A. reticulatus* where most adults emerge in the early morning and mate in the afternoon of the same day (Kainoh 1986). This strategy may maximise the mating success through reducing mate-searching time and risk of predation as reported in other insects (Rymer et al. 2007) and allow time for female parasitoids to mature their eggs because this parasitoid has a pre-oviposition period of 1.9 ± 0.8 days (Rojas et al. 2015).

In many parasitoid species, adults feed on hosts to gain nutrients for egg maturation and production (Jervis and Kidd 1986; Burger et al. 2005; Hanan et al. 2009; Gebiola et al. 2018). I show that *T. triozae* females lay eggs only in the photophase with an oviposition peak between mid-morning and mid-afternoon, which is different with some other parasitoids such as *O. kuwanai* (Weseloh 1972) and *A. ervi* (He et al. 2004) where oviposition occurs throughout the 24-h cycle. However, *T. triozae* females feed on their hosts throughout the day with most host-feeding occurring during the photophase. Host feeding activities have three peaks in the early morning, late afternoon, and dawn, respectively. These results suggest that females have the highest host feeding rate before and after the oviposition peak to acquire necessary nutrients for egg production and somatic maintenance. Similar host feeding and oviposition patterns also occur in *E. warrae* (Hanan et al. 2009). Male harassment during oviposition can reduce female fitness in insects (e.g., Sakurai and Kasuya 2008; Li et al. 2014). However, *T. triozae* has developed a strategy to avoid such harassment because its diel activity peaks for mating and oviposition do not overlap, which could make this parasitoid a more effective biological control agent.

My findings provide useful knowledge for the development of an integrated management programme involving *T. triozae*. For example, sexually mature wasps should be allowed to mate in the early morning of the next day following their emergence to achieve the highest reproductive output and best control of the psyllid. Mated and host-fed females should be released to the field or greenhouses in the early morning of the third day following emergence to achieve better control results because most *T. triozae* females start ovipositing when they are three days old (Rojas et al. 2015) and host feeding plays a significant role in egg production and maturation (Cerón-González et al. 2014). Alternatively, if newly emerged wasps need to be released to the field or greenhouses, the release should occur in the morning when environmental conditions are more favourable and adults are more active. This approach proves to be effective in the application of *G. pennsylvanicum* (Vogt and Nechols 1991). When additional natural enemies are introduced to control the psyllid in the future, their circadian oviposition and host feeding peaks should not completely overlap with those of *T. triozae* to minimize competition that may reduce biological control efficacy (Miranda et al. 2015; Ramírez-Ahuja et al. 2017). Furthermore, overhead irrigations and insecticide sprays may be applied as late in the evening as possible to avoid the activity peaks of *T. triozae* to reduce side effects on this parasitoid because its adults are most susceptible to insecticides. Future studies could focus on testing whether the knowledge of circadian activity patterns would help improve the control efficiency of *T. triozae* under greenhouse or field conditions with natural daylight and photoperiod.

6.3 Effects of adult diets on *Tamarixia triozae* performance

In augmentative release programmes, parasitoid pupae or adults (van Lenteren and Tommasini 1999; Yokoyama et al. 2010; Cancino and López-Arriaga 2016) sometimes need to be shipped from their production sites to the release sites in the absence of hosts, which can last days, depending on distance and transport methods. To maximise its biological control effectiveness, I have investigated the effects of adult diets (honey, water, yeast, and hosts) and timing of their provision on *T. triozae* fitness and oviposition pattern. My study shows that feeding on honey and/or hosts is essential for the survival of *T. triozae* adults because carbohydrates in honey or hosts can provide energy to prolong parasitoid longevity (e.g., Jervis et al. 2008; Picciau et al. 2019). Contradictory to previous findings about the beneficial effect of yeast feeding on parasitoid fitness (Bartlett 1964; Heimpel and Rosenheim 1995), *T. triozae* adults die in about two days if they only feed on yeast solution (Yeast − Host) or water only (Water − Host) after emergence. These findings suggest that honey diet should be provided for *T. triozae* adults immediately after emergence regardless of whether hosts are present, or for pupae during shipment to ensure newly emerged adults can feed on honey in the absence of hosts.

My findings also indicate that honey feeding with host deprivation for three days after mating delays the peaks of daily host feeding, fecundity, and daughter production for three to four days with little or no cost to wasp longevity and lifetime host feeding and fecundity. This suggests that there could be four days for successful shipment of honey-fed and host-deprived females without compromising biological control effectiveness. Moreover, wasps in treatments Water + Host and Honey − Host have the highest fecundity peak, suggesting that the release of wasps that have fed on water for 24 h or on honey for four days in the absence of hosts may achieve quick suppression of pest population when the pest density is high. I also found that wasps feeding on honey or yeast without temporary host deprivation after mating (Honey + Host and Yeast + Host) flatten their daily oviposition curves and spreads their egg laying over a longer period. Therefore, wasps treated this way may reduce the risk of massive removal of hosts and increase their establishment success when the pest population density is low. Furthermore, wasps in treatment Honey − Host have the highest peak of daughter production, suggesting that these wasps can increase their own population soon after released to the field.

Although honey-fed and host-deprived wasps have longest generation time (T) and relatively low intrinsic rate of increase (r) and long doubling time (D_t) due to delayed oviposition, they achieve greatest net reproductive rate (R_0), i.e., produce the highest number of daughters, promoting future population growth (Sabbatini Peverieri et al. 2012). These findings suggest that when the pest population density is low, treatment Honey − Host can lead to better and more sustainable control. Moreover, wasps in treatment Water + Host have the highest r and shortest D_t, the second highest R_0 and relatively short T. Therefore, when the pest population density is high and we wish to achieve fast control, we should feed newly emerged wasps with water for 24 h before release. Producers may also refer to the life table parameters to adjust their mass-rearing programmes depending on whether they aim to yield more parasitoids quickly or to slow production down. Future studies could focus on testing these implications under greenhouse or field conditions.

6.4 Life history strategies of *Tamarixia triozae* in response to host stage and its own age

To help understand the host selection for feeding and oviposition and evaluate the biological control effectiveness of *T. triozae*, I have investigated parasitoid age- and host stage-dependent host feeding, parasitisation, sex allocation and their fitness consequences. The parasitoid females pierce the host integument using ovipositors and make it bleed before feeding on the body fluid. My results show that *T. triozae* females prefer mid-aged to either younger or older nymphs for feeding and older to mid-aged nymphs for parasitisation when all instars of *B. cockerelli* nymphs are present simultaneously, which is the most likely scenario in the field. Because the females neither parasitise fed hosts nor feed on parasitised ones (Cerón-González et al. 2014; CC pers. observ.), they need to rationalise their effort for optimal fitness gain for both mothers and their offspring. Larger or older hosts provide more nutrition (Li and Mills 2004; Liu et al. 2011) but the thicker integument of nymphs (Veronesi et al. 2022a) is more difficult to penetrate by the parasitoids (Kidd and Jervis 1991; Zhang et al. 2015). Therefore, they prefer to feed on younger hosts and parasitise older ones. Such host partitioning strategy may allow *T. triozae* to control the pest effectively by killing hosts of all instars simultaneously because both host feeding and parasitisation destroy the hosts (Martinez et al. 2015; Rojas et al. 2015).

Tamarixia triozae is a solitary species with only one offspring surviving on each parasitised host (Rojas et al. 2015), but females may deposit more than one egg under a host nymph with more eggs allocated to older nymphs. The parasitoid offspring that develop from older nymphs at parasitisation are more likely to develop to adults. Therefore, this host stage-dependent superparasitism may be a strategy to increase parasitoid offspring survival. Moreover, comparing the findings between the current and Rojas et al.'s (2015) studies, I found that *T. triozae* females increase and reduce superparasitism at lower and higher host density, respectively. The host density-dependent superparasitism may help maintain their own population in the field. Because the psyllid populations in field usually increase and then decrease with the seasonal progress (Munyaneza et al. 2009), increasing superparasitism when host density is low may reduce the risk of collapse of the host population and reducing it when the host density is high may maximise the use of available resources.

Furthermore, *T. triozae* females mainly feed on the 3rd instar nymphs in their early life when maximum parasitism occurs on the 4th and 5th instar hosts but their feeding on the 4th instar nymphs increases after they reach mid-age and their parasitism sharply declines. One possible explanation for the increase of their feeding on more nutritious 4th instar nymphs at older age is the combination of declined fecundity and increased demand of nutrition for adult survival. Overall, host feeding and parasitism peak during the first week of female life and decline significantly after two weeks. Because the experimental condition (25°C) in the present study is optimal for the development of both *B. cockerelli* (Lewis et al. 2015) and *T. triozae* (Vega-Chávez et al. 2016), my findings could be realistic in the field and greenhouses when the temperature is around 25°C.

Tamarixia triozae offspring that emerge from parasitised 4th instar nymphs are highly male-biased but those from the 5th instar nymphs are highly female-biased, strongly implying that females allocate more fertilised eggs to the oldest and largest hosts, in accordance with reports in other parasitoids (e.g., Charnov et al. 1981; Favaro et al. 2018; Pérez-Rodríguez et al. 2019). Therefore, using the 5th instar nymphs as hosts during *T. triozae* mass rearing may increase daughter production and reduce rearing cost. Furthermore, female wasps produce most daughters during the first half of adult life, suggesting that young females may maximise the use of sperm (Latham and Mills 2010; Mawela et al. 2021) to ensure the survival and persistence of their next generation. Consequently, their sperm may run out quickly as they age (Pérez-Lachaud and Hardy 1999; Santolamazza Carbone et al. 2007; Hu et al. 2012), as indicated by the male-biased offspring sex ratio after they reach mid-age.

I show that females have longer developmental time and larger body size than males regardless of the host stage at parasitisation, suggesting that females need more time and resources to build up larger body size for reproduction (King 1988, 2000; He et al. 2005). In accordance with other parasitoids (e.g., Liu et al. 2011; Hanan et al. 2015; Luo et al. 2022), the offspring body size of both sexes in *T. triozae* increases with the increase of the host age at parasitisation. Furthermore, newly emerged daughters from hosts parasitised at the 5th instar nymphs have higher egg load than those from hosts parasitised at the 4th instar. Therefore, like other parasitoids (e.g., Hu et al. 2002; Hanan et al. 2015; Li et al. 2018), larger and older hosts increase the fitness of *T. triozae* offspring.

Parasitoids often forage simultaneously with their conspecifics within the same patch in nature (Visser 1995) and during mass rearing (Krüger et al. 2019). Conspecific competition for resources can result in diversification in resource use (Svanbäck and Bolnick 2007) and mutual interference may reduce female searching efficiency (Yang et al. 2015). Therefore, future studies on host feeding and oviposition of *T. triozae* in response to the interactions between foraging parasitoids are recommended. Investigations into potential use of more than one natural enemy in compatible manner may also be warranted. For example, *T. triozae* prefers middle-aged nymphs of TPP for feeding and old-aged ones for parasitism (Rojas et al. 2015; Chen et al. 2023) while *Amblydromalus limonicus* (Acari: Phytoseiidae) prefers first and second instar nymphs for consumption (Xu and Zhang 2015). The combined use of these two natural enemies may thus result in better TPP control. In addition, the combined releases of *T. triozae* and *E. nicotianae* (Veronesi et al. 2022b) or uses of *A. limonicus* and the entomopathogenic fungus *Beauveria bassiana* suspension could also be a good option (Liu et al. 2019).

6.5 Host stage selection behaviours of *Tamarixia triozae* and defence behaviours of *Bactericera cockerelli*

As discussed earlier, host-feeding parasitoids often partition hosts of different stages for feeding and parasitisation but the behavioural mechanisms behind their host stage selections are largely unknown, making it difficult to evaluate parasitoid-host interactions and their effects on biological control success. My study presents the first report on behaviours of the parasitoid-host interactions leading to successful parasitisation and feeding on hosts of different stages by *T. triozae*.

I show that *T. triozae* females are more likely to encounter and evaluate older or larger hosts, probably due to the fact that parasitoids can use visual and chemosensory cues to locate hosts in a short-range (Battaglia et al. 1995, 2000; Mackauer et al. 1996; Powell et al. 1998; Weinbrenner and Volkl 2002; He et al. 2011) and older or larger hosts are more visible and emit stronger semiochemicals (Hanan et al. 2015; Khatri et al. 2016; Bui et al. 2020). After evaluation, female wasps may pierce the hosts of all instars but are more likely to pierce and feed on mid-aged nymphs. However, they perform oviposition probing under the 3rd to 5th instar nymphs with a preference for the 4th and 5th instars nymphs and only lay eggs under the 4th and 5th instars. These findings support the optimal foraging theory (Kishani Farahani et al. 2015) and explain the host partitioning strategy of *T. triozae*, which may minimize the competition for hosts between mothers and their offspring (Lauzière et al. 2001).

My findings reveal that piercing the 1st instar hosts and probing under the 3rd instar hosts do not translate into successful feeding and oviposition, respectively. It is suggested that the wasp continues to assess the host using its ovipositor during piercing or probing because parasitoid ovipositors carry the sensilla that may play a significant role in host stage recognition (Wong et al. 2021). The reduced feeding piercing and feeding on younger and older nymphs may be attributed to the cost-benefit assessment by the wasp. Younger hosts are easier to penetrate due to their thinner integument (Kidd and Jervis 1991; Veronesi et al. 2022a) and have less nutritional values for offspring (Kidd and Jervis 1991; Fellowes et al. 2007; Hanan et al. 2015) while the opposite is the case for older ones. Chapter 4 shows that hosts younger than the 3rd instar do not have sufficient resources to sustain the parasitoid population because no female offspring emerge from hosts of this instar (Chen et al. 2023). These facts may explain why the parasitoid prefers mid-aged hosts for feeding and older ones for oviposition.

In response to feeding attacks, more swaying by the 3rd instar nymphs do not increase the handling time by parasitoids, rather, the wasps are more likely to pierce and feed on them. Moreover, the parasitoids spend more time handling the 5th instar hosts for feeding but they have less success in piercing and feeding. These findings imply that the combination of stage-dependent host integument thickness and defence behaviour governs the success of feeding attacks. Host defence activities and parasitoid handling time in response to oviposition attacks increase with the increase of host stages. However, the parasitoids are still more likely to probe and parasitise the 4th and 5th instar hosts, suggesting that the optimal resource for offspring fitness determines host stage selection for oviposition in *T. triozae*. My results appear to support the prediction that parasitoid females should adopt the best possible host selection strategies to maximise their lifetime fitness gain (Goubault et al. 2003; Danchin et al. 2008). In this study, I have only tested behavioural defense by TPP. Other potential defence mechanisms such as sequestration of plant toxins and encapsulation (Gross 1993) may be investigated in the future. Because conspecific female parasitoids often forage simultaneously within the same patch in nature, future studies on host feeding and oviposition behaviour of *T. triozae* in response to the population size and density of foraging parasitoids are recommended.

6.6 Conclusion

This study provides new insight into the biological control ecology of *T. triozae* on *B. cockerelli*. My results demonstrate that *T. triozae* has developed strategies to maximise its fitness by optimising the circadian rhythms of emergence, host feeding and reproductive activities and adjusting its life history and host selection strategies in response to host stage and parasitoid's age. I also reveal that supplementary adult diets with or without temporary host deprivation may significantly affect female reproductive fitness and lifetime feeding and oviposition patterns. Knowledge generated is useful for the development of effective biological control programmes using this parasitoid, for example, mass rearing, shipment, and release programmes.

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Appendix 1: Three published papers from my PhD studies

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Tamarixia triozae, an important parasitoid of *Bactericera cockerelli*: circadian rhythms and their implications in pest management

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Abstract *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is an important primary parasitoid of *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). This study aimed to unveil the parasitoid's circadian rhythms and provide information for enhancement of its biological control. Most emergence occurred in the morning and most mating took place early next morning. Oviposition only occurred during the daytime, peaking between mid-morning and mid-afternoon while host feeding had three peaks in the early morning, late afternoon and dawn, respectively. We demonstrate that circadian activity peaks for different life functions did not overlap, suggesting that *T. triozae* have developed strategies for maximal fitness gain. These findings have implications in pest management, which can be tested under field conditions. For example, release of sexually mature and host-fed adults in the early morning may help achieve better control. Moreover, insecticide sprays, when necessary, may be applied as late in the evening as possible to reduce side effects on this parasitoid.

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Introduction

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an economically important pest of cultivated solanaceous crops, particularly potatoes, tomatoes, eggplants and peppers, in the USA, Mexico (Cranshaw 1994; Crosslin et al. 2010; Butler and Trumble 2012a; Rojas et al. 2015) and New Zealand (Teulon et al. 2009; Yang and Liu 2009). It has also established in Western Australia since 2017 (FAO 2017) and its economic impact there is under evaluation. Due to its invasive nature, the pest is expected to enter other parts of the world in the future (CABI 2018). TPP damages host plants by direct feeding and transmission of *Liberibacter solanacearum*, the causal agent of zebra chip, and other diseases (Munyaneza et al. 2007, 2008; Butler and Trumble 2012a; Rojas et al. 2015). Soon after its first detection in New Zealand in 2006, the pest cost the potato industry of this country more than NZ$120 million (Ogden 2011). So far, the application of insecticides is the main method for the control of this pest (Munyaneza 2012; Martinez et al. 2015; Wright et al. 2017). However, the psyllid has already developed resistance against commonly used insecticides (Dávila et al. 2012; Cerna et al. 2013; Chávez et al.

2015; Szczepaniec et al. 2019), reducing their effectiveness. Furthermore, the application of insecticides may cause pest resurgence (Prager et al. 2016) due to destruction of natural enemies (Martinez et al. 2015; Morales et al. 2018). Therefore, there is an urgent need to develop more sustainable control methods for this pest including the use of natural enemies (Luna-Cruz et al. 2011; Liu et al. 2012; Cerón-González et al. 2014; Rojas et al. 2015) compatible with integrated pest management programs.

Tamarixia triozae (Burks) (Hymenoptera: Eulophidae) is an important primary ectoparasitoid of TPP (Yang and Liu 2009; Rojas et al. 2015; Yang et al. 2015) with potential for higher population increase than its host (Rojas et al. 2015) and up to 80% parasitism rate (Bravo and López 2007). It is an arrhenotokous synovigenic wasp, killing its hosts by both parasitization and host feeding (Martinez et al. 2015; Rojas et al. 2015). A female consumes an average of 1.4 psyllid nymphs for each egg laid (Cerón-González et al. 2014). Females feed on all instars of host nymphs (Vega Chávez and Valdez 2010) to gain nutrients necessary for egg maturation (Jervis and Kidd 1986; Burger et al. 2005) and prefer to parasitize fourth instar nymphs (Caudillo Ruiz 2010). Each female lays an average of 165 eggs during her lifespan, and the pre-oviposition period and longevity of adult females are about two and 20 days, respectively (Rojas et al. 2015). These features suggest that *T. triozae* can be an effective biological control agent of TPP. Consequently, New Zealand has recently introduced and released the parasitoid for TPP control (Barnes 2017). However, the parasitoid is very sensitive to insecticides which may render it ineffective (Luna-Cruz et al. 2011; Liu et al. 2012), and recommendation for the timing of insecticide sprays to minimize side effects on this natural enemy is yet to be developed.

Most organisms adjust their physiology and behavior through their circadian clock in response to daily photoperiod (Panda et al. 2002; Bertossa et al. 2010; Vaze and Sharma 2013; Saunders 2020). For instance, adults of different parasitoid species have their own unique circadian activity patterns (Brown and Schmitt 2001). Species-specific circadian rhythms may be of adaptive significance, for example different rhythmicity can reduce competition between species consuming the same resources and improve the efficiency of genetic isolation between more closely related species (Saunders 2002; Bertossa et al. 2013). Therefore, understanding circadian activity patterns of pest parasitoid adults may help develop effective laboratory handling and field release programs for biological control. For example, the morning release of the squash bug egg parasitoid *Gryon pennsylvanicum* (Ashmead) appears to be more effective because it is easier for adults to find mates in the morning (Vogt and Nechols 1991). Due to significant overlaps in circadian oviposition patterns of *Diachasmimorpha longicaudata* (Ashmead) and *Doryctobracon crawfordi* (Viereck), two parasitoids of tephritid fruit flies, simultaneous release of both species may result in substantial competition, reducing control efficacy (Miranda et al. 2015).

Knowledge on circadian rhythms of adult parasitoids may also contribute to decisions on the timing of insecticide applications that could reduce impacts on natural enemies. For instance, *G. pennsylvanicum* is most vulnerable to insecticide sprays when adult activity peaks, during which time no insecticide should be applied (Vogt and Nechols 1991). To spare *Encarsia formosa* Gahan, van Lenteren et al. (1992) recommend that irrigation and insecticide sprays be avoided during the peak of adult emergence. However, circadian rhythms of emergence, host feeding and reproduction of *T. triozae* are still unknown, hampering the development of its effective laboratory handling and field releases. Moreover, it may help understand potential competition with other parasitoid species in the field. Without this knowledge, it would be impossible to make recommendations for the timing of insecticide applications or irrigation.

In this study, we simulated New Zealand summer conditions of 25°C and a photoperiod of L:D 14:10 h and carried out a series of experiments to determine the circadian patterns of emergence, host feeding and reproduction in *T. triozae*. Knowledge generated is essential for development of an effective TPP biological control program using this parasitoid and strategies for its integration with other control practices (e.g., the release of other natural enemies, pesticide applications, and watering).

Materials and methods

Breeding colonies and experimental conditions

We established *B. cockerelli* and *T. triozae* colonies with adult insects from BioForce Ltd., Auckland, New

Zealand. We maintained the psyllid colony on five two-month-old bell pepper (*Capsicum annuum* L.) plants, each grown in a pot (9.5 cm in height × 10.5 cm in top diameter × 8.5 cm in bottom diameter) with potting mix. The potted plants were randomly placed in an aluminium-framed cage (43 × 42 × 40 cm) with a fine metal mesh (aperture size = 0.25 mm) on the back and both sides and Perspex on the top and front and aluminium alloy on the bottom. We reared *T. triozae* on 3rd–5th instar psyllid nymphs feeding on five infested potted plants in a cage as before. These two insect colonies were kept in two separate climate rooms to ensure the psyllid colony was parasite-free for experiments. Environmental conditions for colony maintenance were 25 ± 1 °C, 40–60% RH and a L:D 14:10 photoperiod, which normally occur in New Zealand summer. The photophase was set from 08h00 to 22h00 for both colonies.

We carried out experiments in two climate rooms under the above environmental conditions. We set the photophase from 08h00 to 22h00 for one room (a normal light regime) and the scotophase from 10h00 to 20h00 for the other (a reverse light regime). We recorded the data in the normal light room during the period of 08h00 to 22h00 and in the reverse light room during the period of 10h00 to 20h00. Therefore, all data for the photophase were from the normal light room and those for the scotophase were from the reverse light room. We allowed wasps to adapt to the reverse light regime one day before experiments in the reverse light room.

Sex-dependent emergence rhythm and developmental period

We recorded sex-dependent emergence patterns and developmental period of *T. triozae* in both climate rooms. In each room, we set up 15 plastic cylinders made of two identical transparent containers (6.5 cm in diameter × 8.5 cm in height). The bottom container was filled with tap water and covered by a lid bearing a hole (1.0 cm in diameter) at the centre through which we inserted the petiole of a bell pepper leaf infested with 50 3rd–5th instar psyllid nymphs. The top container had three holes, two (3 cm in diameter) covered by a metal mesh (aperture size = 0.25 mm) at the opposite sides for ventilation and one (1 cm in diameter) with a cotton wool plug between the ventilation holes for transferring wasps. A cotton wool ball (0.5 cm in diameter) saturated with 10% honey solution was placed on the lid of the bottom container to provide food for wasps. The two containers were held together with a piece Parafilm®.

At the beginning of the photophase in each room, we released four *T. triozae* females randomly collected from the colony into each plastic cylinder through the hole in the top container using an aspirator, and then plugged the hole with a cotton wool plug. We allowed the wasps to lay eggs during the first three hours of the photophase and then removed them from the cylinder and transferred them back to the colony. We collected the parasitoid pupae seven days after oviposition from each cylinder and individually placed them in glass vials (5 cm in height × 1.5 cm in diameter) in their rooms of origin. Eight days after oviposition, we observed and recorded hourly emergence rhythm of 181 and 195 pupae for three successive days in the normal light and reverse light rooms, respectively. We sexed newly emerged adults according to Vega Chávez and Valdez (2010) and calculated the developmental time of each sex (101 males and 88 females) as the time from the egg deposition to adult emergence.

Sexual maturation and circadian mating rhythm

To determine sexual maturation, we individually paired newly emerged virgin females or males with a one-day-old virgin mate in the above-mentioned glass vials immediately after lights on in the normal light room where these experimental insects originated. We observed the mating events of each pair until mating ended or lights off. We placed a cotton wool ball saturated with 10% honey solution in each vial to provide food for wasps. For each pair, we recorded the pre-mating period from pairing to mating using a stopwatch. We tested 55 males and 52 females, respectively, of which 28 males and 16 females did not mate.

To record circadian mating rhythm, we made observations in both normal light and reverse light rooms with insects from their original rooms. We individually paired one-day-old virgin males with one-day-old virgin females in the above-mentioned glass vials at the beginning of the photophase and scotophase in the normal light and reverse light rooms, respectively, and observed mating events continuously throughout the photophase and scotophase, respectively. We observed 84 and 40 pairs in the normal light and reverse light rooms, respectively, and provided 10% honey solution for each pair as described above. We used red light for observations during the scotophase.

Host feeding and oviposition rhythms

Because most *T. triozae* females begin to lay eggs when they are three day old under conditions similar to those in the present study (Rojas et al. 2015), we used four-day-old mated females for host feeding and oviposition experiments. To obtain four-day-old mated females, we individually paired one-day-old virgin wasps that fed on honey solution before pairing in the above-mentioned glass vials and observed their mating events in each room. In each light regime, we set up five cylinders, each of which held a bell pepper leaf infested with about 80 3rd–5th instar psyllid nymphs and a cotton wool ball saturated with 10% honey solution as described above. We introduced four one-day-old mated females into each cylinder and allowed them to stay for 24 h. We then transferred these females into a new cylinder with psyllids and honey solution as above using an aspirator and allowed them to stay for additional 24 h. We repeated this process until we obtained 20 females at normal and reverse light regimes, respectively, for the following experiment.

We placed a bell pepper leaf upside down with its petiole wrapped with water-saturated cotton wool in a Petri dish (8.5 cm in diameter × 2.4 cm in height) and then gently transferred 20 4th-instar psyllid nymphs onto the leaf and covered the dish with a lid. The lid had three holes, one (1 cm in diameter) covered with metal mesh for ventilation, one (1 cm in diameter) with a cotton wool plug for introducing wasps and one (0.5 cm in diameter) inserted with a cotton wool ball saturated with 10% honey solution as food for wasps. We released one 4-day-old mated female wasp obtained as above into the Petri dish through the introduction hole in the lid, plugged the hole with cotton wool and allowed her to stay for two hours (first feeding and oviposition bouts). We then transferred her to a new Petri dish with 20 fourth-instar psyllid nymphs using an aspirator and allowed her to stay for two more hours. We repeated the process until we achieved seven 2-h bouts in the photophase and five 2-h bouts in the scotophase. We tested 20 female wasps in each light regime and treated each female as a replicate. Since exuded body fluid is the evidence of host feeding and *T. triozae* females deposit their eggs under the host nymphs (Martinez et al. 2015), we counted the number of nymphs fed and then turned over all nymphs to count the number of eggs laid under a stereomicroscope (Leica MZ12, Germany) for each 2-h bout.

Statistical analysis

We carried out all analyses using SAS 9.4 (SAS Institute Inc 2019) with a rejection level set at $\alpha <$ 0.05. We used a χ^2 test (FREQ procedure) to compare differences in proportion of adult emergence between photophase and scotophase and in mating success between males and females. We used non-linear regression models (NLIN procedure) to fit the data of adult emergence, mating, host feeding and oviposition rhythms, and estimated the parameters by a standard least squares method. The estimated parameters in non-linear regression models were significantly different from 0 if both low and up 95% confidence limits (CLs) > 0 or < 0. The coefficients of determination (R^2) for regressions were calculated as the sum of square due to the model divided by the total sum of square.

An exponential decline model (Archontoulis and Miguez 2015) was applied to fit the hourly emergence (y) and mating success (y): $y = a \cdot e^{(-bx)}$, where x (≥ 1) is the hour, a is the maximum y value and b is a decrease rate of y. An asymptotic exponential model (Archontoulis and Miguez 2015) was used to fit the hourly cumulative emergence (y): $y = a\ (1-e^{(-bx)})$, where x ($\geq$ 1) is the hour, a is the maximum y value and b is an increase rate of y. The difference in each estimated parameter (a or b) in a model was compared between sexes according to Julious (2004): if the 95% CLs overlap, then there is no significant difference.

A Gaussian functional model (Archontoulis and Miguez 2015) was modified to fit the data on oviposition, which occurred exclusively during the photophase: $y = a\ e^{\{-b[\ln(x/x_0)/c]^2\}}$, where x is the hour, a is the oviposition peak at time x_0 (h) and b (default = 0.5 for the Gaussian function) and c are coefficients controlling the width of the peak. Because host feeding took place throughout 24 h cycle, we improved the above Gaussian functional model to fit the data on host feeding in the photophase: y = $1/(a\, e^{\{-b[\ln(x/x_0)/c]^2\}})$, where $1/a$ is the minimum of hosts fed at time x_0. We then used an exponential increase model to estimate the number of hosts fed in the scotophase: $y = e^{[a(x-x_0)]}$, where a is the maximum of hosts fed at time x_0.

We used a Shapiro–Wilk test (UNIVARIATE procedure) to check whether data on the pre-mating duration and developmental time followed a normal distribution. We analyzed those on pre-mating duration using an ANOVA due to their normal distribution. Because data on the developmental time of both sexes were not normally distributed even after transformation, they were analyzed using the non-parametric Kruskal–Wallis test (NPAR1WAY procedure).

Results

Sex-dependent emergence rhythm and developmental period

We show that the vast majority of emergence (91% of males and 79% of females) occurred during the photophase (x_1^2 = 58.91 and 34.47 for males and females, respectively; $P < 0.0001$) (Fig. 1a). Emergence of both sexes was highest during the early photophase and then significantly decreased (95% CLs of coefficient = 0.43 – 0.91 for males, and 0.13 – 0.22 for females) (Fig. 1a). Male emergence declined significantly faster (coefficient = 0.67) than female emergence (coefficient = 0.17) (non-overlapped 95% CLs of coefficients). Cumulative emergence reveals that 50% of males and females emerged two hours and four hours after lights on, respectively (95% CLs of coefficient = 0.33–0.60 for males, and 0.17–0.18 for females) (Fig. 1b). The cumulative emergence increased significantly faster in males (coefficient = 0.47) than in females (coefficient = 0.17) (non-overlapped 95% CLs of coefficients). Our results indicate that the developmental period from egg to adult in males (mean ± SE, 9.90 ± 0.03 days) was significantly shorter than that in females (10.12 ± 0.04 days) (x_1^2 = 49.73, $P < 0.0001$).

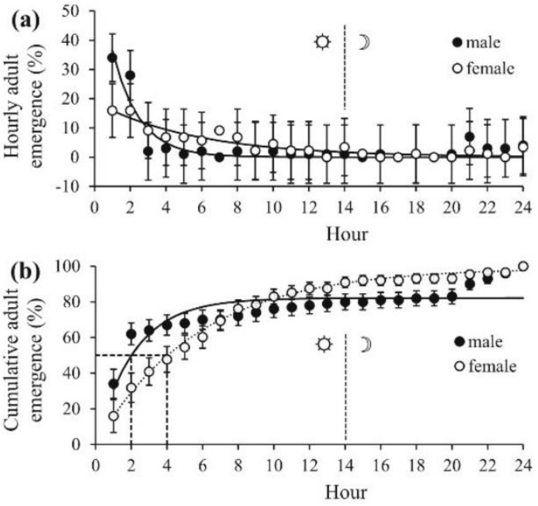

Fig. 1 Hourly (**a**) and cumulative (**b**) emergence patterns in *T. triozae* (photophase = 0–14 h and scotophase = 14–24 h). **a** Hourly emergence of males (%): $y = 71.48\ e^{(-0.67x)}$, $R^2 = 0.8754$; hourly emergence of females (%): $y = 18.97\ e^{(-0.17x)}$, $R^2 = 0.9285$. **b** Cumulative emergence of males (%): $y = 82.16(1\text{-}e^{(-0.47x)})$, $R^2 = 0.9915$; cumulative emergence of females (%): $y = 99.13(1\text{-}e^{(-0.17x)})$, $R^2 = 0.9996$; the points where the dotted horizontal and vertical lines intersect indicate 50% of cumulative emergence for males and females, respectively. Lines on dots are SE

Sexual maturation and circadian mating rhythm

Significantly more newly emerged females (69.2 ± 6.4%) mated successfully than newly emerged males (49.1 ± 6.7%) when they were offered one-day-old mates ($x_1^2 = 4.48$, $P = 0.0343$). Similarly, females became sexually receptive (1.3 ± 0.1 h) significantly earlier than males (2.3 ± 0.6 h) ($F_{1,61} = 4.18$, $P = 0.0456$).

About 70% of one-day-old adults mated during the first hour of the photophase and then mating success significantly declined within four hours into the photophase (95% CLs of coefficient = 1.18–1.55) (Fig. 2). Very few matings occurred after four hours into the photophase and during the scotophase (Fig. 2).

Host feeding and oviposition rhythms

Adult females fed on hosts throughout the day but laid eggs only in the photophase (Fig. 3). In the photophase the number of hosts fed on significantly decreased in

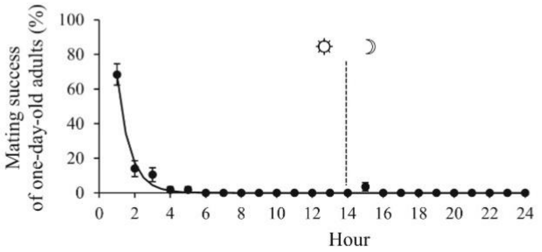

Fig. 2 Mean hourly mating success of one-day-old adults (photophase = 0–14 h and scotophase = 14–24 h): $y = 266.20\ e^{(-1.36x)}$, $R^2 = 0.9874$. Lines on bars are SE

the first three 2 h feeding bouts and then significantly increased (95% CLs of coefficient = 0.45–1.95) but in the scotophase this significantly increased over time (95% CLs of coefficient = 0.03 – 0.42). The number of eggs laid significantly increased in the first three 2 h bouts and then significantly declined (95% CLs of coefficient = 0.45–0.91).

Discussion

The present study shows that *T. triozae* adults emerged throughout the day with the vast majority of emergence occurring in the photophase, particularly during the first few hours after the onset of the photophase (Fig. 1). Such emergence patterns may be beneficial to this primarily diurnal insect because the lower temperature and higher humidity in the early morning are ideal for wing expansion, initial dispersal and search for mates with less water loss (Lankinen 1986; Fantinou et al. 1998; Marchand and McNeil 2000; Karpova 2006; McClure et al. 2007). Similar circadian emergence patterns take place in some other parasitoid wasps such as *Ascogaster reticulatus* Watanabe (Kainoh 1986), *Bathyplectes curculionis* (Thomson) (England 1995), *Aphidius ervi* Haliday (He et al. 2004) and *Eretmocerus warrae* Nauman and Schmidt (Hanan et al. 2009).

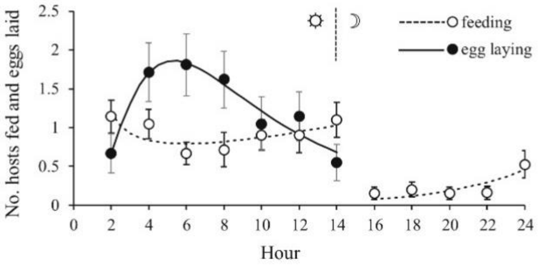

Fig. 3 Mean number of hosts fed and eggs laid by *T. triozae* females (photophase = 0–14 h and scotophase = 14–24 h): number of hosts fed in the photophase: $y = 1/(1.26\ e^{\{-0.5[\ln(x/5.86)/1.20]^2\}})$, $R^2 = 0.5117$; number of hosts fed in the scotophase: $y = e^{[0.22(x-27.46)]}$, $R^2 = 0.2323$; number of eggs laid in the photophase: $y = 1.86\ e^{\{-0.5[\ln(x/5.31)/0.68]^2\}}$, $R^2 = 0.4336$. Lines on bars are SE

Similar to some earlier studies on hymenopteran parasitoids (e.g., Kainoh 1986; He et al. 2004; Rojas et al. 2015), *T. triozae* males had significantly shorter developmental period than did females. Consequently, most males emerged significantly earlier than females (Fig. 1), indicating a protandrous nature, which is a strategy to increase male mating success and reduce the risk of female pre-reproductive death (Waage and Ming 1984; Quicke 1997; Morbey and Ydenberg 2001). Furthermore, *T. triozae* males became sexually mature significantly later than females, and when offered sexually mature one-day-old mates, females were more likely than males to mate during the photophase of their emergence. However, when we paired one-day-old wasps during the entire photophase, 95% of pairs mated during the first four hours of the photophase with $> 65\%$ of mating occurring in the first hour of the photophase (Fig. 2). These findings suggest that, unlike *A. reticulatus* where emergence occurs early in the morning and mating in the afternoon of the same day (Kainoh 1986), most *T. triozae* adults may not mate on the day of emergence, rather they are more likely to mate in the early morning of the following day when both sexes are sexually mature. This strategy may reduce mate-searching time, mating failure, and risk of predation as reported in other insects (Rymer et al. 2007) and allow time for *T. triozae* females to mature their eggs because most females begin to lay eggs in the third photophase following emergence (Rojas et al. 2015).

Adults of many parasitoid species including a congeneric species *T. radiata* (Waterston) (Gebiola et al. 2018) feed on their hosts to gain nutrients necessary for egg maturation and production (Jervis and Kidd 1986; Burger et al. 2005; Hanan et al. 2009). We show that *T. triozae* females laid eggs only in the photophase but fed on hosts throughout the day with most host feeding happening during the photophase (Fig. 3). In some parasitoids such as *Ooencyrtus kuwanai* (Howard) (Weseloh 1972) and *A. ervi* (He et al. 2004), oviposition occurs throughout the 24 h cycle while in others such as *E. warrae* (Hanan et al. 2009), host feeding and oviposition take place in both photophase and scotophase. In the photophase, host feeding and oviposition in *T. triozae* had reverse patterns where maximum oviposition and minimum host feeding happened four to eight hours into the photophase (Fig. 3). Although host feeding activity was generally low during the scotophase, it reached a peak just before its end (Fig. 3). These findings reveal a clear strategy that female *T. triozae* take to maximize their fitness, i.e. they have highest host feeding rate before and after the oviposition peak to acquire nutrients for egg production and somatic maintenance. *E. warrae* females probably use the same strategy to achieve the best fitness because their circadian oviposition and host feeding activity peaks do not overlap (Hanan et al. 2009).

In general, male harassment can reduce female fitness in insects (e.g., Sakurai and Kasuya 2008; Li et al. 2014). In the present study, we found that circadian mating (Fig. 2) and oviposition (Fig. 3) peaks mostly did not overlap in *T. triozae*, suggesting that males are not able to harass females after oviposition starts because males are active in the early morning. This clearly defined circadian activity pattern for host feeding and reproduction in *T. triozae* indicates that this parasitoid species has developed a highly adaptive strategy for maximum fitness, which may make it an effective biological control agent.

Our findings provide important knowledge for development of laboratory handling and field release programs for biological control of TPP. For example, to achieve the highest reproductive output and best control of TPP, we may allow sexually mature wasps to mate in the early morning of the next day following their emergence. Because most *T. triozae* females start ovipositing when they are three day old (Rojas et al. 2015) and host feeding is essential for egg production and maturation (Cerón-González et al. 2014), we may achieve better results if we carry out field or greenhouse release of mated and host-fed females in the early morning of the third day following emergence. Alternatively, if growers decide to release newly emerged wasps in the field or greenhouses, they may do so in the morning when adults are more active and environmental conditions are more favorable. This approach has proved to be effective in the application of another diurnal parasitoid, *G. pennsylvanicum* (Vogt and Nechols 1991). When we introduce new natural enemies to control TPP in the future, we need to ensure that their circadian oviposition and host feeding peaks do not completely overlap with those of *T. triozae* to minimize competition that may reduce biological control efficacy (Miranda et al. 2015; Ramirez-Ahuja et al. 2017). Furthermore, a recent report shows that circadian rhythms of predatory mites may vary in different seasons (Pérez-Sayas et al. 2018). It is thus worth exploring whether those of *T. triozae* differ seasonally.

Non-selective pest control measures including application of insecticides and crop production procedures such as overhead irrigation can make natural enemies ineffective. Therefore, to reduce impact on biological control agents, particularly parasitoids, the timing of these operations is important (Vogt and Nechols 1991; van Lenteren et al. 1992; Martinez et al. 2015). In the present study, we demonstrate that most emergence and mating occurred in the morning (Figs. 1 and 2), and oviposition concentrated from mid-morning to mid-afternoon and host feeding peaked in the early morning, late afternoon and dawn (Fig. 3). Therefore, we suggest that field trials be conducted to test whether insecticide sprays and overhead irrigations outside the activity peaks of *T. triozae* would reduce their impact on the parasitoid. Because *T. triozae* pupae are less susceptible to insecticides than other immature stages (Morales et al. 2018) and the population peak of *T. triozae* is 2–3 weeks behind that of TPP in the field (Butler and Trumble 2012b), the difference in the phenology of these two organisms should be taken into consideration when integrating biological control and other cultural practices. In addition, biopesticides with short residual activity and low toxicity to natural enemies (Liu and Stansly 1995; Gharalari et al. 2009; Buteler and Stadler 2011) should be considered in the integrated management program involving *T. triozae*.

In summary, most adult *T. triozae* emerge in the morning with males emerging earlier than females and females becoming sexually mature earlier than males. Most individuals mate at the very beginning of the morning of the second photophase following emergence. Oviposition only occurs during the daytime, peaking between mid-morning and mid-afternoon. Host feeding has three peaks taking place in the early morning, late afternoon and dawn, respectively. Our findings demonstrate that most mating, host feeding and oviposition activities do not overlap over the 24 h cycle, suggesting that *T. triozae* have developed strategies to perform different life functions at the particular times for maximal fitness gain.

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Compliance with ethical standards

Conflict of interest All authors declare that we have made all efforts to meet the standard and believe that we have met the standard.

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Diets for *Tamarixia triozae* adults before releasing in augmentative biological control

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Abstract The effectiveness of augmentative biological control using parasitoids often depends on their physiological state and the pest population density at the time of release. *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is a primary host-feeding parasitoid of a serious invasive pest *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). Here we investigated the effects of adult diets (honey, water, yeast, and hosts) and timing of their provision on *T. triozae* fitness and oviposition patterns, providing knowledge for enhancement of its biological control potential. Adults fed with honey for four days with no access to hosts or with water or yeast for one day followed by host feeding for three days had similar longevity and lifetime pest killing ability. Adults fed with only water for one day before release had significantly greater intrinsic rate of increase, shorter doubling time, and higher daily fecundity peak. Adults fed with honey or yeast for one day followed by host feeding for three days significantly flattened their daily oviposition curves. These findings have several implications for augmentative biological control using *T. triozae*. First, honey diet may allow at least four days for successful shipment of host-deprived adults without compromising biological control effectiveness. Second, the release of host-deprived adults with one-day water feeding may achieve rapid pest suppression when the pest population density is high. Finally, releasing host-deprived adults with one-day honey or yeast feeding followed by three-day host feeding can increase their establishment success and reduce the risk of massive removal of hosts when the pest population density is low.

Keywords Adult diet · Longevity · Reproduction · Augmentative release

Introduction

Augmentative release of natural enemies, particularly parasitoids, is one of the most widely used approaches to pest biological control in annual crops and greenhouses (van Lenteren and Bueno 2003; Hoy 2008; Amadou et al. 2019; Bueno et al. 2020; Kazak et al. 2020). It is also commonly applied to control resurgent pests resulting from disruptions of natural enemy populations by seasonal factors and harvesting activities (Hajek and Eilenberg 2018; Stenberg et al. 2021) or insecticide applications (Coppel and Mertins 1977; DeBach and Rosen 1991). Evidence shows that

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adult host feeding is important for synovigenic parasitoids (Jervis and Kidd 1986; Giron et al. 2004; Burger et al. 2005; Jervis et al. 2008; Kapranas and Luck 2008; Liu et al. 2015; Benelli et al. 2017; Hanan et al. 2017; Gebiola et al. 2018), and supplementary adult diets such as honey and honeydew (Bezemer et al. 2005; Wade et al. 2008; Hossain and Haque 2015; Benelli et al. 2017; Dong et al. 2018; Picciau et al. 2019) and yeast (Bartlett 1964; Heimpel and Rosenheim 1995) can increase parasitoid fecundity and longevity. Therefore, provision of adult parasitoid diets before release can not only improve parasitoids' ability to establish at release sites (Tena et al. 2017; Stahl et al. 2019) but also increase their biological control effectiveness after release (Hougardy et al. 2005; Hougardy and Mills 2006, 2007; Benelli et al. 2017).

Several studies report that adult diets can change parasitoids' lifetime oviposition patterns (Bai and Smith 1993; Wade et al. 2008; Hill et al. 2020). This suggests that we may be able to modify parasitoids' lifetime oviposition trajectory by tailoring their adult diets before release to achieve effective augmentative biological control under different circumstances. For example, when the pest population density is high, we may aim to achieve quick pest suppression (Karacaoğlu et al. 2018) where the parasitoids are expected to perform maximum host killing through host feeding and parasitization immediately after release. When the pest population density is low, we may want to delay their oviposition peaks and flatten their oviposition curves (Stahl et al. 2019) where the released parasitoids are expected to spread their oviposition and host feeding more evenly or move these activities toward their later life. Nevertheless, it is not clear how alteration of timing and type of diets provided for adult parasitoids before release can change their life history traits towards our advantage. It is also unclear whether such manipulation would compromise their overall pest killing ability in terms of fecundity and host feeding. This knowledge is crucial for the development of successful augmentative biological control programs using parasitoids.

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a severe invasive pest of solanaceous crops such as potatoes, tomatoes, eggplants and peppers in the USA, Mexico and New Zealand (Cranshaw 1994; Teulon et al. 2009; Crosslin et al. 2010; Butler and Trumble 2012; Rojas et al. 2015), causing significant economic losses. It has also invaded Australia, Canada and Central and South America where its economic importance is under evaluation (FAO 2017; WADPIRD 2018; Castillo Carrillo et al. 2019; Olaniyan et al. 2020). *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) is an important synovigenic parasitoid of TPP (Bravo and López 2007; Rojas et al. 2015; Yang et al. 2015). It kills the pest by both host feeding and parasitization (Martinez et al. 2015). However, it is still unknown how provision of different adult diets and temporary deprivation of hosts could affect reproductive fitness and lifetime feeding and oviposition patterns in *T. triozae*. This information is of vital importance for development of strategies to optimize augmentative biological control of TPP using *T. triozae*.

In the present study, we tested the effects of adult diets with or without temporary host deprivation on survival, lifetime fecundity, host feeding, oviposition patterns and life table parameters in *T. triozae*. Knowledge presented in this work could help enhance the success of augmentative biological control using *T. triozae*.

Materials and methods

Insects

We obtained *B. cockerelli* and *T. triozae* from Bio-Force Limited, Auckland, New Zealand, and established the colonies in the laboratory. We maintained the psyllid colony on five two-month-old bell pepper plants, each of which was grown in a pot (9.5 cm in height × 10.5 cm in top diameter × 8.5 cm in bottom diameter) with potting mix. The potted plants were placed in an aluminium-framed cage (43 × 42 × 40 cm) with a fine metal mesh (aperture size = 0.25 × 0.25 mm) on the back and both sides and Perspex on the top and front and aluminium alloy on the bottom. We kept the parasitoid colony on the 3rd–5th instar psyllid nymphs feeding on five potted plants in another aluminum-framed cage of the same size. We provided 10% honey solution in a plastic tube (7.5 cm in length × 1 cm in diameter) fitted with cotton wick (3.5 cm in length × 1 cm in diameter) for parasitoid adults as supplementary food in the cage. To ensure the psyllid colony was parasite-free for experiments, we kept psyllid and parasitoid colonies separate in two climate rooms at 25 ± 1 °C, 40–60% RH and a L:D 14:10 photoperiod. We carried out all experiments under the above environmental conditions.

We transferred about 400 psyllid adults from the colony onto four two-month-old un-infested pepper plants in an aforementioned cage for oviposition. We transferred the infested plants into another cage and replaced them with the same number of un-infested plants once every two days. We obtained a total of 84 infested plants, observed them daily, and collected the 4th instar nymphs for experiments. We used transparent plastic cylinders consisting of two identical containers for production of experimental parasitoids. Briefly, we transferred about 100 fourth instar nymphs onto a bell pepper leaf, inserted the leaf petiole through a hole (1.0 cm in diameter) in the lid of a container (6.5 cm in diameter × 8.5 cm in height) filled with tap water. We then introduced five female wasps from the colony to the infested leaf and covered the container with another container upside down. We allowed these wasps to oviposit in the cylinder for 24 h before removing them. The top container had two holes (3 cm in diameter) covered by a metal mesh (aperture size = 0.25 mm) at the opposite sides of the wall for ventilation. We collected parasitoid pupae seven days later and placed them individually in glass vials (5 cm in height × 1.5 cm in diameter) plugged with cotton wool until emergence. We set up 20 such cylinders to obtain sufficient wasps for experiments.

Treatments

To determine how adult diets and temporary host deprivation affected parasitoid longevity, lifetime fecundity, daughter production, oviposition and host feeding patterns, and life table parameters, we carried out two phases of treatments. In the first phase, we provided one of the following three diets for wasps: (1) Honey—10% honey solution made of 10 g honey + 90 ml distilled water, (2) Yeast—5% yeast solution consisting of 5 g yeast extract + 95 ml distilled water, and (3) Water—distilled water only. For each diet treatment, we individually introduced 60 newly emerged female and 60 newly emerged male wasps into glass vials (5 cm in height × 1.5 cm in diameter). Each vial was provided with a diet saturated in a cotton ball (0.5 cm in diameter) and plugged with cotton wool. Because one-day-old wasps can successfully copulate within one hour after encountering a mate (Chen et al. 2020), we kept wasps in their vials for 24 h, and then individually paired males and females that fed on the same diet in glass vials and allowed 2 h for mating to occur. We obtained 28, 30, and 28 mated pairs that fed on honey, yeast, and water diets, respectively, for the second phase of treatments.

In the second phase, we divided the above pairs into two groups and maintained them in one of the following two conditions: (1) no host deprivation (+ Host)—each pair was released into a Petri dish (8.5 cm in diameter × 2.4 cm in height) containing the same adult diet saturated in a cotton ball and 24 4th instar psyllid nymphs feeding on a bell pepper leaf (5 cm in diameter, upside down) with its petiole embedded in water-saturated cotton wool and wrapped with parafilm. The lid of the Petri dish had two holes (1 cm in diameter), one plugged with a cotton wool for introducing wasps and one covered with metal mesh for ventilation. We allowed the pair to stay in the dish for 24 h, and then transferred them to another dish with the same diet and fresh hosts once every day until both male and female died. We replaced the adult diet with fresh one once every day. (2) Temporary host deprivation (− Host)—each pair was released into a Petri dish of the same size containing the same adult diet but no hosts. Three days later (live wasps were now four days old), we provided them with the same adult diet and 24 4th instar psyllid nymphs as above and allowed each pair to stay in the Petri dish for 24 h. We then transferred the pair into another Petri dish with the same adult diet and fresh hosts once every day until both male and female died. We replaced the adult diet with fresh one once every day. We tested 15, 13, 13, 13, 17 and 15 pairs for treatments Honey + Host, Yeast + Host, Water + Host, Honey − Host, Yeast − Host and Water − Host, respectively.

Effect of adult diets and temporary host deprivation on survival, reproduction, host feeding and life table parameters

We started recording daily survival of *T. triozae* adults after we transferred one-day-old, mated wasps to Petri dishes. We commenced to count the daily number of hosts fed and parasitized one day after hosts were provided, i.e., when wasps were two days old for + Host treatments and five days old for − Host treatments. We did not record reproduction data for treatments Yeast − Host and Water − Host because all females died before we provided them with hosts. We examined host feeding and parasitism under a stereomicroscope (Leica MZ12, Germany). Host feeding was identified by bleeding that occurred from the nymph (Martinez et al. 2015) or an inverted V-shape mark on the hollowed body (Morales et al. 2013). Because *T. triozae* females deposit eggs under the nymphs (Martinez et al. 2015), we turned all nymphs over and placed them on the surface of 1% agar to determine parasitism. We then transferred all parasitized nymphs from each dish onto a fresh bell pepper leaf in an above-mentioned plastic cylinder. We collected the parasitoid pupae seven days after parasitization and individually placed them in above-mentioned glass vials plugged with cotton wool until emergence. We recorded the developmental time from egg to adult and sex of each emerged adult. We used the data collected for life table calculations.

Statistical analysis

We conducted all data analyses using SAS software (SAS 9.3, SAS Institute Inc., NC, USA). We analyzed the data on adult survival using the Kaplan–Meier method. We compared the survival curves between treatments with a non-parametric Wilcoxon test (LIFETEST procedure). Data on lifetime host feeding were normally distributed (Shapiro–Wilk test, UNIVARIATE procedure) and analyzed using an ANOVA (GLM procedure) followed by a Tukey's Studentized (HSD) range test for multiple comparisons. Data on lifetime fecundity were not normally distributed even after transformation, and thus analyzed using a Kruskal–Wallis test.

We developed two Gaussian functional models according to Archontoulis and Miguez (2015): $y = a\, e^{(-0.5\{[(x-c)-(x_0-c)]/b\}^2)}$ to fit the daily host feeding and $y = a\, e^{(-0.5\{\ln[(x-c)/(x_0-c)]/b\}^2)}$ to fit the daily oviposition and daughter production for each treatment, where x is the age of female wasps (days), a is the peak at time x_0 (wasp age), b is the coefficient controlling the width of the peak, and c is the period (days) before the females were exposed to hosts (i.e., $c = 1$ and 4 days for the + Host and − Host treatments, respectively). These models allowed us to compare the host feeding and reproduction patterns between treatments. If the 95% CLs of a given parameter overlap, then there is no significant difference between treatments.

To estimate how adult diets and temporary host deprivation affected population growth, we calculated the life table parameters (Jervis et al. 2005) using the data on daily survival and daughter production of each *T. triozae* female. The intrinsic rate of increase (r, daughters per female per day) was calculated by solving the Lotka-Euler equation: $\sum e^{-rx} \mathrm{l}_x \mathrm{m}_x = 1$, where x is the pivotal age, l_x is the proportion of females surviving to age x, and m_x is the number of daughters produced per female at age x. Other life table parameters included the net reproductive rate ($R_0 = \sum \mathrm{l}_x \mathrm{m}_x$, daughters per female per generation), doubling time [$D_t = \log_e(2)/r$, days] and generation time [$T = \log_e(R_0)/r$, days]. For each treatment, we used the bootstrap method with 100,000 bootstrap samples to calculate the pseudo-values of life table parameters (Huang and Chi 2012; Yu et al. 2013). We then employed a paired-bootstrap test (MULTTEST procedure) for multiple comparisons between treatments (Efron and Tibshirani 1993; Mou et al. 2015; Reddy and Chi 2015). Because multiple comparisons raise the Type I error (Noble 2009), the overall P value was adjusted by the Bonferroni correction (MULTTEST procedure) (Gravandian et al. 2022).

Results

Effect of adult diets and temporary host deprivation on survival and lifetime reproductive outputs

Our results reveal that most wasps in treatments Yeast − Host and Water − Host died within two days after emergence. These wasps lived significantly shorter than those in other treatments ($x_5^2 = 94.56$ and 93.35 for females and males, respectively; $P < 0.0001$) (Fig. 1). Wasps in other treatments including either hosts or honey lived for about 21 days (Fig. 1), where females had similar survival curves and males in treatment Honey + Host lived significantly longer. As shown in Fig. 2, female wasps in treatments Honey + Host, Yeast + Host, Water + Host and Honey − Host had similar lifetime host feeding and fecundity ($F_{3,50} = 0.36$, $P = 0.7802$ for host feeding; $x_3^2 = 2.97$, $P = 0.3961$ for fecundity).

Treatments significantly affected all life table parameters measured in this study (Table 1). Wasps in

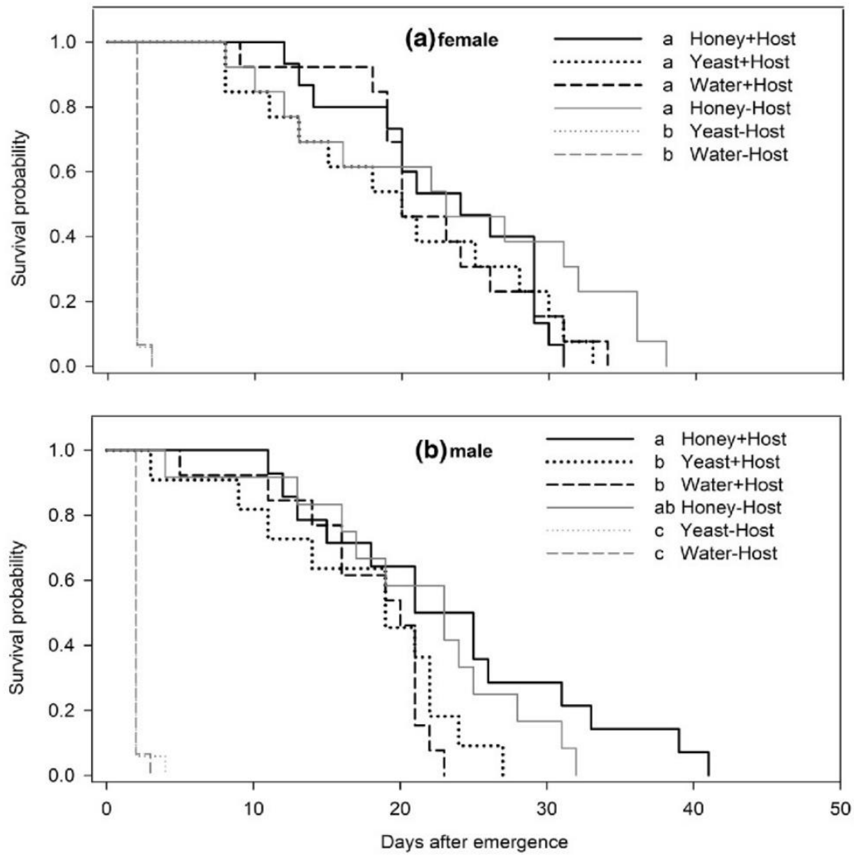

Fig. 1 Survival probability of *Tamarixia triozae* females (**a**) and males (**b**) feeding on different diets with (− Host) and without temporary host deprivation (+ Host). Treatments followed by different letters are significantly different ($P < 0.05$)

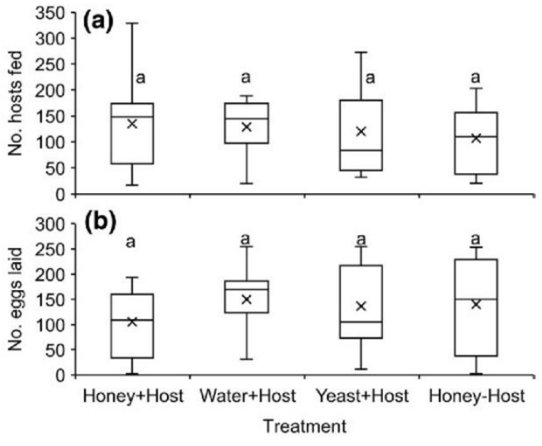

Fig. 2 Number of hosts fed (**a**) and eggs laid (**b**) by *Tamarixia triozae* females in different treatments. Each box plot shows the mean (×), median line, the upper and lower quartiles (i.e., the range where 25% of scores fall above and 25% fall below the median), and scores outside the 50% middle (upper '⊤' and lower '⊥'). Columns followed by the same letters are not significantly different ($P > 0.05$)

treatment Honey − Host had greatest net reproductive rate (R_0) and longest generation time (T) while those in treatment Water + Host had highest intrinsic rate of increase (r) and shortest doubling time (D_t).

Effect of adult diets and temporary host deprivation on lifetime host feeding and reproductive patterns

When we provided one-day-old wasps with hosts immediately after their mating, daily host feeding (Fig. 3a) peaked between eight and 13 days after emergence, and daily fecundity (Fig. 3b) and daughter production (Fig. 3c) peaked between five and eight days after emergence. Host deprivation for three days after mating delayed the peaks of daily host feeding, fecundity and daughter production for three to four days (Fig. 3). There was no significant difference in the width of daily feeding (Fig. 3a) and daughter production (Fig. 3c) peaks between treatments. However, daily fecundity peak in Yeast + Host

Table 1 Effects of different diets with (−Host) or without (+Host) temporary host deprivation on life table parameters (mean ± SE)

Diet	R_0 (daughters per female per generation)	r (daughters per female per day)	T (days)	D_t (days)
Honey + Host (H)	16.85 ± 0.19d	0.1596 ± 0.0008d	17.70 ± 0.08b	4.35 ± 0.02a
Yeast + Host (Y)	19.91 ± 0.30c	0.1797 ± 0.0007b	16.64 ± 0.04d	3.86 ± 0.02c
Water + Host (W)	26.43 ± 0.34b	0.1909 ± 0.0001a	17.14 ± 0.06c	3.63 ± 0.00d
Honey − Host (H1)	30.91 ± 0.56a	0.1705 ± 0.0007c	20.11 ± 0.06a	4.07 ± 0.02b
$P_{(Bon.)}$	< 0.0378	< 0.0001	< 0.0001	< 0.0001

Estimated values in columns followed by different letters are significantly different based on the paired-bootstrap test with Bonferroni correction

$P_{(Bon.)}$ Bonferroni corrected P value

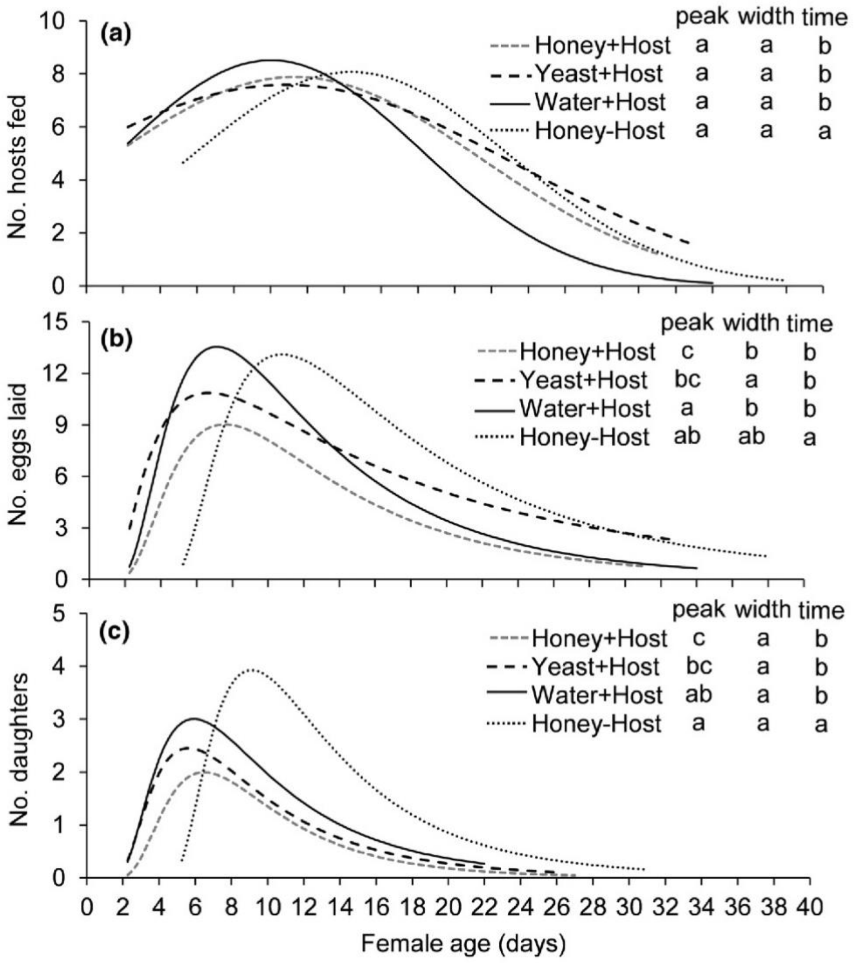

Fig. 3 Daily number of hosts fed (**a**), eggs laid (**b**) and daughters produced (**c**) in *Tamarixia triozae* females that fed on different diets with or without host deprivation. Diet + Host = both adult diets and hosts were provided after mating, and Diet − Host = adult diets were provided but hosts were unavailable for three days after mating. For each parameter, treatments followed by the same letters in a column are not significantly different (overlapped 95% CLs of coefficients)

was significantly wider than in Honey + Host and Water + Host (Fig. 3b). Treatment had no significant effect on the height of daily host feeding peaks (Fig. 3a). Daily fecundity peak in Water + Host was significantly higher than in Honey + Host and Yeast + Host, and that in Honey − Host significantly higher than in Honey + Host (Fig. 3b). Daily daughter production peak was the highest in Honey − Host (Fig. 3c). Detailed statistical data are provided in Supplementary Table S1.

Discussion

In augmentative release programs, entomologists sometimes ship parasitoid pupae or adults (e.g., van Lenteren and Tommasini 1999; Yokoyama et al. 2010; Cancino and López-Arriaga 2016) from their production sites to the release sites in the absence of hosts, which can last days, depending on distance and transport methods. Like other synovigenic parasitoids (e.g., Giron et al. 2004; Liu et al. 2015; Gebiola et al. 2018), adult feeding on honey and/or hosts was essential for the survival of *T. triozae* because carbohydrates in these diets provided energy to prolong their longevity (e.g., Jervis et al. 2008; Picciau et al. 2019). However, our results do not support previous findings about the beneficial effect of yeast feeding on parasitoid fitness (Bartlett 1964; Heimpel and Rosenheim 1995) because *T. triozae* adults died in about two days if they only fed on yeast solution (Yeast − Host) or water (Water − Host) after emergence. Our findings suggest that honey diet should be provided for *T. triozae* adults immediately after emergence regardless of whether hosts are present, or for pupae during shipment to ensure newly emerged adults can get access to honey food in the absence of hosts.

We demonstrate that adult diets and temporary host deprivation altered daily feeding and oviposition patterns of *T. triozae* females. This information can be used to develop strategies for TPP biological control. For example, we could have four days to successfully ship honey-fed and host-deprived females from production to release sites without compromising their longevity and lifetime host-killing ability. Wasps in treatments Water + Host and Honey − Host had highest fecundity peaks, suggesting that when the pest density is high, we can quickly suppress the pest population by releasing wasps that have fed on water for 24 h or on honey for four days in the absence of hosts. The current study shows that wasps that fed on honey or yeast without temporary host deprivation (Honey + Host and Yeast + Host) flattened their daily oviposition curves, which may reduce the risk of massive removal of hosts and increase establishment success of wasps in the field when the pest population density is low (Eggenkamp-Rotteveel Mansveld et al. 1982). Furthermore, wasps that fed on honey for four days without access to hosts (Honey − Host) had highest peak of daughter production, suggesting that soon after release to the field (hosts become available), these wasps are not only able to quickly suppress the pest population but also increase their own.

In the absence of hosts for four days after emergence, honey-fed wasps had longest generation time (T) and relatively low intrinsic rate of increase (r) and long doubling time (D_t) due to delayed oviposition. However, these host-deprived and honey-fed adults achieved greatest net reproductive rate (R_0), i.e., produced highest number of daughters, promoting future population growth (Sabbatini Peverieri et al. 2012). These findings suggest that treatment Honey − Host can lead to better and more sustainable control when the pest density is low at the time of release. On the other hand, wasps in treatment Water + Host had the highest r and shortest D_t, the second highest R_0 and relatively short T. Therefore, if we aim to achieve immediate control of the pest when its population density is high, we should feed newly emerged wasps with water for 24 h and then immediately release them into the infested crops. The released wasps can kill more pest individuals within a shorter time period and quickly build up their own population. The life table parameters also provide references for producers to adjust their mass-rearing programs depending on whether they want to yield more parasitoids quickly or to slow production down.

In conclusion, adult diets can affect survival and reproductive patterns of *T. triozae*. In the absence of hosts at emergence, wasps feeding on water or yeast diet cannot live for more than two days, which is not long enough for successful shipment. However, adult *T. triozae* feeding on honey solutions for four days or other diets for one day followed by provision of hosts can survive for about 21 days with similar lifetime pest killing ability. Furthermore, wasps feeding on water for one day before access to hosts have greater intrinsic rate of increase, shorter doubling time, and higher daily fecundity peak while those fed with honey or yeast for one day followed by host feeding for three days flatten their daily oviposition curves. These findings have three implications: (1) honey diet can allow at least four days for successful shipment of host-deprived adults without compromising host-killing ability; (2) releasing host-deprived wasps with one-day water feeding can achieve rapid pest suppression when the pest population density is high, and (3) releasing host-deprived adults with one-day honey or yeast feeding followed by three-day host feeding can increase the establishment success and reduce the risk of massive removal of hosts when the pest population density is low. These implications can be tested under field conditions.

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Author contributions CC, XZH and QW conceived and designed the study; CC and PZ collected the data. All author contributed to data analysis and manuscript preparation. All authors read and approved the manuscript.

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Data availability The datasets from the current study are available from the corresponding author on request.

Declarations

Conflict of interest All authors declare that they have no conflicts of interest.

Research involving human participants and/or animals No humans and/or animals were used in this study that required informed consent or submission to animal welfare committee for evaluation.

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Life history and behavior of *Tamarixia triozae* parasitizing the tomato-potato psyllid, *Bactericera cockerelli*

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HIGHLIGHTS

- *Tamarixia triozae* preferred early instar hosts for feeding, and later ones for parasitism.
- Most feeding and parasitism occurred in the week after emergence and declined sharply after two weeks.
- Progeny developing in fourth and fifth instar nymphs were male-biased and female-biased, respectively.
- Offspring developing in later host instars were larger with higher egg loads.

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ABSTRACT

Tamarixia triozae is an important primary parasitoid of the tomato-potato psyllid, *Bactericera cockerelli,* a serious cosmopolitan pest of solanaceous crops. However, without better information about its life history and behavior, it will be difficult to use this parasitoid in effective biological control programs. We carried out a series of experiments to characterize its parasitism, adult feeding, and oviposition behaviors and its sex allocation in response to different life stages of its host, and their fitness consequences. We show that *T. triozae* females fed on all host instars with a preference for mid-aged ones, and preferred to parasitize later instars, thus inflicting mortality on all instars simultaneously. Host feeding and parasitism peaked during the first week of female life and declined markedly after two weeks. Parasitoids allocated more fertilized eggs to older and larger nymphs, and superparasitism declined with increasing host density. The oviposition rate of fertilized eggs peaked when females were four to five days old, with $>$ 90 % of daughters produced during the first half of adult life. The body size and egg loads of progeny increased with increasing host instar at parasitism, demonstrating a positive relationship between the host size and offspring fitness.

1. Introduction

The tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a serious pest of solanaceous crops native to the United States and Mexico, causing significant losses (Cranshaw, 1994; Crosslin et al., 2010; Butler and Trumble, 2012; Rojas et al., 2015). It has invaded New Zealand, Australia, Canada, and Ecuador, where the assessment of its economic impact is under way (Teulon et al., 2009; Ogden, 2011; FAO, 2017; WADPIRD, 2018; Castillo Carrillo et al., 2019; Olaniyan et al., 2020). The psyllid damages plants by feeding and transmission of zebra chip and other diseases (Munyaneza et al., 2008; Butler and Trumble, 2012; Rojas et al., 2015; Chen et al., 2020). To date, insecticides are still the main tactic for the control of this pest in the field (Munyaneza, 2012; Martinez et al., 2015; Wright et al., 2017). *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae), which has recently been introduced from Mexico to New Zealand for TTP control (Barnes, 2017), is an important primary ectoparasitoid of the pest (Rojas et al., 2015; Yang et al., 2015). It has a higher population growth potential than its host (Rojas et al., 2015) and about 80 % parasitism rate (Bravo and López, 2007). Furthermore, it is a synovigenic wasp, killing its hosts by both parasitization and host feeding (Martinez et al., 2015; Rojas et al., 2015; Chen et al., 2020, 2022). It can overwinter successfully and establish self-sustaining populations in New Zealand (Anderson, 2020). This parasitoid can thus be highly effective in TTP control, particularly

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in greenhouse crops.

Parasitoids foraging on host plants normally encounter hosts of different stages and sizes. The optimal foraging theory predicts that parasitoid females should adopt the host selection strategies that maximize their lifetime fitness gain (Goubault et al., 2003; Danchin et al., 2008). Many studies show that parasitoids gain better reward in offspring fitness by parasitizing larger hosts because these hosts provide more resources for their offspring (e.g., Liu et al., 2011; Hanan et al., 2015; Luo et al., 2022). However, *T. triozae* neither feed on parasitized hosts nor parasitize fed ones (Cerón-González et al., 2014; CC pers. observ.), suggesting that they must have developed a host partitioning strategy for feeding and parasitization to maximize their offspring's and their own fitness. For example, like other host-feeding parasitoid species (e.g., Kidd and Jervis, 1991; Choi et al., 2001; Ebrahimifar and Jamshidnia, 2022), *T. triozae* females may select smaller hosts for feeding and larger ones for oviposition. Although several authors have examined host stage selections in this parasitoid (Morales et al., 2013; Yang et al., 2015; Ramírez-Ahuja et al., 2017), it is still not clear how *T. triozae* females partition their hosts of different stages for feeding and parasitization when *B. cockerelli* nymphs of all instars are present simultaneously, which is the most likely scenario in the field. This knowledge may contribute to better understanding of host selection for feeding and ovipositon and evaluation of biological control effectiveness of the parasitoid.

Due to the nature of haplodiploidy, female parasitic wasps can control offspring sex ratio by adjusting the proportion of fertilized eggs at oviposition (King, 1988). Previous studies show that they usually allocate more fertilized eggs to larger hosts (e.g., Charnov et al., 1981; Favaro et al., 2018; Pérez-Rodríguez et al., 2019) because their offspring developing from larger hosts have larger body size (e.g., Hanan et al., 2015; Luo et al., 2022) and higher reproductive fitness (e.g., Hanan et al., 2015; Khatri et al., 2016). However, it is not clear how *T. triozae* females allocate the sex of their offspring based on the host stage they attack and whether host stage at parasitization has any effect on their offspring fitness. This knowledge can help design mass rearing programs for effective parasitoid production. Furthermore, although only one offspring survives on each parasitized host, *T. triozae* females often deposit more than one egg under a host (Rojas et al., 2015). To date, it is unknown whether their egg and sex allocation to each host varies with the host stage and density, making it difficult to understand the mechanisms behind their superparasitism.

Parasitoids of different ages may adjust their daily feeding, oviposition and sex allocation patterns in response to host stage, knowledge of which is currently lacking for *T. triozae* but important for the development of effective pest control programs and decision on the timing of releases. In some parasitoids, younger females may have maximal feeding (Zhang et al., 2014) and oviposition (Wade et al., 2008; Bodino et al., 2019), achieving greatest pest suppression soon after release to the field but these activities decline when they are older, reducing biocontrol effectiveness. Mated females may maximize the use of sperm and produce more daughters in their early life (Latham and Mills, 2010; Mawela et al., 2021) to ensure the survival and persistence of their next generation in the field while this reproductive feature may lead to sperm depletion over age, reducing daughter production in their later life (e.g., Pérez-Lachaud and Hardy, 1999; Santolamazza Carbone et al., 2007; Hu et al., 2012).

The aim of this study was to determine parasitoid age- and host stage-dependent host feeding, parasitization and sex allocation and their fitness consequences in *T. triozae*. We exposed mated females of *T. triozae* to *B. cockerelli* nymphs of all instars daily, recorded their lifetime feeding, parasitization, superparasitism and sex allocation on each instar, and established their offspring fitness. Knowledge generated here provides essential information for the development of effective rearing and releasing programs for the control of TTP.

2. Material and methods

2.1. Breeding colony and environmental conditions

We established the colonies of *B. cockerelli* and *T. triozae* using adults from BioForce Limited, Auckland, New Zealand. We utilized the bell pepper (*Capsicum annuum* L.) as host plants for colony maintenance and experiments. To ensure the psyllid colony was parasite-free, we kept psyllid and parasitoid colonies separate in two climate rooms. We reared psyllids on five 2-month-old plants, each in a pot (9.5 cm in height × 10.5 cm in top diameter × 8.5 cm in bottom diameter) with potting mix, in an aluminium-framed cage (43 × 42 × 40 cm) with a metal mesh (aperture size = 0.25 mm × 0.25 mm) on the back and both sides and Perspex on the top and front and aluminium alloy on the bottom. We maintained the parasitoid colony on the 3rd–5th instar psyllid nymphs feeding on five potted, 2-month-old plants in another aluminium-framed cage of the same size. Colonies were maintained and experiments carried out at 25 ± 1 °C, 40–60 % RH and a photoperiod of 14L:10D hours.

2.2. Preparation of insects for experiments

To obtain psyllids of different instars (1st–5th instars) for experiments, we randomly collected about 200 male and female adults from the colony, released them onto two potted, clean, 2-month-old pepper plants in an aluminium-framed cage mentioned above. We allowed adults to lay eggs for 24 h, and then removed all adults from the infested plants using an aspirator and transferred those plants into a nylon mesh-framed cage (65 × 50 × 50 cm, aperture size: 0.075 mm × 0.075 mm). We placed two clean plants of similar conditions into the aluminium-framed cage and repeated the above process daily until we obtained sufficient nymphs of desirable instars for experiments. During this period, we examined the aluminium-framed cage daily and added new adults from the colony to assure there were about 200 adults in the cage. When the nymphs reached the 5th instar on the first two infested plants in the nylon mesh-framed cage, we harvested all nymphs from infested plants in all cages to obtain nymphs of different instars for experiments. We determined nymph instars under a stereomicroscope (Leica MZ12, Germany) according to Vega-Chávez (2010).

To acquire parasitoids for experiments, we randomly collected five females of *T. triozae* from the colony, released them into a plastic cylinder with a bell pepper leaf infested by 100 4th-instar psyllid nymphs and allowed them to stay in the cylinder for 24 h. The cylinder consisted of two identical transparent containers (6.5 cm in diameter × 8.5 cm in height). The bottom container was filled with tap water and covered by a lid bearing a hole (1.0 cm in diameter) at the centre through which we inserted the petiole of the infested leaf. The top container had two holes (3 cm in diameter) covered by a metal mesh at the opposite sides of the wall for ventilation. We set up a total of 10 such cylinders. We collected parasitoid pupae seven days after oviposition and individually placed them in glass vials (5 cm in height × 1.5 cm in diameter) plugged with cotton wools until adult emergence. We individually introduced the newly emerged wasps to the glass vials with a cotton ball saturated with 10 % honey solution as food and maintained them there for 24 h. Because both sexes become sexually mature on the day they emerge (Chen et al., 2020), we individually paired 1-day-old virgin females and males in the glass vials with honey food until mating ended. Mating usually occurs within 90 mins after pairing and lasts about 15 min (CC pers. observ.).

2.3. Effect of host stage on lifetime host feeding and parasitization

To determine lifetime parasitism and host feeding in relation to host stage, we transferred 30 psyllid nymphs of five different instars (6 individuals × 5 instars) onto a pepper leaf with its petiole embedded in water-saturated cotton wool wrapped with parafilm in a Petri dish (8.5 cm in diameter × 2.4 cm in height). We covered the dish with the lid bearing two holes (1 cm in diameter), one covered with metal mesh for ventilation and one with a cotton wool plug for introducing wasps. We introduced a 1-d-old mated female parasitoid into the dish and allowed it to stay for 24 h. We then transferred the wasp to another dish with a leaf and 30 fresh psyllid nymphs as above and allowed it to stay for 24 h. We repeated this process until the wasp died, which lasted about 20 days. During the experiment, psyllid nymphs were the only food for the wasp and each wasp was exposed to more than 600 nymphs in its lifetime. A cotton ball saturated with water was provided to the wasp and refreshed every day. We tested 18 female wasps, each of which was considered a replicate.

For each replicate, we examined all dishes after removing the wasp and recorded the number and instar of hosts fed and parasitized, and number of eggs laid under the stereomicroscope. Host feeding was determined by the presence of body fluid (Martinez et al., 2015) or an inverted V-shape mark on the hollowed body (Morales et al., 2013). During oviposition female wasps paralyse psyllid nymphs and then deposit eggs on the ventral side of the hosts and secret adhesive substance to attach the eggs to the host cuticle (Martinez et al., 2015). Therefore, we turned over all host nymphs and put them on the surface of 1 % agar to determine oviposition and counted the number of eggs laid under each parasitized nymph.

2.4. Effect of host stage at parasitization on parasitoid offspring fitness

We separated the parasitized nymphs according to their instars in each dish. We then transferred those of the same instar onto a randomly selected leaf on a potted, 2-month-old pepper plant in a nylon mesh-framed cage. Immediately after the transfer of psyllid nymphs, we enclosed the leaf with a transparent resealable plastic bag (17.5 cm in length × 10 cm in width) bearing small holes made by insect pins for ventilation and filled the space between the leaf petiole and the zip seal of the bag with cotton wool. Parasitoid pupae were collected seven days after parasitism and individually placed in the glass vials plugged with cotton wool until emergence. For a given host instar, the emergence rate was calculated as the number of emerged offspring divided by the number of hosts parasitized. The developmental time from egg to adult emergence and sex of emerged wasps were recorded.

To measure the effect of host instar at parasitization on the body size of parasitoid offspring at emergence, we randomly sampled 19 and 30 newly emerged females from the 4th and 5th instar nymphs (no females emerged from the 3rd instar – see Results), and 12, 30 and 28 newly emerged males from the 3rd, 4th and 5th instar nymphs (no 1st and 2nd nymphs were parasitized – see Results), respectively. We measured the hind tibial length of these adults as an index of body size (Khatri et al., 2016) under the stereomicroscope. To determine the egg load of newly emerged females, after tibial measurement we dissected these females in 70 % alcohol on a slide under the stereomicroscope. Because immature eggs absorb the stain while mature eggs prevent the stain (Edwards, 1954), we added one droplet of stain (1 % acetocarmine) into the alcohol. The ovaries were covered with a slide cover, spread by gently pressing 3–5 min after staining and eggs counted under the stereomicroscope. The number of mature eggs in the ovaries was recorded as egg load at emergence.

2.5. Statistical analysis

We performed all analyses using SAS 9.4 (SAS Institute Inc., NC, USA). Because our data were not normally distributed (Shapiro-Wilk test, UNIVARIATE Procedure), we used a generalized linear model (GLIMMIX procedure) to analyze them with the instar as a fixed factor and the replicate as a random factor in the model. A Gamma distribution and a log function were applied to the number of eggs laid under each parasitized nymph, developmental time and hind tibial length; a Poisson distribution and a log function to the lifetime number of hosts fed and parasitized, number of eggs laid, and egg load, and a Binomial distribution and a *logit* function to the emergence rate and proportion of daughters produced. A Tukey-Kramer test was used for multiple comparisons between the instars.

According to Archontoulis and Miguez (2015), we developed two Gaussian functional models, $y = a\,e^{(-0.5\{[(x-c)-(x_0-c)]/b\}^2)}$, to fit the daily host feeding on nymphs of different instars, and $y = a\,e^{(-0.5\{\ln[(x-c)/(x_0-c)]/b\}^2)}$, to fit the daily number of parasitism on and daughter emergence from nymphs of different instars, where x is the age of female wasps (days), a is the peak at age x_0, b is the coefficient controlling the width of the peak, and c (=1 day) is the time before the females got access to hosts. The difference in each estimated parameter in the Gaussian functional models was compared between treatments: if the 95 % confidence limits (CLs) overlap, then there is no significant difference. We did not fit the data on host feeding for the 1st and 5th instar nymphs, and on parasitism and daughter emergence for 3rd instar nymphs because only a few 1st and 5th instar nymphs were fed, few 3rd instar nymphs were parasitized, and no daughter emerged from the 3rd instar nymphs.

3. Results

3.1. Effect of host stage on lifetime host feeding and parasitization

The female parasitoids fed on hosts of all instars but significantly preferred the 2nd, 3rd and 4th to the 1st and 5th instar nymphs for feeding with the highest number of the 3rd instar nymphs fed ($F_{4,68} = 241.19$, $P < 0.0001$) (Fig. 1a). They significantly preferred the 4th and 5th to the 3rd instar nymphs for oviposition with the highest number of parasitism and eggs laid on the 5th instar nymphs ($F_{2,34} = 213.56$, $P < 0.0001$ for parasitism; $F_{2,34} = 314.12$, $P < 0.0001$ for eggs laid) but did not parasitize the 1st and 2nd instar nymphs (Fig. 1b). Furthermore, the parasitoids deposited significantly more eggs under the 4th and 5th instar nymphs (1.34 and 1.47 eggs per nymph, respectively) than under the 3rd instar nymphs (1.11 eggs per nymph) ($F_{2,27} = 6.46$, $P = 0.0051$).

Daily host feeding and parasitization patterns varied with host stage and parasitoid age (Fig. 2, Table S1). Daily number of hosts fed by parasitoids on the 3rd and 2nd instar nymphs peaked about six and seven days after emergence, respectively, significantly earlier than that

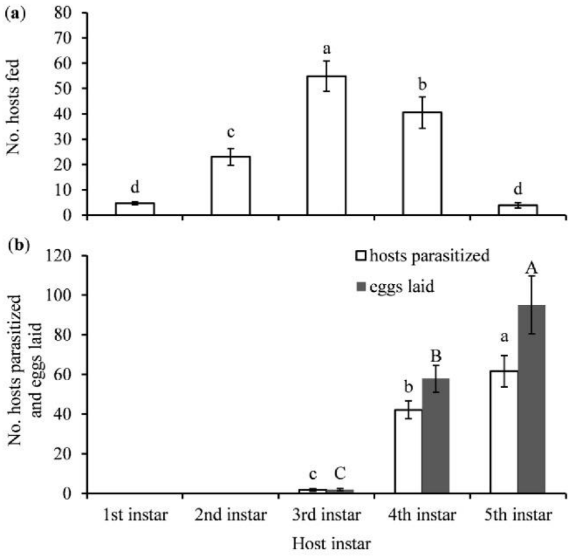

Fig. 1. Mean (±SE) lifetime number of hosts fed (**a**), parasitized and eggs laid (**b**) by *T. triozae* on *B. cockerelli* nymphs of different instars. Columns with different letters are significantly different (P < 0.05).

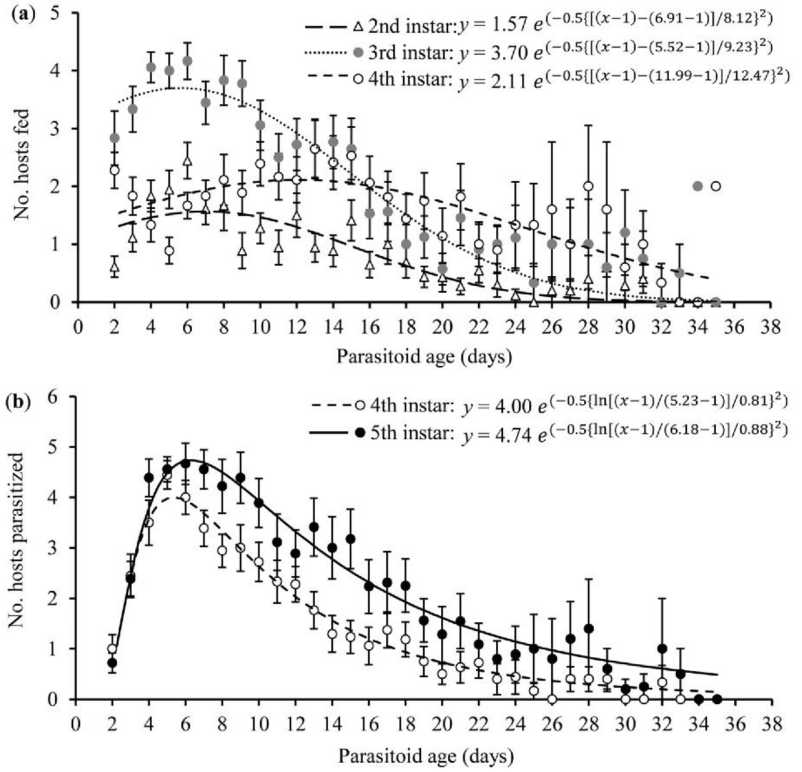

Fig. 2. Mean (±SE) daily number of hosts fed (**a**) and parasitized (**b**) by *T. triozae* females on *B. cockerelli* nymphs of different instars.

on the 4th instar nymphs which peaked about 12 days after emergence (non-overlapping 95 % CLs). The host feeding peak on the 3rd instar nymphs was significantly higher than that on other nymph stages (non-overlapping 95 % CLs). Daily parasitism on the 4th instar nymphs peaked about five days after emergence, significantly earlier than that on the 5th instar nymphs which peaked about six days after emergence (non-overlapping 95 % CLs). The parasitism peak on the 5th instar nymphs was significantly higher than that on the 4th instar nymphs (non-overlapping 95 % CLs). However, there was no significant difference in the width of daily host feeding and parasitism curves between instars (overlapping 95 % CLs).

3.2. *Effect of host stage at parasitization on parasitoid offspring fitness*

The emergence rate of parasitoid offspring significantly increased with the increase of host instars at parasitization ($F_{2,27} = 21.75$, $P < 0.0001$) (Fig. 3a). Both sons and daughters emerged from hosts parasitized at the 4th and 5th instars while only sons emerged from those parasitized at the 3rd instar. Emerged offspring were male-biased if the parasitoids parasitized the 4th instar nymphs but female-biased if parasitization occurred at the 5th instar ($F_{2,23} = 71.55$, $P < 0.0001$) (Fig. 3b). We show that both host stage and parasitoid age at parasitization affected parasitoid daughter emergence patterns (Fig. 4, Table S2). The highest number of daughters emerged from the 4th instar nymphs parasitized by 4-d-old parasitoids while that occurred from the 5th instar nymphs parasitized by 5-d-old parasitoids (non-overlapping 95 % CLs). Daughter emergence peak from the 5th instar nymphs was significantly higher than that from the 4th instar nymphs (non-overlapping 95 % CLs). However, there was no significant difference in peak width between the two instars (overlapping 95 % CLs). Furthermore, 90 % of daughters were produced from the 4th and 5th instar nymphs by the parasitoids of 3–11 days old.

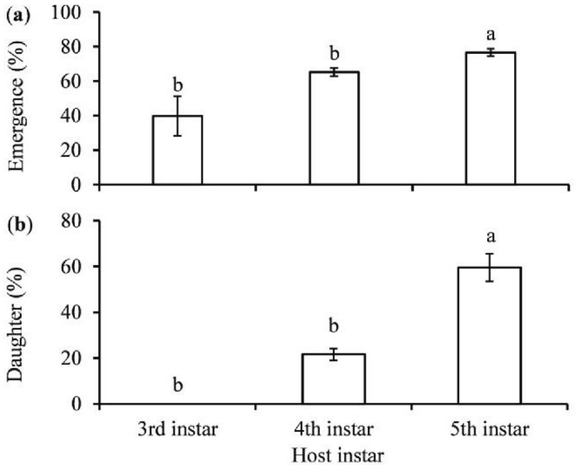

Fig. 3. Mean (±SE) emergence rate (**a**) and daughter percentage (**b**) of *T. triozae* offspring that developed from *B. cockerelli* nymphs parasitized at different instars. Columns with different letters are significantly different ($P < 0.05$).

Sons required significantly longer time to complete development on the 3rd instar than on the 4th and 5th instar nymphs (Developmental time: mean ± SE = 10.07 ± 0.05, 9.58 ± 0.04 and 9.74 ± 0.07 days for the 3rd, 4th and 5th instars, respectively; $F_{2,23} = 8.13$, $P = 0.0021$) but

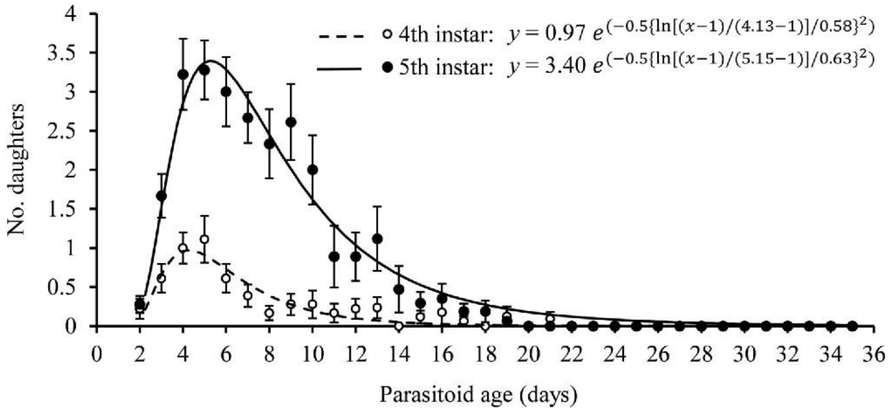

Fig. 4. Mean (±SE) number of daughters emerging from *B. cockerelli* nymphs of different instars parasitized by *T. triozae* over lifetime.

the host stage at parasitization had no impact on daughter developmental time (Developmental time: mean ± SE = 10.00 ± 0.05 and 10.06 ± 0.05 days for the 4th and 5th instars, respectively; $F_{1,17} = 0.83$, $P = 0.3749$). Daughters needed significantly longer time to complete development than sons regardless of the host stage parasitized ($F_{1,17} = 53.15$, $P < 0.0001$ for the 4th instar; $F_{1,17} = 11.04$, $P = 0.0040$ for the 5th instar). There was no significant interaction between the host stage parasitized and sex of emerged offspring in their effects on developmental period ($x_1^2 = 1.02$, $P = 0.3115$).

Offspring body size of both sexes significantly increased with the increase of the host stage at parasitization (Tibial length of sons: mean ± SE = 129.07 ± 3.47, 160.87 ± 1.67 and 190.82 ± 3.00 μm for the 3rd, 4th and 5th instars, respectively; $F_{2,38} = 86.24$, $P < 0.0001$. Tibial length of daughters: mean ± SE = 170.06 ± 1.98 and 203.45 ± 1.82 μm for the 4th and 5th instars, respectively; $F_{1,18} = 99.53$, $P < 0.0001$), and regardless of host stage at parasitization, daughters were significantly larger than sons ($F_{1,18} = 13.52$, $P = 0.0017$ for the 4th instar; $F_{1,27} = 13.38$, $P = 0.0011$ for the 5th instar). There was also no significant interaction between the host stage parasitized and sex of emerged offspring in their effects on offspring body size ($x_1^2 = 0.11$, $P = 0.7420$). In addition, newly emerged daughters from hosts parasitized at the 5th instar had significantly higher egg load than those from hosts parasitized at the 4th instar (Egg load: mean ± SE = 0.05 ± 0.05 and 1.67 ± 0.27 for the 4th and 5th instars, respectively; $F_{1,18} = 13.19$, $P = 0.0019$).

4. Discussion

We demonstrate that *T. triozae* females partitioned hosts of different instars for feeding and parasitization. When all instars of *B. cockerelli* nymphs were present, which should be the most common scenario in the field, the parasitoids preferred mid-aged nymphs for feeding and older for parasitization. Because the parasitoids need to pierce the hosts with ovipositors before feeding or parasitization (Martinez et al., 2015; Rojas et al., 2015) and do not parasitize fed hosts (Cerón-González et al., 2014), they rationalize their effort for optimal fitness gain for both mothers and their offspring. Older hosts are more nutritious for parasitoid offspring development (Li and Mills, 2004; Liu et al., 2011) but more difficult to punctuate (Kidd and Jervis, 1991; Zhang et al., 2015) due to their thicker integument (Veronesi et al., 2022) and defensive behaviour (Liu et al., 2011). Therefore, they choose to feed on younger hosts and parasitize older ones, consistent with the optimal foraging theory (Kishani Farahani et al., 2015). As both adult feeding and parasitization destroy the hosts (Martinez et al., 2015; Rojas et al., 2015), the host partitioning behavior may allow the parasitoids to control the pest effectively by killing hosts of different life stages simultaneously.

Although only one offspring survives on each parasitized host (Rojas et al., 2015), *T. triozae* females deposited more than one egg under a host with more eggs allocated to older nymphs. This host stage-dependent egg investment may help increase parasitoid offspring survival because the offspring from older nymphs at parasitization were more likely to develop to adults. Comparing the findings between the current and Rojas et al.'s (2015) studies, we found that the egg allocation was also host density dependent. In the current study, the parasitoids deposited an average of 1.4 eggs per host of the 4th or 5th instar while they laid about 1.15 eggs under each host when the density of the same stage nymphs was twice as that in our study (Rojas et al., 2015). These findings suggest: (1) when the number of nymphs suitable for parasitization is lower, the parasitoid may increase superparasitism to avoid collapse of the host population for the survival of future generations, but (2) when the number of nymphs suitable for parasitization is higher, the parasitoid may reduce superparasitism to maximize the use of available resources. This behavior may make the parasitoids more effective and persistent in the field.

Our results indicate that *T. triozae* females adjusted their daily feeding and parasitization patterns in response to host stage and their own age. They mainly fed on the 3rd nymphs in their early life when they had maximum parasitism on the 4th and 5th instar hosts but they increased their feeding on the 4th instar nymphs after they reached mid-age and their parasitism sharply declined. The increase of feeding on more nutritious 4th instar nymphs at older age is probably due to the combination of declined fecundity and increased demand of nutrition for adult survival. Overall, *T. triozae* completed 90 % of their lifetime feeding and parasitism when they were 17 days old. Because our experimental condition (25 °C) is optimal for the development of both *B. cockerelli* (Lewis et al., 2015) and *T. triozae* (Vega-Chávez et al., 2016), our findings may provide important knowledge for development of field release programs for biological control of TPP under similar conditions.

The present study reveals that although *T. triozae* preferred to parasitize the 4th and 5th instar nymphs, adults emerged from the 4th instar nymphs at parasitization were highly male-biased and those from the 5th instar nymphs were highly female-biased. These results strongly imply that the parasitoids allocate significantly more fertilized eggs to the oldest and largest nymphs, which was also reported in other parasitoids (e.g., Charnov et al., 1981; Favaro et al., 2018; Pérez-Rodríguez et al., 2019). Using the 5th instar nymphs as hosts for *T. triozae* during mass rearing may thus increase daughter production and reduce rearing cost. The sex allocation patterns also shifted with the age of parasitoids, i.e., females laid the highest number of fertilized eggs per day when they were four to five days old, with > 90 % of daughters produced during their first half of life. We suggest that the females may maximize the use of sperm in their early life to ensure the survival and persistence of their next generation in the field. As a result, they may quickly run out of sperm as they age (Pérez-Lachaud and Hardy, 1999; Santolamazza Carbone et al., 2007; Hu et al., 2012) and after they reach mid-age, they produce male-biased offspring.

We show that regardless of the host stage at parasitization, females had longer developmental time and larger body than males, suggesting that females need more resources and time to build up larger body size for reproduction (King, 1988, 2000; He et al., 2005). Like other parasitoids (e.g., Liu et al., 2011; Hanan et al., 2015; Luo et al., 2022), the host stage affected offspring body size of both sexes in *T. triozae*, which increased with the increase of the host age at parasitization. Furthermore, newly emerged daughters from hosts parasitized at the 5th instar carried more mature egg than those from hosts parasitized at the 4th instar. Although we have not measured male reproductive potential, other studies show that larger parasitoid males mate more times (Ji et al., 2004; Lacoume et al., 2006) and produce more sperm (He and Wang, 2006; Kant et al., 2012). In accordance with other parasitoids (e.g., Hu et al., 2002; Hanan et al., 2015; Li et al., 2018), larger and older hosts increase the fitness of *T. triozae* offspring.

In conclusion, *T. triozae* females adjust their behaviors in response to host stage and their own age. They feed on hosts of all instars with a preference for mid-aged ones and almost always parasitize late instar hosts, thus inflicting mortality on all instars simultaneously. The parasitoids increase and reduce superparasitism at lower and at higher host density, respectively. This may help maintain their own population in the field. Maximum feeding and parasitization occur during the first week of their life and these activities sharply decline when they are two weeks old. The parasitoids allocate more fertilized eggs to the oldest and largest nymphs. They lay the highest number of fertilized eggs per day when they are four to five days old, with > 90 % of daughters produced during their first half of life. Parasitoids' offspring body size and egg load increase with the increase of host instar at parasitization, demonstrating a positive relationship between host size and offspring fitness.

CRediT authorship contribution statement

Chen Chen: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Xiong Z. He:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Peng Zhou:** Investigation, Formal analysis, Writing – review & editing. **Qiao Wang:** Conceptualization, Methodology, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and material

The datasets from the current study are available from the corresponding author on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2023.105152.

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Appendix 2: A list of other published papers I co-authored during my PhD studies at Massey University

Zhou P, He XZ, Chen C, Wang Q (2021) Reproductive strategies that may facilitate invasion success: evidence from a spider mite. J Econ Entomol 114:632–637

Zhou P, He XZ, Chen C, Wang Q (2021) Resource relocations in relation to dispersal in *Tetranychus ludeni* Zacher. Syst Appl Acarol 26:2018–2026

Zhou P, He XZ, Chen C, Wang Q (2020) No evidence for inbreeding depression and inbreeding avoidance in a haplodiploid mite *Tetranychus ludeni* Zacher. Syst Appl Acarol 25:1723–1728