THE UNIVERSITY of ADELAIDE

Foraging behavior of *Dolichogenidea tasmanica*
&
patterns of parasitism in light brown apple moth

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Thesis submitted for degree of Doctor of Philosophy in the

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This thesis is dedicated

To the memory of my father,

I miss him every day, and I know he would be glad and proud to see me fulfilling this ambitious process to its completion.

To my Mom,

For dealing with me being world away, offering the support to make it possible, as well as plenty of friendly encouragement.

Abstract

Dolichogenidea tasmanica (Cameron) (Hymenoptera: Braconidae) is a solitary endoparasitoid that is native to Australia. I examined its response to a key pest, light brown apple moth (LBAM), *Epiphyas postvittana* (Lepidoptera: Tortricidae). LBAM is a native species in south-eastern Australia. *D. tasmanica* is known to parasitise larvae of several tortricid moths. The use of insecticides after flowering has been restricted in Australian vineyards to minimize the likelihood of pesticide residues in wine. Therefore biological control is crucial to the effective management of LBAM populations on grapes. With the long term aim of enhancing biological control in vineyards, factors that influence behaviour and population dynamics of the pest and their natural enemies must be taken into account. So in this thesis, I focused on the foraging behaviour and functional response of *D. tasmanica* to elucidate aspects of the biological control potential of this parasitic wasp.

An understanding of the host stage preference of *D. tasmanica* is crucial to elucidate its role in biological control. Therefore, the first objective of my study was to determine the larval stages of LBAM that are parasitised by *D. tasmanica*. This study was conducted in four identical wind tunnels, using choice and no-choice tests. Here I showed that *D. tasmanica* parasitises the 3rd instar of LBAM, however, it was previously reported as a parasitoid of only the 1st and 2nd instars. So, *D. tasmanica* is capable of parasitising early larval instars, which is important for minimising pest populations through biocontrol.

The searching behaviour of female *D. tasmanica* responding to plants infested with susceptible larval stages of LBAM was studied next. Behavior was continuously recorded with event-recorder software. The elapsed time before and after taking flight from the release point until landing on an infested leaf, and the mean duration,

frequency and proportion of time devoted to each type of behaviour on the leaf were analysed for each instar. These observations gave insight into how *D. tasmanica* interacts with hosts. I found that female *D. tasmanica* effectively responds to cues associated with all susceptible larval stages of LBAM to locate hosts. These experiments showed that first instar LBAM is more susceptible to parasitism by *D. tasmanica*.

Superparasitism is an important factor as it affects the stability of host-parasitoid interactions and the shape of the functional response curve. To assess the rate of superparasitism avoidance by *D. tasmanica,* female wasps were given choices between (i) unparasitised hosts versus freshly parasitised hosts, (ii) unparasitised hosts versus hosts at 24 h post-parasitisation, and (iii) freshly self-parasitised hosts versus hosts freshly parasitised by a conspecific female. So I investigated the frequency of superparasitism in order to evaluate whether *D. tasmanica* deposits its eggs in a random or non-random fashion. Experiments demonstrated that host discrimination frequently occurs in *D. tasmanica*. However, it appears that females are not able to discriminate the host parasitisation status prior to contacting a host. So *D. tasmanica* contacts hosts randomly but host acceptance is not random.

The functional response of a parasitoid influences its capacity to control pests. I conducted a study to determine the type of functional response of *D. tasmanica* to varying densities of larval LBAM. The results showed that *D. tasmanica* displays a Type III functional response to varying low densities of LBAM. This suggests that it should operate in a density-dependent manner in contributing to population regulation of LBAM. Subsequently experiments were conducted to determine how recent experience affects the functional response of *D. tasmanica*. Results showed that *D.*

tasmanica responds to its experience in a habitat, which influences the searching behaviour in the process of host location

Parasitoids do not always forage alone. Individuals of a given species have similar needs for survival, growth, and reproduction, and thus will often compete for similar resources (e.g., hosts, food, nests). This may lead to a flexible patch exploitation strategy compared to the situation of a solitary forager. The foraging behaviour of single and multiple female *D. tasmanica* in the presence of patchily-distributed hosts was observed and analysed. Results showed that females *D. tasmanica* assessed patch quality instantaneously while foraging. A searching female might deposit a marking pheromone on a visited patch, which inhibits further searching and contributes to mutual interference among competitors.

In this study key aspects of the individual and population behaviour of *D. tasmanica* were investigated. It seems that *D. tasmanica* can play a valuable role in the biocontrol of LBAM. The outcomes of my study should also provide insights into parasitism of other leafrollers that are susceptible to *D. tasmanica*. Finally the results of this research provide a foundation for understanding the biological control potential of *D. tasmanica* against LBAM in vineyards. Further research is needed to investigate how factors such as host plant preference, alternative host species, the availability of floral resources and interspecific competition influence patterns of parasitism by this species.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Maryam Yazdani Date

Acknowledgment

A major research project like this is never the work of anyone alone. The contributions of many different people, in their different ways, have made this possible. I would like to extend my appreciation especially to the following people.

First and foremost I offer my sincerest gratitude to my supervisor, Associate Professor Mike Keller, who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my PhD degree to his encouragement and effort and without him this thesis, too, would not have been completed or written. You have been a tremendous mentor for me and your advice on both research, as well as on my career, have been priceless. Your kindness and extensive support have been ever present in this important time of my life, for which I am eternally grateful.

I would like to thank my co-supervisor Dr. Richard Glatz for encouraging and supporting me to grow as a research scientist. Special appreciation to Professor Eileen Scott, she has been always my support in the moments when there was no one else to answer my queries. I thank staff at SARDI (South Australian Research and Development Institute) for supplying information on rearing LBAM and also Latif Salehi and Cate Paull for their support during my project. I would also like to thank Dr. Farid Christo for his guidance and encouragement.

I would especially like to thank my officemates, Arthur Selwyn Mark, Feng Yi, Kala Bhandari, Mary Retallack, Kay Anantanawat, Ahmad Chatha and Tom Wang. In my daily work I have been blessed with a friendly and cheerful group of fellow students. I would like to thank you all for supporting me in writing, and encouraging me to strive towards my goal. All of you have been there whenever I needed you.

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Last but not least, I would like to thank The University of Adelaide which has provided the support and equipment I have needed to produce and complete my thesis and the ***Adelaide Scholarship International (ASI)*** has funded my studies and living allowance.

Preface

The research discussed in this thesis has led to the generation of five journal papers and three conference papers.

Journal papers

- **M. Yazdani**, F. Yi, R. Glatz & M.A. Keller. Host stage preference of *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae). *Austral Entomology*.

  DOI: 10.1111/aen.12130
- **M. Yazdani**, R. Glatz & M.A. Keller. "Searching behaviour of *Dolichogenidae tasmanica* in response to susceptible stages of *Epiphyas postvittana*". Submitted: *Australian Journal of Zoology.*
- **M. Yazdani**, R. Glatz & M.A. Keller. "Host discrimination by the solitary endoparasitoid *Dolichogenidea tasmanica* (Hymenopotera: Braconidae)*". Biocontrol Science and Technology.*

  DOI: http://dx.doi.org/10.1080/09583157.2014.964663
- **M. Yazdani** & M.A. Keller. "The sigmoid functional response of *Dolichogenidea tasmanica* (Hymenoptera: Braconidae) is affected by experience". Submitted: *Oecologia.*
- **M. Yazdani** & M.A. Keller. "Foraging for Patchily-Distributed Light Brown Apple Moth by *Dolichogenidea tasmanica* (Cameron) (Hym.: Braconidae)". Submitted: *Biological control.*

Conference papers

- **M. Yazdani**, M.A. Keller. Understanding the mechanisms that influence the response of the parasitic wasp *Dolichogenidea tasmanica* (Hym.: Braconidae) to the density of Light Brown Apple Moth. 61st Annual Meeting of the

Entomological Society of America (ESA). Austin, Texas, 10-13 November 2013.

- **M. Yazdani**, M.A. Keller. Host location and host stage preference of *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae). Australian Entomological Society (AES) 44th AGM & Scientific Conference Adelaide 29 September-2 October 2013.
- **M. Yazdani**, M.A. Keller, F. Yi. Searching behaviour of *Dolichogenidea tasmanica* in response to different larval instars of LBAM. Australian Society for the Study of Animal Behaviour (ASSAB). Deakin University, Geelong, 26-28 June 2012.
- **M. Yazdani**, M.A. Keller, How easy is for parasitoid wasp to locate and parasitise its host in vineyard? Postgraduate Symposium. School of Agriculture, Food & Wine, 19-20 September 2012.

CHAPTER 1

INTRODUCTION

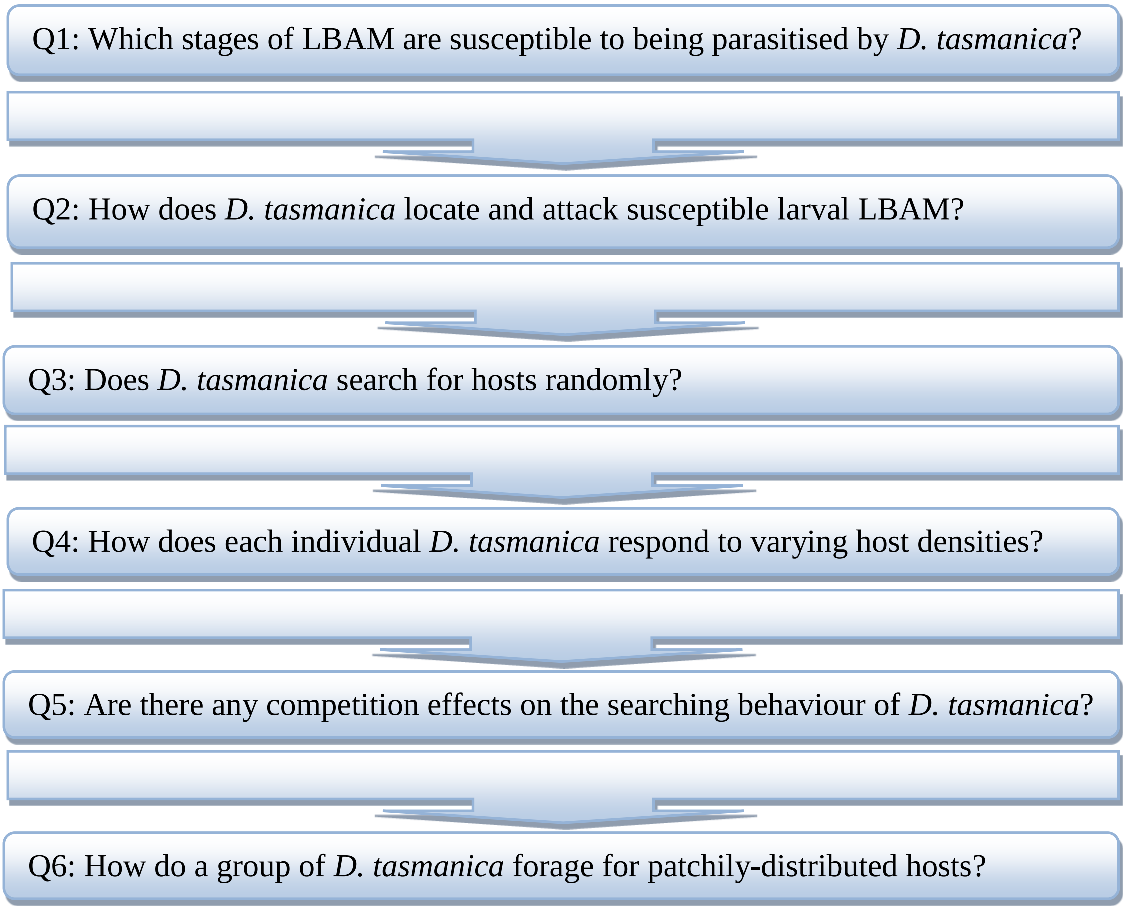
1. General Introduction

Behavioural ecological studies of parasitoids may provide useful insights and measurements that can contribute to modelling host-parasitoid interactions, because they can refine understanding of the intricacies of such phenomena as the functional response and the limitations of efforts to model these interactions. Ecological and behavioural models provide a scientific means of understanding the successes and failures of natural enemies in biological control (Hochberg and Ives 2000). When a wasp parasitises a host, it is deciding its potential contribution to future generations. This potential is tightly linked to the number and quality of hosts that it chooses to parasitise, since these hosts provide the resources for the parasitoid's offspring. However, most population models involving host-parasitoid interactions assume that hosts are equally acceptable regardless of their quality and the rate at which they are encountered. Such homogeneity in host populations under field conditions is unlikely (Luck 1990). The analysis of functional and numerical responses within a parasitoid-host interaction is often used to determine the potential effects of parasitoids on the host population (Oaten and Murdoch 1975). Functional responses are an essential constituent of any predator-prey and host-parasitoid system, especially when attempting to model them from elementary behavioural processes (Casas et al. 1994). It is believed that a natural enemy is more likely to be effective if the functional response is density-dependent (Solomon 1949; Nicholson 1958), although there are various ideas about the role of density-dependent factors in controlling pest populations (Stiling 1987, 1989).

This study focuses on the parasitoid *Dolichogenidea tasmanica* (Cameron 1912) (Hymenoptera: Braconidae). In particular, it investigates the factors which influence its behaviour at different densities of its hosts, and attempts to model the functional response them observations of elementary behavioural processes (Figure 1). The wasp exists naturally in southern Australia where it attacks a damaging pest host, the light brown apple moth, *Epiphyas postvittana* (Walker 1863) (Lepidoptera: Tortricidae; LBAM). It has not been utilised in a managed biocontrol program. Therefore we need to understand important aspects of the parasitoid-host interaction to develop the foundation for a successful biocontrol program using *D. tasmanica* for managing populations of *E. postvittana*. It is a highly polyphagous native Australian species and the most destructive insect pest in Australian vineyards (Scholefield and Morison 2012).

This study addresses the most important factors that influence the behaviour of *D. tasmanica* when it searches for and parasitises *E. postvittana*, including host stage preference, host discrimination, functional response, learning and population response. Study of the functional response is essential to determination of the potential stability of parasitoid-host interactions and other aspects of population responses of *D. tasmanica*.

In this chapter I provide a brief and critical review of the literature relating to my research on *D. tasmanica* and LBAM. The emphasis of this chapter is on the biological control of LBAM, so some important aspects of biocontrol are reviewed first. The biology and ecology of light brown apple moth and its economic impact are considered next. The importance of this insect has prompted development of classical biological control programs in other countries, together with a wide variety of other practices that can be used in integrated pest management or integrated pest eradication programs. Finally there is a detailed review of the literature on *D. tasmanica* as it is the most abundant natural enemy that is known to attack LBAM.



Q1: Which stages of LBAM are susceptible to being parasitised by *D. tasmanica*?

Q2: How does *D. tasmanica* locate and attack susceptible larval LBAM?

Q3: Does *D. tasmanica* search for hosts randomly?

Q4: How does each individual *D. tasmanica* respond to varying host densities?

Q5: Are there any competition effects on the searching behaviour of *D. tasmanica*?

Q6: How do a group of *D. tasmanica* forage for patchily-distributed hosts?

Figure 1. Main objectives of the thesis.

1.1 Format

The thesis has been submitted as a portfolio of the publications according to the formatting requirements of The University of Adelaide. The printed and online versions of this thesis are identical. The online version of the thesis is available as a PDF. The PDF version can be viewed in its correct fashion with the use of Adobe Reader 9.

2. Some important aspects of Biological Control

Biological control using natural enemies has long been recognized as a fundamental component of insect pest management (Dent 2000). Biological control in practice is a tactical, empirical procedure. There are three approaches to biological control: classical (or introduction), augmentation, and conservation (Van Driesche and Bellows 1996). Historically most emphasis has been placed on classical biological control, although in the recent decades a great deal more effort has been directed at inundative and augmentative control (Dent 2000). In classical biological control, natural enemies from a pest's area of origin are released, together or in sequence, in the hope that one or more will establish breeding populations that suppress the pest’s populations and regulate them successfully (Koul 2004).

Parasitoids have a central role in the application of biological control (Hawkins and Cornell 1999). They comprise some 10% or more of all metazoan species and most of them belong to two orders, the Diptera (flies) and the Hymenoptera (Hassell 2000). An insect parasitoid completes its larval development on or inside a host. The adults lay one or more eggs on or inside a particular life stage (egg, larva or pupa) of its host. When the parasitoid egg hatches, its larva feeds on the host’s tissues, ultimately killing the host as it matures and becomes free living as an adult. Endoparasitoids develop inside the host, while ectoparasitoids develop outside the host’s body. In some species, only one parasitoid will develop in or on each host (solitary parasitoids); in others, several to hundreds of young larvae may develop from a single host (gregarious parasitoids) (Godfray 1994).

The success of biological control has stimulated the development of analytical models that explore the dynamics of natural enemies and their hosts or prey. These models seek to identify those general characteristics of the natural enemy, host or prey population that lead to economically acceptable levels of pest control (Luck 1990). The challenge, then, for a general theory of population dynamics as it relates to biological control is two-fold: (1) to explain when and how natural enemies regulate their host or prey populations, especially in light of the several alternative hypotheses that density dependent processes are essential for population regulation and (2) to develop techniques by which effective natural enemies can be detected and evaluated for use in introduction, augmentation or inoculation release programs, or by which indigenous natural enemies can be identified for purposes of conservation. Two general approaches have evolved to address these challenges: (1) the development of mathematical models as metaphors of the parasitoid-host interaction, with the purpose of identifying processes that regulate the host population, and (2) studies of laboratory or field populations using life tables and analytical techniques that seek to identify density-dependent processes associated with the regulation of these populations, especially those involving natural enemies. The latter approach assumes a linkage between effective biological control, density dependence and host population regulation (Luck 1990).

2.1 Host-Parasitoid Models

Many models of parasitoid population dynamics and diversity have been applied to biological control (Mills and Getz 1996). For the most part, modelling studies have focused on which ecological characteristics of host-parasitoid interactions contribute to the suppression and stability of host populations. Most studies about the behaviour and ecology of parasitoids have been performed in the laboratory (Godfray 1994; Quick 1997), while behavioural studies of parasitoid species in the field are relatively rare (Hochberg and Ives 2000).

A major challenge for population ecology studies is to integrate an understanding of population characteristics and processes together with the characteristics and behaviour of individuals. Although we can use models as predictive analytical tools, they should not be considered an ultimate goal in ecological studies (Sharov 1996). One of the basic approaches to modelling predatory-prey interactions is derived from the Lotka-Volterra equations for continuously breeding species and the Nicholson-Bailey Model for populations that have discrete generations (Nicholson and Bailey 1935).

Models of host-parasitoid population dynamics have incorporated the effects of numerous parasitoid behaviours (Hassell 1978; Walde and Murdoch 1988; Hochberg and Ives 2000). In 1924, Thompson published a model intended to explain and guide decisions about biological control of insect pests by parasitoids. He centred his model on the probability of a given host escaping parasitism. His basic assumptions were that parasitoids distribute their attacks at random and that they are unable to actively avoid superparasitism (Hochberg and Ives 2000). Thompson's model always leads to extinction of both populations. To build his model, he made the restrictive assumption that parasitoid reproduction was independent of number of hosts encountered. Nicholson and Bailey (1935) overcame this limitation by assuming that parasitised hosts become parasitoids in the next generation, and that each host attacked produces a single adult parasitoid. They also assumed that each healthy host produces a constant number of offspring. In the realm of host-parasitoid interactions, the pioneering work of Thompson (1924) and Nicholoson and Bailey (1935) were developed under the general assumption that natural populations are stable. Twenty-five years after Nicholoson and Bailey, C. S. Holling (1959) studied predation of small mammals on pine sawflies. He found that predation rates increased with increasing prey population density. This resulted from two effects: (1) each predator increased its rate of consumption, and (2) predator density increased. Holling considered these effects as two kinds of responses of predator population to prey density: (1) the functional response and (2) the numerical response.

The functional response refers to the relationship between the numbers of parasitised/consumed targets and the host/prey density over time (Holling 1959). The numerical response refers to a change in the numbers of predators or parasitoids in response to changing prey or host density. Numerical responses can be caused by non-random movement or reproduction. The analysis of functional and numerical responses of the parasitoid-host interaction is often used to determine the potential efficiency of parasitoids to regulate host populations (Oaten and Murdoch 1975). The three types of functional responses have distinctive curves that reflect the relationship between the numbers of hosts or prey attacked versus the numbers present. These models may be modified by parameters such as length of exposure to the prey, attack rate, or handling time (Hassell et al. 1977).

With a Type I model (“Linear or Straight”), the numbers of hosts/prey killed rise linearly to a plateau. A type II model (“Crytoid or C- shaped”) shows a curvilinear rise to a plateau under the influence of handling time or satiation. A Type II functional response is typical of many species and corresponds to the Holling (1959) disk equation. Predators displaying this type of relationship with the density of their prey cause maximum mortality rates at low prey density. In a type III (“Sigmoid or S-Shape”) response, there is a sigmoid increase in numbers of prey/hosts attacked (Hassell 2000; Mills and Lacan 2004; Moezipour 2008). Type III functional responses occur in predators which increase their search activity with increasing prey or host density.

Mortality first increases when prey or host density is low, and then declines as handling time reduces the time available for searching (Figure 2).

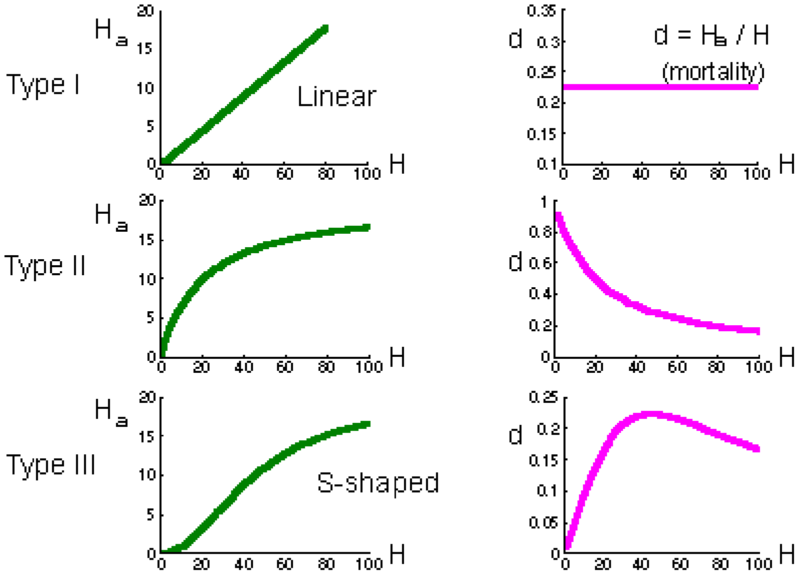


Figure 2. Three major types of functional responses. The relationships between number of prey eaten/parasitised host (H_a) and the number of prey/host present (H) are depicted on the left side. The corresponding relationships between proportion eaten/parasitised (H_a/H) and number of prey/host present (H) are depicted on the right side (Sharov 1996).

2.2 Host Discrimination

Another important factor for an effective parasitoid is its ability to distinguish between parasitised and non-parasitised hosts (van Lenteren et al. 1978). Sometimes a parasitoid oviposits a second time in or on the one host, which is called "superparasitism". There are two different kinds of superparasitism, "self" and "conspecific" superparasitism. The difference between "conspecific" and "self" superparasitism is that when eggs are deposited in a parasitised host by another conspecific female there is potential for competition between offspring of each female, whereas eggs deposited in a host parasitised by the same female will increase competition among siblings (Van Alphen 1990).

The ability to discriminate between parasitised and non-parasitised hosts enables a parasitoid to avoid superparasitism and minimize time and energy associated with searching behavior (Godfray 1994; Mackauer 1990). In other words, this ability allows a parasitoid to decide to oviposit in or reject parasitised hosts depending on the conditions. For example, many braconids have evolved an ability to discriminate between unparasitised and parasitised hosts (e.g. Cloutier et al. 1984; Moore and Ridout 1987; Outreman et al. 2001; Wang et al. 2010; Benelli et al. 2013). The ability to avoid oviposition in parasitised hosts was readily recognized as an adaptive trait (Van Alphen 1990). On the other hand, the knowledge that parasitoids are able to avoid super-parasitism raises the question why superparasitism is so common in nature (Salt 1961).

Some parasitoids are able to recognize hosts that are parasitised by conspecifics or by themselves and to reject such hosts, but they still often lay a second egg, or clutch of eggs. This distinction between self and conspecific superparasitism is most obvious in solitary parasitoids, where only one of the eggs deposited in a host will be able to develop into an adult. Self superparasitism in solitary parasitoids therefore often means a “waste” of the time and the egg, whereas conspecific superparasitism can be advantageous under a wider range of conditions because of the probability of elimination of the non-sibling competitor from the parasitised host. In theoretical model, Hubbard et al (1987) showed that the ability to discriminate between cues related to prior self and conspecific parasitism can enable the wasps to exhibit patterns of host discrimination in an evolutionarily stable strategy (ESS).

Van Alphen (1988) has shown that superparasitism increases in solitary parasitoids that are simultaneously searching in a patch when the number of females increases. This increase in superparasitism results in aggregation and mutual interference, both factors that can stabilize host-parasitoid models. In some models it has been assumed that parasitoids have perfect knowledge about the distribution of unparasitised and parasitised hosts within the habitat when predicting whether parasitoids should superparasitise or not (Van Alphen 1990).

Van Alphen (1990) assumed that parasitoids have perfect knowledge about the distribution of unparasitised and parasitised hosts within the habitat when predicting whether parasitoids should superparasitise or not. In nature, they have to accumulate information while foraging, and so foraging decisions may be influenced by previous experience. There is ample evidence that parasitoids retain information and that their behaviour can be modified by experience (Arthur 1966; van Alphen & van Harsel 1982; van Dijken et al. 1986). The decision to superparasitise may likewise be influenced by previous experience. In the past host discrimination and the avoidance of superparasitism were held to be synonymous, so the observation that inexperienced females of some parasitoid species readily oviposit in parasitised hosts has been explained as an inability of inexperienced females to recognise parasitised hosts (Klomp et al. 1980; Suzuki et al. 1984; van Lenteren 1976). In some experiments, it was shown that parasitoids avoid oviposition in parasitised hosts after oviposition in a number of unparasitised hosts, which led to the conclusion that host discrimination has to be learned (Suzuki et al. 1984; van Lenteren 1976). However, a better functional explanation for this behaviour can be given, i. e., a patch already exploited by conspecifics signals a poor habitat and, since the inexperienced female has certainty that all the eggs she lays in parasitised hosts represent conspecific superparasitism, superparasitism may be advantageous (van Alphen et al. 1987; 1982). A reanalysis of older data (Klomp et al. 1980; Suzuki et al. 1984) and new experiments has shown that inexperienced parasitoids are well able to discriminate between unparasitised and parasitised hosts (van Alphen et al. 1987). Hence Van Alphen (1990) concluded that host discrimination does not have to be learned, but the tendency to superparasitise can be influenced by experience.

3. The light brown apple moth

The light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) was first described as *Teras postvittana*, in 1863, but Bradley (1956) reassigned the species to a new genus *Austrotortrix*. Later systematic studies led Common (1961) to assign *postvittana* and 31 related species to the genus *Epiphyas* (Danthanarayana 1975). Now *Epiphyas* includes 40 species, all of which have been described from Australia (Brown et al. 2010).

Although LBAM is indigenous to south-eastern Australia, it has invaded Tasmania and New Zealand (Bradley et al. 1973; Danthanarayana 1975), New Caledonia, Hawaii, England, and Ireland It has also been found elsewhere in Europe including in the Netherlands (Wolschrijn et al. 2006) and southern Sweden (Svensson 2009). In 2007, it was confirmed as established in the USA in California in Los Angeles, the Napa Valley and San Francisco (Johnson et al. 2007; Fowler et al. 2009; Gutierrez and Mills 2010; Suckling and Brockerhoff 2010).

LBAM is an herbivorous generalist pest that is believed originally to have infested local evergreens such as acacias (Danthanarayana 1975; Geier and Bries 1981; Suckling and Brockerhoff 2010). The long list of recorded host plants includes over 120 species found in Australia, of which 23 are Australian natives (Danthanarayana 1975, Geier and Briese 1980) and 250 plant species found in New Zealand (Thomas and Shaw 1982). *E. postvittana* may attack over 500 plant species in 363 genera (Suckling and Brockerhoff 2010). Larvae feed on many types of crops such as grapes, apples, cranberries, stone fruits, citrus, ornamentals, vegetables, glasshouse crops, and occasionally young pine seedlings (Thomas 1989). I It is a pest of numerous ornamentals in Australia and New Zealand, and is considered to be an important invasive pest in North America and elsewhere (Varela et al. 2008; Brown et al. 2010; Suckling and Brockerhoff 2010; Wang et al. 2012). The pest status of LBAM has led to extensive investigation of its biology, ecology and behaviour (Dumbleton 1932, 1939; Evans 1937; Geier 1965; Lawrence and Bartell 1972; 1973; MacLellan 1973; Danthanarayana 1975; 1976 a, b; Buchana 1977; Bartell and Lawernce 1973, 1976, 1977).

3.1 Damage and economic impact of LBAM

LBAM larvae typically conceal themselves by tying plant material with silk, often rolling leaves around them to produce characteristic “leaf rolls”. Like most Archipini, early instars feed on the undersides of leaves within a silk shelter. Late instars may fold individual leaves, create a nest of several leaves webbed together, or web leaves to fruit and then feed on the surface of the fruit (Danthanarayana 1975). Larvae can feed on leaves, flowers and fruit, which can damage seedlings, ornamental plants, deciduous fruit-trees, and fruits such as grapes and citrus (Geier et al. 1981). This damage can lead to reduction in the rate of photosynthesis and growth, deformity of the host plant, and reduction of fruit quality, which may become unsuitable for sale (Irvin 2009). If vineyards are left unchecked, infestations can cost thousands of dollars per hectare (Scholefield and Morison 2010). Feeding by larvae on mature grapes leads to opportunistic growth and damage caused by *Botrytis cinerea* (Nair 1985, Bailey et al. 1996, Lewis and Hodges 2010). LBAM has various impacts on agriculture, horticulture, and natural and urban environments. Direct economic damage caused by LBAM in Australia is mainly been limited to pome fruits, stone fruits, grapes, and citrus

(Danthanarayana 1975, Danthanarayana et al. 1995). Direct damage to fruit occurs on a wider range of crops in New Zealand where it is not native (Suckling and Brockerhoff 2010). Moreover, in exported fruit there is a nil tolerance of live LBAM, which significantly raises the requirement for control in the field, in comparison to non-export crops (Varela et al. 2008; Suckling and Brockerhoff 2010). In unsprayed crops, up to 70% direct damage levels have been recorded (Wearing et al. 1991; Suckling and Brockerhoff 2010). The estimated annual economic impact of direct damage cause by LBAM in the Australian wine grape industry is $18 million. The indirect damage, mainly caused by promoting *Botrytis spp.*, is reported to be about $52 million per year giving an estimated average industry-wide economic impact of about $70 million per year (Scholefield and Morison 2010).

3.2 Morphology of LBAM

Adults LBAM has folded wings that are approximately 10 mm long. The colour varies from rust-brown to pale yellow, with brown to dark brown markings. The forewing pattern of *E. postvittana* is sexually dimorphic and variable within each sex. Males are distinguished by dark spots on the hind margin of forewings (Figure 3) (Danthanarayana 1975; Lewis and Hodges 2010; Brown et al. 2010).

Figure 3. Dorsal view of an adult male (left) and female (right) *Epiphyas postvittana*.

Eggs are flat and broadly oval, around 0.84 - 0.95 mm long. They are laid in an overlapping pattern, shingle-like (imbricate), in rows or oval patches, without scaling or debris applied by the female (Powell and Common 1985, Wearing et al. 1991). Newly laid eggs are pale yellow to white and translucent, and the embryos are visible when incubation proceeds (Brown et al. 2010).

Larvae LBAM typically has six instars, and reaches 10 to 20 mm in length. They are generally yellowish green with paler subdorsal, subventral and ventral lines (Danthanarayana1975). First instars are ~1.5 mm long with a dark head and light-colored body. Succeeding instars have a darker body colour that is green. All instars are darker dorsally, and the pinacula of later instars are slightly paler than the surrounding integument (Brown et al. 2010).

Pupae Newly formed pupae appear green, but quickly harden and darken to become brown. A typical pupa is 10-15 mm long and often found within a "leaf roll," or a silken cocoon spun and woven between two leaves. The female pupae are typically larger than males, and can be distinguished through various morphological characteristics on the ventral surface of the abdomen (Danthanarayana 1975). Males have four abdominal sutures while females have three.

3.3 Life cycle and Biology

LBAM completes 2-5 generations annually over much of its range, depending on temperature and latitude (Wearing 1999).The life cycle of LBAM from egg to adult (Figure 4) is longer at cooler temperatures so only two generations typically occur in cooler regions (Danthanarayana 1975). In warmer regions, four to five overlapping generations may occur. Overwintering typically occurs between the second and forth larval instars (Espinosa and Hodges 2009; Lewis and Hodges 2010).

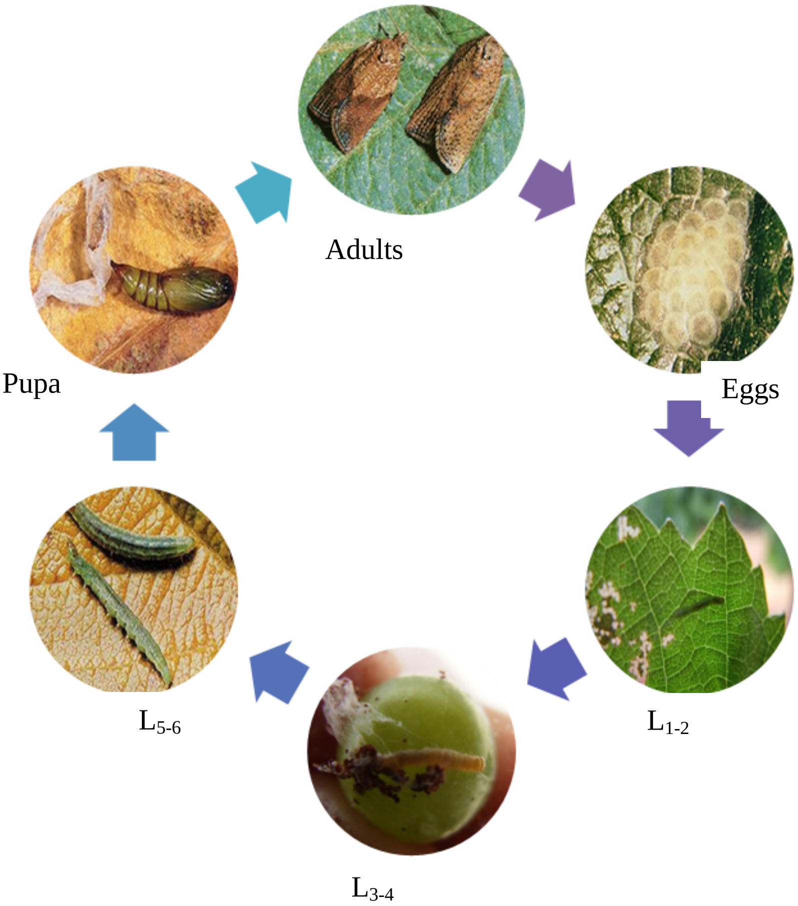


Figure 4. Life cycle of *Epiphyas postvittana*.

Adults are nocturnal, and females attract males with a sex pheromone (Espinosa and Hodges 2009). About 24 h after emerging, mating begins with nearly all females being mated within 4 days (Gu et al. 1990 a, b, 1992). Plant-derived stimuli are the main factors that affect oviposition site (Foster et al. 1997), however female moths prefer oviposition on smooth surface textures of host plant foliage, and often select ridges, and upper surfaces (Foster et al. 1997). Egg masses vary from 4 to 150 eggs under laboratory conditions, the largest mass usually is deposited first, with successively smaller numbers per oviposition over several days (Dumbleton 1932, Powell and Common 1985). In the field, females deposit fewer eggs per oviposition event. Geier and Briese (1980) tallied 23-26 eggs per mass over four seasons, and Wearing et al.

(1991) recorded a mean of 35 (range 4-96) (Brown et al. 2010). Moths of summer generations produce significantly fewer eggs than those of spring and autumn-winter generations (Danthanarayana 1975).

Depending on the temperature, eggs will hatch after 5 to 30 days. The mean duration of the egg stage is 5.7 days at 28ºC with no eggs hatching above 31.3ºC (Danthanarayana et al. 1995; Gu et al. 1990 a, b; 1992 a, b; USDA 2008). When larvae emerge from eggs, they disperse and migrate across the surface of their plant hosts, and then spin a fine-web on the underside of a leaf to make a protective cover where they settle and feed. Sometimes larvae move to feed on flowers and fruits, leaving brown pits and scars. Larvae on deciduous trees and shrubs feed as long as leaves remain on the host plant and then drop to the ground, where they may feed on understory vegetation or survive in leaf litter. Pupation occurs within the concealed feeding refuge, and metamorphosis takes about 10 d at 20ºC (Danthanarayana 1975; Suckling and Brockerhoff 2010). There is not any diapause in their life cycle when temperatures are above ~7ºC. The biology of LBAM varies somewhat among populations and locations (Geier et al. 1976, 1980).

3.4 Pest Management

A number of control strategies is available to manage LBAM, including cultural control practices, using pheromones for mating disruption, chemical control and biological control (Loch 2007). Integrated pest management (IPM) combines different control methods and aims to minimize the use of pesticides, which can contribute to sustainable agriculture and maintains current yields (Kogan 1998).

3.4.1 Insecticides and Biopesticides

Insecticides are the dominant tactic for suppression of LBAM. A range of insecticides is used against it on wine grapes in Australia. These include chlorantraniliprole, methoxyfenozide, spinetoram, emamectin benzoate, and indoxacarb (Essling and Longbottom 2013). *Bacillus thuringiensis kurstaki* (*Btk*), abacterial insecticide specific to Lepidoptera, is also effective in vineyards, on kiwifruit and, to a limited extent, on organic apples (Suckling et al. 2010).

Resistance has occurred to several classes of insecticide, suggesting that different control methods should be integrated for management of this pest (Suckling et al. 2010; O'Neil et al. 2003).

3.4.2 Pheromones and mating disruption

Sex pheromones can be used for monitoring and mating disruption in pest management (Suckling et al. 1990). Pheromone trap thresholds are using to justify interventions in orchards, and the combination of using pheromone traps for monitoring, and rigorous using of chemicals, has helped fruit growers to increase market access in practice (Suckling et al. 2010).

3.4.3 Postharvest Treatments

Although different types of postharvest treatments have been tested for fruits, few have become commercialized. Some stresses have been examined as single and combination treatments, such as hot water treatment, high-temperature controlled-atmosphere storage, and high-temperature low-oxygen pulse followed by cold storage (Suckling et al. 2010) however, LBAM has proved more tolerant to some treatments than the fruit (Lay-Yee et al. 1997). In order to prevent citrus infestation, both postharvest oil and spray oil, can be applied as dips (Tavener et al. 1999).

3.4.4 Biological Control

Natural enemies can contribute to both natural biological control of LBAM in Australia and introduction biological control in other countries. Since the use of insecticides after flowering has been restricted in Australian vineyards to minimise the likelihood of pesticide residues in wine (Essling and Longbottom 2013), biological control is crucial to providing effective management options for LBAM populations on wine grapes. Biological control has been demonstrated to be capable of suppressing *E. postvittana* in New Zealand, where the pest has been successfully managed through a combination of biological control and threshold-based applications of selective insecticide (Varela et al. 2010).

A review of LBAM parasitoids in Australia provided a key for 25 species, including five hyperparasitoids (Paull and Austin 2006). In Australian the most common larval and pupal parasitoids of LBAM are the braconids *Dolichogenidea tasmanica* and *Therophilus unimaculatus* (previously described as *Bassus* sp.); the bethylid *Gonozius jacintae*; the ichneumonids *Australoglypta latrobei*, *Exochus sp.*, and *Xanthopimpla rhopaloceros*; the chalcid *Brachymeria rubripes*, and the tachinid *Voriella uniseta*. Some species appear to be common over much of LBAM's range (e.g., *D. tasmanica* and *X. rhopaloceros*), but others are less common and more geographically restricted (Suckling and Brockerhoff 2010).

Although it is difficult to compare the relative importance of each natural enemy in affecting LBAM populations, *D. tasmanica* is the most commonly collected parasitoid of *E. postvittana* in New Zealand and Australia (Charles et al. 1996; Suckling et al. 1998; Paull and Austin 2006). The significance of *D. tasmanica* is evident in the relatively high percentage of parasitism of young larvae (about 50% of instar stages two to four) compared with parasitism of later immature stages by other species (about 20%)

(Varela et al. 2010). Paull et al. (2013) found that *D. tasmanica* had an inversely density-dependent response to host density with over 90% of the collected data. So, they argued that this inverse density-dependence does not change as the season progresses, which strongly suggests a lack of aggregation. This parasitoid can locate and sting other species of leafrollers, three of which are economically damaging to apples in Australia and New Zealand (Suckling et al. 2001; 2010).

4. *Dolichogenidea tasmanica*

D. tasmanica is an arrhentokous, solitary, koinobiont endoparasitoid of *E. postvittana* (Dumbleton 1935; Early 1984). *D. tasmanica* attacks early instars of tortricids, depositing its egg inside host larvae. The parasitoid larva develops and emerges from the third or fourth host instar (Figure 5).

Figure 5. Female *D. tasmanica* parasitising larval LBAM

Parasitoid larvae emerge from the host and spin a white cocoon around themselves next to their dying leafroller host, emerging as an adult between 13 and 24 days later (Dumbleton1935; Paull and Austin 2006). One generation takes approximately two months in late summer, about 10 days fewer than the generation time of its host

(Dumbleton 1935). There are probably 2-3 generations per summer and parasitoids overwinter as larvae in the overwintering host larvae (Dumbleton 1935; Early 1984).

D. tasmanica is associated with a range of host plants and insects. Parasitism by *D. tasmanica* of first instar larvae of the LBAM varies significantly among larval host plants (Suckling et al. 2001). And, in addition to E. postvittana, *D. tasmanica* attacks native leafroller species, but there is some evidence that it prefers LBAM in New Zealand, apparently because of the defensive behaviour of larger native leafrollers (Wearing et al. 1991; Suckling et al. 2001).

Parasitoids like D. tasmanica feed on nectar.Irvin N.A. et al. (1999) showed that a combination of buckwheat and coriander resulted in greater parasitoid longevity compared with either plant species alone. Incorporating flowering plants, such as coriander and buckwheat into the orchard understorey as part of an Integrated Fruit Production programme, may contribute to leafroller biocontrol by enhancing the abundance and longevity of their parasitoids. Later a Laboratory experiments assessed the effects of floral food resources on the longevity, fecundity, and sex ratio of *D. tasmanica*. Alyssum (Lobularia maritima (L.), Brassicaceae) plants with flowers were compared with to plants without flowers, with water available in both treatments (Irvin et al. 1999; Berndt and Wratten 2005). Both female and male parasitoids with access to alyssum flowers lived longer than those without flowers. The lifetime realised fecundity of *D. tasmanica* was also significantly increased in the presence of flowers, although this was a consequence of the increase in longevity, rather than an increase in daily fecundity. Without flowers, offspring sex ratios were strongly male biased, but when females had access to flowers, an approximately equal sex ratio was produced. These results are discussed in relation to the use of flowers in agro-ecosystems for the conservation biological control of leafroller pests (Berndt and Wratten 2005).

Incorporating flowering plants, such as coriander and buckwheat into the orchard or vineyard understorey as part of an integrated pest mangement program may contribute to leafroller biocontrol by enhancing the abundance and longevity of their parasitoids (Irvin et al. 1999; Berndt et al. 2006).

5. Aims and significance of the project

Natural enemies can have an important impact in keeping populations of LBAM below the economic threshold (Berndt et al. 2006). Therefore if broad-spectrum insecticides are avoided, natural enemies should play a greater role in the management of LBAM population in vineyards. Even though much is known about the biology and ecology of LBAM (Geier 1965; Lawrence et al. 1972; Bartell et al. 1973; MacLellan 1973; Dumbleton 1932, 1939; Evans 1937; Danthanarayana 1975 a; 1976 a, b), very little information is available about its parasitoids and their efficiency of control.

Because the levels of parasitism of LBAM by *D. tasmanica* are reported to be high and it is a native parasitoid in Australia, there should be greater potential for using *D. tasmanica* for biological control in Australian vineyards than other species. But little research has been carried out on the behaviour and ecology of *D. tasmanica*, and greater understanding of this parasitoid species is needed in order to make the most of its capacity to deliver greater biological control of LBAM.

In my project (Figure 6), I addressed the question, “How does the behaviour of *D. tasmanica* influence the mortality it causes to LBAM?” In order to answer this question, experiments sought to answer six related questions. (Chapter 2) Which stages of LBAM are susceptible to being parasitised by *D. tasmanica*? (Chapter 3) How does *D. tasmanica* locate and attack the susceptible instars of LBAM? (Chapter 4) Does *D. tasmanica* parasitise hosts randomly? (Chapter 5)

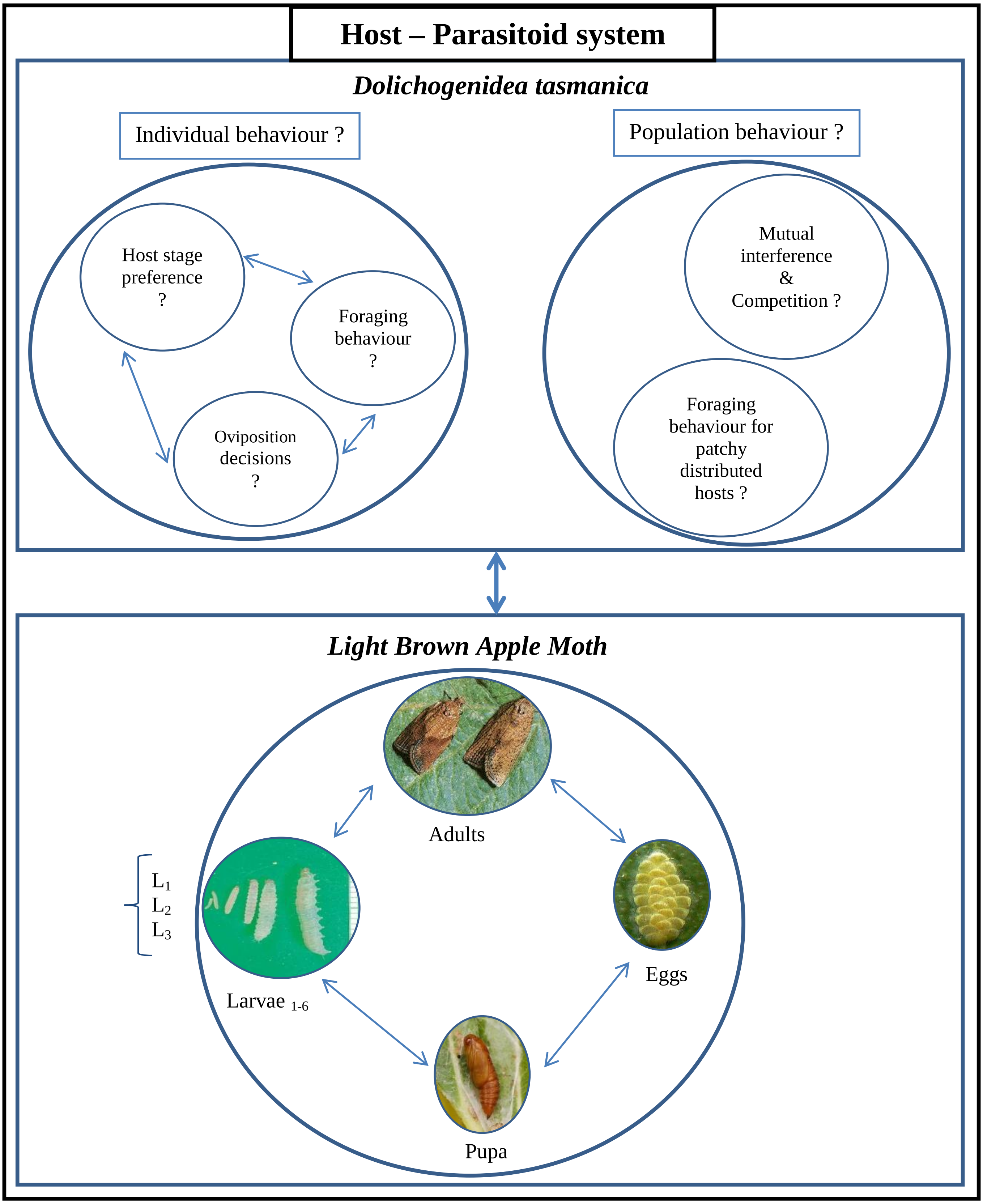


Figure 6. Host-parasitoid interactions between *D.tasmanica* and the susceptible larval instars of LBAM.

How does each individual *D. tasmanica* respond to the local abundance of hosts? (Chapter 6) Are there any effects of interspecific competition on the searching behaviour of *D. tasmanica*? (Chapter 6) How do groups of *D. tasmanica* forage for patchily-distributed hosts? The results of this research provide a better understanding of the contribution that *D. tasmanica* can make to biological control of LBAM. In this research most of the experiments were carried out in wind tunnels to provide standardised conditions for the experiments that allowed observations to be made efficiently

6. Scope and structure of thesis

The main body of this thesis is written as a series of manuscripts for publication. As each is intended to be read independently, it is inevitable that there is some repetition in the introductory and discussion sections of the chapters. The major results of this study are reviewed and integrated in the final general discussion. It contains the conclusions from the body of work presented here along with recommendations for future work in this area of research.

CHAPTER 2

HOST STAGE PREFERENCE OF *DOLICHOGENIDEA TASMANICA* (CAMERON) (HYMENOPTERA: BRACONIDAE)

Statement of Authorship

Host stage preference of *Dolichogenidea tasmanica* (Cameron, 1912) (Hymenoptera: Braconidae), a parasitoid of *Epiphyas postvittana* (Walker, 1863) (Lepidoptera: Tortricidae)

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MY designed and performed the experiments, interpreted data and wrote the manuscript; YF help in developing the idea and conducted experiment; RG contributed to manuscript production; other conceptual input; MK provided guidance throughout and assistd with statistical analysis.

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ABSTRACT

Epiphyas postvittana (Walker, 1863) (Lepidoptera: Tortricidae), or light brown apple moth (LBAM), is a highly polyphagous native Australian species and a major insect pest in Australian vineyards. *Dolichogenidea tasmanica* (Cameron, 1912) (Hymenoptera: Braconidae) is the most abundant parasitoid of the larval stages of LBAM. The prime objective of our study was to determine the parasitism success of *D. tasmanica* on different larval stages of *E. postvittana*. Additionally, we aimed to find out if larval head capsule width could be used to determine the instar number by examining the variability in head capsule size of each instar of LBAM, and the effect of rearing temperature on larval head capsule size. To determine which of the first three larval stages of LBAM parasitised is most effectively by *D. tasmanica*, choice and no-choice tests were conducted in a wind tunnel. Head capsule widths varied with rearing temperature, especially in the final fifth or sixth instar. We showed that the ranges of head capsule widths overlaps between successive instars 3 through 6, which makes it impossible to distinguish these instars of LBAM using head capsule size alone. We showed that first, second and third instars of LBAM are parasitised by *D. tasmanica*. No-choice and choice tests revealed that there are significant differences in parasitism

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among the three susceptible instars, with the highest percentage of parasitism found in second instars in no-choice and choice tests (65.1 and 65.8%, respectively), compared to first instars (61.1 and 45.1%) and third instars (27.3 and 37.5%). Wasps developed faster in third instars, but the wasp female's size was not significantly different among the instars attacked. These data confirm that *D. tasmanica* is capable of parasitising early larval instars, which is important for minimising damage through biological control.

Key words

biological control, light brown apple moth, host stage preference, wind tunnel, head capsule

INTRODUCTION

Many insect species are parasitoids in their larval phase, whereby a parasitised host serves as a resource for the developing parasitoid larva, eventually leading to host death. The host's stage can influence the foraging behaviour, reproductive success and survival of parasitoids, and also can affect the developmental rate and size of their offspring (Weisser 1994; Mackauer 1997; Harvey & Strand 2002). Hence many parasitoids exhibit an apparent preference for a specific host larval stage.

Host preference has been defined as the relative frequency of parasitised host types compared with the frequency of host types available (Hopper & King 1984). Most parasitoids have the ability to determine host quality during the parasitism process, and hosts will often be accepted or rejected according to their species, size and developmental stage (Sait *et al.* 1997; Li *et al.* 2006). For a specific parasitoid, host stage preference can occur for a range of reasons. Preference may be based on increased survival of offspring or ease of parasitism (Mattiacci & Dicke 1995; McGregor 1996).

There are differences in host quality associated with the age of the host that influence the developmental performance of the larval parasitoid. Also, as the developing host grows in size, it increases its capacity for physical and behavioural defences, which would potentially increase risks for the parasitoid (Mattiacci & Dicke 1995).

Parasitoids, through their host selection behaviour, can influence the timing of their offspring's life history events (McGregor 1996; Li *et al.* 2006). In this case host stage preference can have considerable consequences for the population growth of both a parasitoid and its host if the development and reproduction of the parasitoid are affected by the host stage at the time of parasitism (Harvey & Strand 2002). The close relationship between oviposition behaviour and fitness gains, at least for solitary parasitoids, provides a unique opportunity for linking individual behaviour and population dynamics to evolutionary processes (Wajnberg *et al.* 2008). It may also have practical importance with respect to the techniques used in the mass rearing of a given parasitoid and also to the choice of individual species used for experimental studies of parasitoids (Hagvar & Hofsvang 1991). Consequently, it is important to determine the host stage most effectively parasitised by a parasitoid. With this information, it should be possible to predict the most effective time for management of a pest by a particular wasp. Hence an understanding of the host stage preference of key parasitoid species is crucial to elucidating their role in biological control.

The major insect pest in Australian vineyards and many viticultural regions is *Epiphyas postvittana* (Walker 1863) (Lepidoptera: Tortricidae), or light brown apple moth (LBAM) (Scholefield and Morison 2010). LBAM is indigenous to south-eastern Australia (Danthanarayana 1975) and has invaded other countries, including the relatively recent introduction into California (Suckling *et al.* 2010). The use of insecticides after flowering has been restricted in Australian vineyards to minimise the likelihood of pesticide residues in wine (Essling & Cuijvers 2011). Therefore biological control plays an important role in management of LBAM populations on grapes.

The specific objective of our study was to determine the parasitism success of *Dolichogenidea tasmanica* (Cameron 1912) (Hymenoptera: Braconidae) on different larval stages of *E. postvittana*. *D. tasmanica* has been reported as the most abundant parasitoid of LBAM in Australia and New Zealand, comprising 66-97% of the parasitoids reared from LBAM consuming various crops (Charles *et al.* 1996; Paull & Austin 2006). It is an arrhenotokous, solitary, koinobiont endoparasitoid (Dumbleton 1935), however, very little information is available about its efficiency as a biocontrol agent for LBAM.

Despite a previous study by Danthanarayana (1975), the number of instars of *E. postvittana* and the mean size of each instar has been unclear. This information is essential in studying age-specific biology and behavioural responses of *D. tasmanica* to this host. Before starting experiments in this study, it was essential to enumerate the instars of LBAM and to identify them accurately. Thus, we also determined the number of instars of LBAM and described the effects of temperature on the size of the instars.

MATERIALS AND METHODS

Rearing *E. postvittana*

The culture of LBAM was obtained from the South Australian Research and Development Institute, where it was maintained since 1994 with annual additions of wild moths (approx. 200 generations). A laboratory colony of *E. postvittana* was reared at 22 ± 2 ºC and a photoperiod of 12L: 12D on an artificial diet. The diet consisted of dried lima beans (250 g), which were soaked overnight in cold water, brewer's yeast (80 g), sorbic acid (2.5 g), methyl-p-hydroxy benzoate (5 g) and water (600 ml). The mixture was sterilised in an autoclave for 20 minutes at 120 ºC. When the bean mixture cooled to 70 ºC, ascorbic acid (8 g), formaldehyde (4 ml) and agar (32 g) dissolved in water (1000 ml) were added to it and mixed well in a blender. The hot diet was poured to depth of 1-2 cm into 100 ml plastic cups that served as rearing containers. Three to five individual egg masses were cut from the adult holding cups (see below) and inserted into the diet layer in the rearing containers. Development from egg to pupa was completed in 30-35 days, within the last week of development the pupae were harvested. Pupae were sexed by examining the anatomy of the abdominal segments. Female and male pupae were placed in separate emergence cages and supplied with 10% honey solution on a wick. Six newly emerged adults of each sex were placed in a 285 ml plastic cup with vertical ridges that contained a dental wick soaked in honey solution and was covered with a sheet of voile. Cups were left in natural light at room temperature for at least three days to allow adults to lay eggs along the ridges of cups. Eggs are laid in flat overlapping masses that contain 20-50 eggs.

Rearing *D. tasmanica*

A culture of *D. tasmanica* was established from individuals collected in 2012 in South Australian vineyards. The wasps were reared on larval LBAM infesting plantain, *Plantago lanceolata* L., at 23 ± 2 ºC, 14L: 10D. When wasp cocoons formed, they were isolated in 100 ml containers together with a drop of honey. Every morning the newly emerged females were collected, caged overnight with 5 males, and provided with water and honey. Females were subsequently re-isolated in 18 mm diam. × 50 mm glass vials 1 h before being used in experiments.

Instar number and head capsule widths of *E. postvittana*

The effect of temperature on the size of immature LBAM was determined at three constant temperatures. Individual LBAM egg masses containing 50 to 60 eggs each were placed into 100 ml plastic cups containing artificial diet. A total of 15 egg masses were used, with five egg masses each reared at 15 ºC, 20 ºC and 28 ºC in incubators. When ≥ 80% of eggs had hatched, five larvae were selected randomly from each cup to rear individually in CSIRO 32-well plastic trays (10 ml/well) on artificial diet. The width of the head capsule was used as the index of larval size in all observations, as this is the only significant body part that is sclerotised. Every day the head-capsule width was measured under a dissecting microscope at a magnification of 40X, using a calibrated ocular micrometer (precision = ± 0.0125 mm). Measurements ceased when larvae pupated, and they were kept at the same temperature until adult emergence. Only healthy larvae that developed to the adult stage were included in the analysis.

Host stage preference

Although it has been reported that *D. tasmanica* is a parasitoid of first and second instars of leaf rolling Lepidoptera (Dumbleton 1935; Charles *et al.* 1996; Paull & Austin 2006), a preliminary experimental test showed that it is also capable of parasitising the third instar of LBAM. Therefore, the experimental treatments included the first three instars and host stage preference was determined by choice and no-choice preference tests as described below.

The capacity of laboratory-based host specificity tests to predict the ecological host ranges of parasitoids is limited by reduced complexity of the experimental "habitat", the fact that the full set of host finding behaviours cannot be expressed, and that only the final stage of the host selection process, host acceptance, is usually assessed (Kitt and Keller 1998; Murray *et al.* 2010). In order to diminish the influence of these limitations on results, a series of experiments was carried out in wind tunnels to present conditions in which a parasitoid could detect host cues more naturally because of air flow.

Four identical wind tunnels were used in experiments (Figure 1). Each wind tunnel had inside dimensions of 35 cm (H)× 50 cm (L)× 30 cm (W). The framework and floor of the wind tunnels were wood that was painted white. Each was covered on two sides and the top with clear acetate, and the two ends were covered in organza (12 x 22 threads/cm^2). At one end, a duct connected the wind tunnel to two 8 cm^2 computer fans (KD1208PTS2, Sunon, Taiwan) that pulled air through the tunnel. The duct was made of balsa wood covered with rice paper and coated with clear model aeroplane “dope”. The wind speed was measured in one of the wind tunnels using a thermo-anemometer (Compuflow GGA-65P, Alnor, Turku, Finland). Ten readings were taken every 20 seconds. The mean wind speed was 29 ± 0.67 cm/s (mean ± SD).

Influence of host stage on parasitism by *D. tasmanica*

No-choice and choice experiments were conducted to determine if the instars of LBAM vary in their susceptibility to parasitism by *D. tasmanica.* Host larvae were exposed to a 1-2 day-old mated female in a wind tunnel for 2 h. Each wind tunnel contained 15 small grape leaves (*Cabernet;* 3.5-4.5 cm L, 4-4.5 cm W), each infested with one host larva of a selected instar, which were placed on the leaves 24 h before the experiment. Each leaf was placed in a 10 mm diam. × 50 mm glass vial filled with water and vials were placed 6 cm apart in three rows in the wind tunnel. In the no-choice tests, all 15 larvae in a wind tunnel were the same instar, first, second or third. In the choice tests, 5 larvae each of first, second and third instars were randomly placed on the leaves. In order to stimulate the naive wasps before starting the experiment, each wasp was exposed to a second instar LBAM larva and allowed to sting it once. The wasp was then released in the wind tunnel 10 cm downwind from the first row of leaves. Choice and no-choice experiments were conducted for the same 2 h time period, and were replicated 8 times. After exposure to wasps, the leaves were collected and placed in 100 ml plastic cups and kept at room temperature for 4 days. On the forth day after each experiment, the larvae were dissected in water under a dissecting microscope to determine the frequency of parasitism of first- third instars of LBAM by *D. tasmanica*.

Effects of host stage on developmental time of *D. tasmanica*

To determine the effects of host stage on the development of *D. tasmanica*, 1-2 day-old mated female parasitoids were exposed to host larvae in a wind tunnel for 2 h. Each wind tunnel contained 2 female parasitoids that had no previous access to any live host and 2 plantain leaves that were each infested by 15 larval LBAM, either first, second or third instars 24 h before the experiment. The infested leaves were hung from the ceiling of the wind tunnel and separated by 7 cm. Wasps were released in the wind tunnel 10 cm downwind from each of the leaves. Trials with each instar were replicated 7 times. After exposure, the host larvae were placed individually in 100 ml plastic cups containing fresh plantain leaves, which were renewed daily. Plantain was used as a food source because parasitoid survival is poor when LBAM are fed on an artificial diet. Larvae were checked daily until they pupated, died, or parasitoid cocoons were produced. The date of cocoon spinning and the number of parasitoid cocoons were recorded. The developmental time from egg to spinning the cocoon, and then from cocoon to adult wasp emergence, as well as the emergent wasp's sex, were recorded. To assess the influence of host larval stage on allometric relationships, the different parasitised instars were reared with excess food under controlled conditions in the laboratory. Then the head-capsule widths of adult male and female *D. tasmanica* were measured as an indicator of adult wasp size under a dissecting microscope at a magnification of 20X.

Data analysis

All data were analysed with IBM SPSS Statistic 20. The data were subjected to analysis of variance to determine differences between means where appropriate. Where significant differences occurred, the LSD test was applied for mean separation. Data were analysed with Mann-Whitney U test, where they were not normally distributed. Differences were considered significant at $P < 0.05$; also the 0.05 probability level was used for rejection of all null hypothesises. Differences in head capsule size of larval LBAM were analysed with Kruskal-Wallis one way ANOVA. Differences were considered significant at $P < 0.01$.

RESULTS

Instar number and head capsule widths of *E. postvittana*

Larval LBAM passed through four to six instars (Table 1). There were no detectable differences in larval sizes among temperatures or developmental phenotypes (df_1=2, df_2=261, F=0.175, P=0.840), however, in later instars larval head capsule width was affected by temperature and was more variable. With the exception of first and second instars, there was overlap in the range of head capsule widths between individuals of consecutive instars. Head capsule width can be used in practice to assign the likely instar of a larva in the first three instars. In order to choose the different instars accurately for subsequent experiments, larvae with head capsule widths of 0.225, 0.325 and 0.500 mm were identified as first, second and third instars of LBAM, respectively.

Influence of host instar on parasitism by *D. tasmanica* in no-choice and choice tests

There were significant differences in parasitism by *D. tasmanica* between instars of *E. postvittana* in both the no-choice and choice tests (Fig. 2). When given a choice, *D. tasmanica* parasitised first and second instars more frequently than third instars.

Effects of host stage on developmental time of *D. tasmanica*

After oviposition by *D. tasmanica* into first instar LBAM, the time taken until cocoon formation took longer than for the other two instars, but there was no significant difference when second and third instars were parasitised. Mean time from cocoon to adult emergence was not significantly different across all instars parasitised. However, the development time from parasitism to adult female emergence was significantly longer from first and second instars than third instars (Table 2). There were not any significant differences among total development times for males.

The head capsule widths of female and male *D. tasmanica* were 0.75 ± 0.003 and 0.70 ± 0.003 mm, respectively, which was significantly different (χ^2 =71.123, *df*=1, *P*=0.000) but it did not differ statistically among host instars (df_1=2, df_2=106, F=0.805, P=0.450).

The proportion of female *D. tasmanica* did not vary significantly among instars (χ^2 =3.02, *df*=2, *P*=0.22; Table 2). However, statistical power was low and more replication is needed to confirm this.

DISCUSSION

For koinobiont species, parasitoid larval development rate and the nutritional resources available to the developing larva depend on the host age or stage of development, rather than on its size, at the time of parasitism. Also host quality is not a linear function of parasitised host size for koinobiont parasitoids, but is influenced by the host age and development stage during development, which in turn determine the host's potential tosupport parasitoid growth and development (Harvey & Strand 2002; Harvey 2005).

D. tasmanica was previously reported as a parasitoid of only the first and second instars of LBAM (Dumbleton 1935; Paull & Austin2006). However, here we showed that it also parasitises the third instar. The ability of *D. tasmanica* to parasitise the first three instars is important with respect to its potential to reduce LBAM damage in crops because the attack of earlier instars would be more likely to reduce host larval feeding damage and minimize the potential secondary damage from pathogens like *Botrytis sp.*.

There were significant differences in percentage parasitism among the first three instars. The apparent preference for first and second instars, in comparison to third instars, was reflected in the results of the no-choice and choice experiments. Babendreier *et al.* (2005) reported general agreement between no-choice and choice test results in most insect control studies that have included both, and our results reflect this. It seems that in choice experiments, because of the stronger olfactory cues produced by some of the larger larval LBAM present, wasps had greater stimulation to search and attack larvae on infested plants. However, in no-choice tests, responses to the plants infested with first instars were not as strong as those to second and third instars, probably due to less damage and associated cues produced by first instars. It seems that defensive behaviours of third instar LBAM reduced vulnerability to parasitism compared to first and second instars, and here the preference is based at least partly on ease of oviposition.

The results of choice tests indicate that when an adult female *D. tasmanica* reaches an infested plant containing a range of instars, it is capable of parasitising considerable numbers of first and second instars. This is a key finding in terms of the potential role of this parasitoid as a biological control agent of LBAM, because LBAM populations overlap during the year. If early instars of LBAM are efficiently parasitised, then the local population of LBAM should be less likely to increase, or this may be delayed leading to reduced costs of control.

Parasitoid wasps have long been considered as model organisms for examining optimal resource allocation to different fitness functions, such as body size and developmental time (Harvey 2005). Various studies have shown diverse relationships between parasitoid developmental time and host age at parasitism (Colinet *et al.* 2005). Here, we have shown that female parasitoid developmental time is dependent on the host instar that is parasitised, being longer for the first and second instars and shorter for the third instar. Also other study showed that both female and male *D. tasmanica* with access to alyssum flowers lived longer than those without flowers (Berndt & Wratten 2005).The developmental strategy of *D. tasmanica* favours a constant adult body size at the expense of increased development time in smaller hosts. This is consistent with other solitary parasitoids that feed on concealed hosts (Harvey & Strand 2002; Harvey 2005). As larval LBAM construct a silken shelter and later a leaf roll (Danthanarayana 1975), this lessens the risk that the host will die before *D. tasmanica* matures.

Progeny allocation models designed with parasitoids in mind assume that offspring develop by consuming most or all of the resources available from a single host, and that size is the most important factor affecting offspring fitness (e.g., Mackauer & Sequeira 1993). Many parasitoids exhibit host usage patterns consistent with these assumptions, but Harvey (2000) found that endoparasitic wasps in the family Braconidae often do not. Some species consume most of their host, while others feed exclusively on host hemolymph and consume a relatively small proportion of available host resources (Wharton 1993). Harvey (2000) demonstrated that the relative importance of offspring size and development time will be influenced by host ecology and the effects of selected traits on parasitoid survival. Our data indicate that the size of adult female *D. tasmanica* is unaffected by the instar parasitised. This suggests that the same developmental outcome occurs for all parasitoid larvae despite the instar into which the parasitoid egg is placed, and the total resource availability provided by the first three host instars is not limiting.

Host-stage dependent sex ratio theory by Charnov *et al.* (1981) assumes that solitary species of parasitoids are expected to oviposit more females in high quality hosts, when host size and quality vary. Host quality is generally assumed to be determined by size

(Charnov *et al.* 1981; Mackauer 1986; King 1988), but the data on adult size suggested that host instar is not an indicator of quality. In our study the number of females that emerged from first and second instar hosts was greater than 55%, but for third instar about 40% females were produced. This non-significant difference may be a due to natural random variation or a type II statistical error, which requires further study.

It would be practical if there was a simple method to determine the instar of a larva of LBAM. However, our results and those of Danthanarayana (1975) indicate that there is both plasticity in the total number of instars and the sizes of head capsules for a given pattern of development. Danthanarayana (1975) reported that LBAM can complete between five and seven instars, with the most typical number being six. In our study, we found that larvae completed four to six instars. The head capsule sizes of the first three instars are virtually the same across the laboratory and field studies of Danthanarayana (1975) and our results, with the exception of the third instar of one larva that only completed four instars. As the head capsule widths of the first three instars do not overlap, they can be used to determine instar numbers on through three. But in succeeding instars there is variation in the reported sizes of head capsules between laboratory and field (Danthanarayana 1975) and the level of variation among different temperatures. As a result of this natural variation, there is no practical way to determine the instar of LBAM based on head capsule size in instars 4-6.

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Table 1 Head capsule widths of larval *E. postvittana* that were reared at three constant temperatures. One larva completed only four instars at 20 °C (Head capsule widths 0.225, 0.325, 0.950 and 1.425 mm).

	15 °C		20 °C		28 °C	
Instar	Mean	Range	Mean	Range	Mean	Range
			Five instars			
	n = 2		n = 16		n = 10	
1	0.225	0.225	0.225	0.225	0.225	0.225
2	0.325	0.325	0.341	0.325 - 0.500	0.325	0.325
3	0.550	0.550	0.538	0.500 - 0.700	0.500	0.500
4	1.125	1.125	0.867	0.700 - 1.050	0.833	0.750 - 0.875
5	1.275	1.275	1.286	1.050 - 1.500	1.268	1.200 - 1.375
			Six instars			
	n = 8		n = 4		n = 7	
1	0.225	0.225	0.225	0.225	0.225	0.225
2	0.325	0.325	0.325	0.325	0.325	0.325
3	0.478	0.425 - 0.550	0.488	0.400 - 0.550	0.500	0.500
4	0.603	0.550 - 0.700	0.763	0.625 - 0.825	0.789	0.750 - 0.875
5	0.919	0.850 - 1.125	0.988	0.950 - 1.050	1.054	0.825 - 1.200
6	1.316	1.275 - 1.500	1.394	1.350 - 1.425	1.232	1.125 - 1.300

Table 2 Mean (±SE) developmental time (day) from stinging to cocoon formation and from cocoon formation to adult emergence of *D. tasmanica* parasitising *E. postvittana* in the no-choice test.

Host instars	Egg-Cocoon (d)		Cocoon-Adult (d)		Total (d)	
	Male	Female	Male	Female	Male	Female
1	17.8 ± 0.6^{a}	16.7 ± 0.4^{a}	9.0 ± 0.3^{a}	9.9 ± 0.2^{a}	26.9 ± 0.5^{a} (n=31)	26.5 ± 0.4^{a} (n=41)
2	15.8 ± 0.4^{b}	15.6 ± 0.3^{b}	10.2 ± 1.0^{a}	10 ± 0.2^{a}	26 ± 0.1^{a} (n=37)	25.5 ±0.3^{a} (n=46)
3	15.5 ± 0.3^{b}	15.5 ±0.2^{b}	9.1 ± 0.4^{a}	9.2 ± 0.5^{a}	24.6 ± 0.4^{a} (n=30)	24.5 ± 0.4^{b} (n=22)
ANOVA	*F=7.7, P<.01* *df =2, 95*	*F=4.7, P=0.01* *df = 2, 106*	*F=0.9, P=0.4* *df =2, 95*	*F=1.9, P=0.1* *df = 2, 106*	*F=2.2, P=0.1* *df =2, 95*	*F= 4.8, P=0.01* *df = 2, 106*

Means followed by same letter in columns do not differ statistically (*P*>0.05; LSD test).

Figure legends

Fig 1. Wind tunnel used in experiments. Arrow indicates direction of air flow.

Fig. 2. Mean percentage parasitism of first three instars of *E. postvittana* by *D. tasmanica* in (A) no-choice ($F = 11.49; df = 2, 14; P < 0.01$) and (B) choice experiments ($F = 3.87; df = 2, 14; P = 0.04$). Means labelled with the same letter in graph bars do not differ statistically ($P \geq 0.05$).

Fig. 1

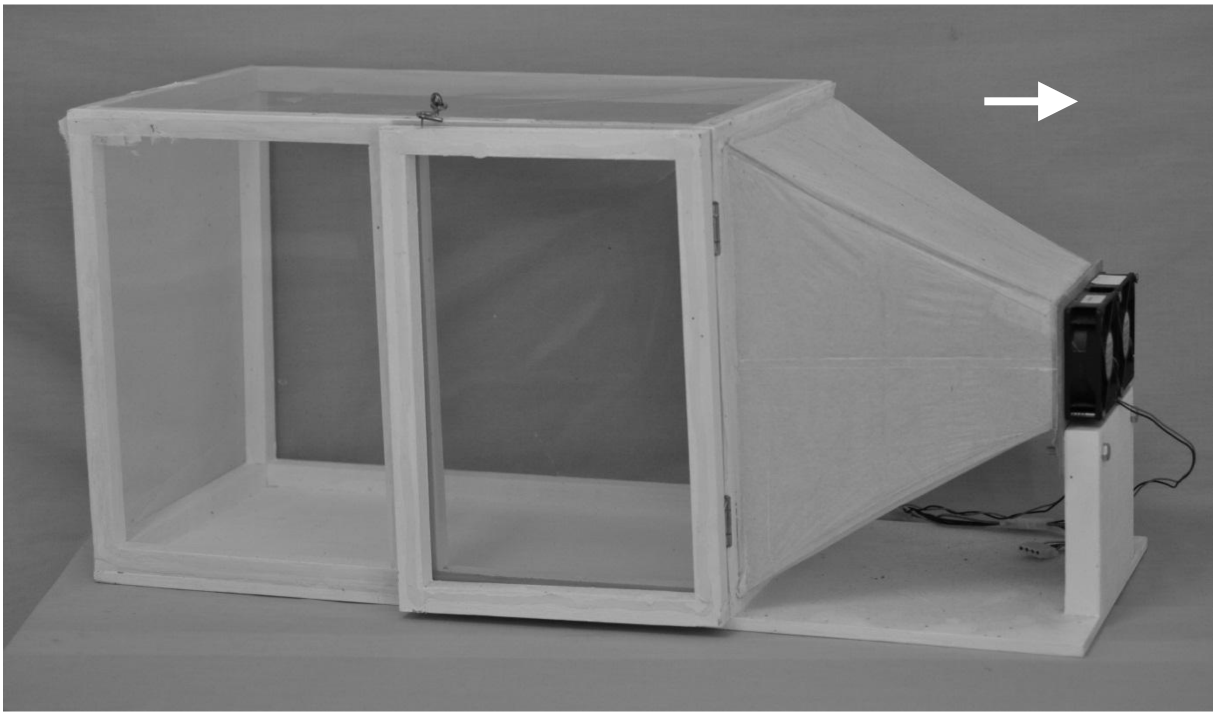
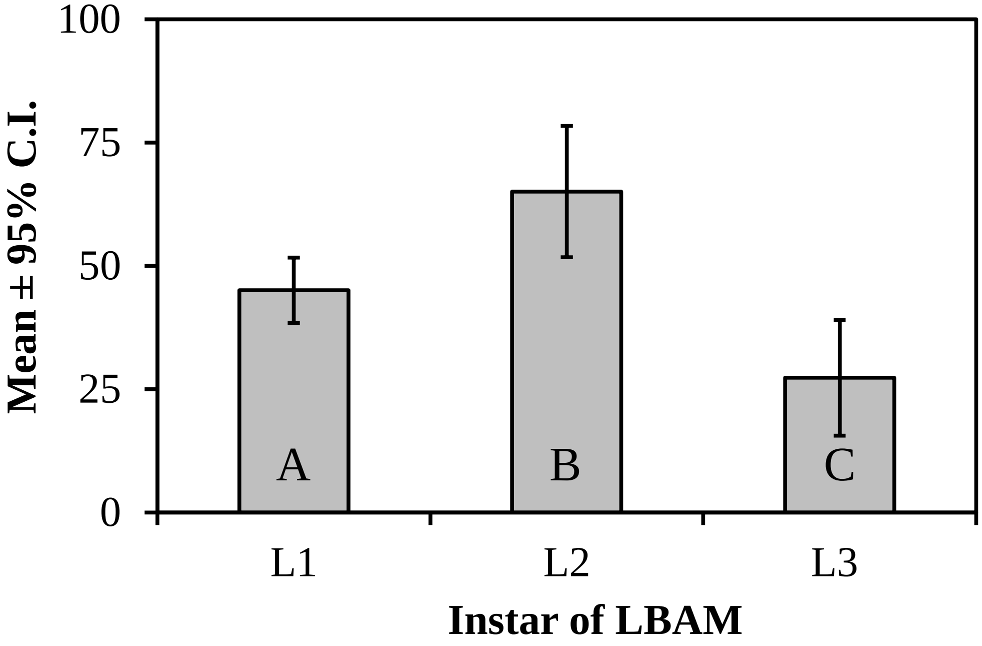
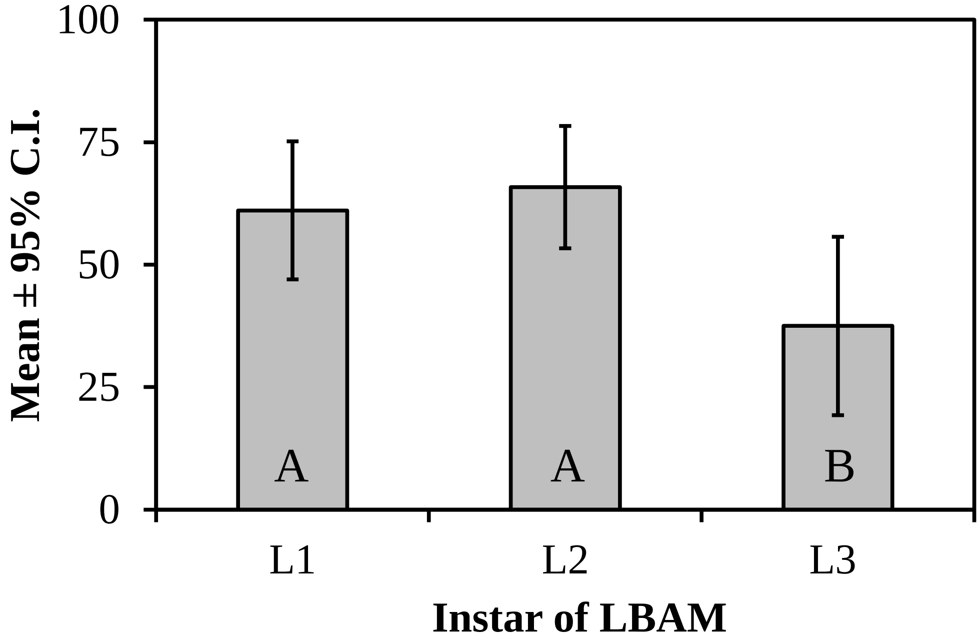


Fig. 2.

A.


Mean ± 95% C.I.
100
75
50
25
0
A
B
C
L1
L2
L3
Instar of LBAM

B.


Mean ± 95% C.I.
100
75
50
25
0
A
A
B
L1
L2
L3
Instar of LBAM

CHAPTER 3

SEARCHING BEHAVIOR OF *DOLICHOGENIDEA TASMANICA* IN RESPONSE TO SUSCEPTIBLE STAGES OF *EPIPHYAS POSTVITTANA*

Statement of Authorship

Searching behaviour of *Dolichogenidae tasmanica* in response to susceptible instars of *Epiphyas postvittana*

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MY designed and performed the experiments, interpreted data and wrote the manuscrip; RG contributed to manuscript production; other conceptual input; MK provided guidance throughout and assistd with statistical analysis.

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Abstract

Dolichogenidea tasmanica (Hymenoptera: Braconidae) is the most commonly reared parasitoid from larval light brown apple moth (LBAM), *Epiphyas postvittana* (Lepidoptera: Tortricidae), feeding on grapevines in Australia. In order to evaluate the efficiency of searching behaviour of *D. tasmanica*, a laboratory study was undertaken to determine how this wasp responds to the susceptible stages of larval LBAM. Observations of searching behaviour were made in a wind tunnel, which allowed the wasp to express its full range of behaviour. The behaviour of *D. tasmanica* and susceptibility of LBAM to parasitism varies significantly among instars. The wasp most readily parasitises newly hatched larvae, but can parasitise the first three of the six instars. The first instars cause less damage and also produce less faeces and silk than later instars, so they are associated with less volatile cues able to be detected by the parasitoid. The tendency to initiate flight to an infested leaf was lower in the presence of first instars compared to second and third instars. The flight duration was shortest when females were exposed to plants infested by third instars. An analysis of the sequence and timing of searching behaviour indicated that females respond differently to each of the instars of LBAM.

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Key words: foraging behaviour, wind tunnel, host location, host acceptance, host stage selection, larval stages, parasitoid, Braconidae, Light brown apple moth

Introduction

When a wasp parasitises a host, it is making a decision that affects its potential contribution to future generations. This potential is tightly linked to the number and quality of hosts that it chooses to parasitise, since these hosts provide the resources for the parasitoid's offspring (Luck 1990). Thus, understanding host selection behaviour is critical to explaining when and how natural enemies might regulate their host populations.

Successful parasitism of herbivorous insect hosts is preceded by several phases of host searching that lead females into the vicinity of their potential hosts. This sequential process can be divided in host habitat location, host location, and host acceptance (Vinson *et al.* 1975; Vinson 1976). In each of these phases, host-searching behaviour is characterised by responses to various types of environmental stimuli (Price 1981; Wackers and Lewis 1994; Geervliet *et al.* 1994). Godfray (1994) categorised these cues into three broad groups. They are (1) stimuli arising from the host itself, (2) stimuli arising from the host's microhabitat or food plant, and (3) stimuli indirectly associated with the presence of the host (Consuelo *et al.* 1999). In general, plant cues are most important at long distances while herbivore associated cues are more important at short distances (Vinson 1985). For long-range detection of hosts, parasitoids most often depend on indirect cues associated with the presence or activity of the host (Vet and Dicke 1992; Vet *et al.* 1995; Perfecto and Vet 2003).

Insect parasitoids may be specialized with respect to the species that can serve as hosts, or specialized on certain stages within a host species, and even on individuals of a certain age within a stage (Mattiacci and Dicke 1995b). They might be able to discriminate between old and young instars without contacting them, by exploiting instar-specific cues. Moreover, in some species, parasitoids are known to distinguish the suitability of the herbivore feeding on the plant through perception of herbivore-induced plant volatiles (HIPVs) (e.g., Mattiacci and Dicke 1995b). Plants respond differently to attacks by different herbivore species and even to different herbivore stages, thus potentially providing parasitoids and predators with specific signals (Gouinguene *et al.* 2003). So far, with the exception of a few studies (e.g., Takabayashi *et al.* 1995; Mattiacci and Dicke 1995 a and b; Canale and Loni 2006), little attention has been paid to the ability of parasitoids of herbivores to respond to cues associated with different herbivore stages.

In a biological control program that involves a parasitoid, it is important to establish which stages of the target species are suitable for parasitism (Canale and Loni 2006). Furthermore, since host densities are generally low under a successful biological control programme, the ability that a natural enemy has in locating hosts for parasitism and host feeding is important. Parasitoids with relatively poor searching ability would not frequently encounter potential hosts. Therefore, it is more effective to select parasitoids with good searching capabilities (Drost *et al.* 2000; Hudak *et al.* 2003).

In this paper we report on an investigation of the foraging behaviour of *Dolichogenidea tasmanica* Cameron (Hymenoptera: Braconidae). It is an arrhenotokous, solitary, koinobiont endoparasitoid of larvae of tortricids (Dumbleton 1935; Charles *et al.* 1996). *D. tasmanica* is the most abundant parasitoid of the light brown apple moth (LBAM), *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) (Charles *et al.* 1996; Paull and Austin 2006), but it also attacks other leafrollers (Suckling *et al.* 2001). LBAM is an herbivorous generalist pest, which is indigenous to

South-eastern Australia (Danthanarayana 1975; Thomas 1989) and has invaded other countries (Suckling and Brockerhoff 2010). It is the most destructive insect pest in Australian vineyards (Scholefield and Morison 2010). In order to evaluate the efficiency of searching behaviour of *D. tasmanica*, the objective of this study was to investigate whether the behaviour of female *D. tasmanica* differs toward the susceptible instars of LBAM feeding on plants. Drost *et al.* (1986) conducted the first study of parasitoid host-searching behaviour in a wind tunnel with *Microplitis croceipes* Cresson (Hymenoptera: Braconidae). Their results demonstrated that oriented flight responses of parasitoids to airborne host odours could be studied under laboratory conditions. Also, female *D. tasmanica* are known to fly to apple foliage that is infested with LBAM but not to uninfested foliage (Suckling *et al.* 2012). Thus, we observed foraging behaviours of female *D. tasmanica* using wind tunnel experiments and here report on the responses of this wasp to susceptible larval stages of LBAM. This knowledge should prove useful in understanding parasitoid-host interactions, and studies aimed at elucidating the capacity of *D. tasmanica* to contribute to biological control of LBAM.

Materials and methods

Rearing E. postvittana

A laboratory colony of *E. postvittana* was maintained at 22 ± 2 ºC and a photoperiod of 12 L: 12 D on an artificial diet. The diet consisted of dried lima beans (250 g), which were soaked overnight in cold water, brewer’s yeast (80 g), sorbic acid (2.5 g), methyl-p-hydroxy benzoate (5 g) and water (600 ml). For further information see Yazdani *et al.* (2014).

Rearing D. tasmanica

A culture of *D. tasmanica* was established from individuals collected in 2012 from South Australian vineyards. The wasps were reared on larval LBAM infesting plantain, *Plantago lanceolata* L., at 23 ± 2 ºC, 14 L: 10 D. When wasp cocoons formed, they were isolated in 100 ml containers together with a drop of honey to provide sustenance to emergent adults. Every morning newly emerged females were collected, caged overnight with 5 males, and provided with water and honey. Females were subsequently re-isolated in 18 mm diam. × 50 mm glass vials 1 h before being used in experiments.

Responses of D. tasmanica to susceptible larval stages of LBAM

An experiment was conducted to examine the response of *D. tasmanica* to plants infested with different larval stages of LBAM. In our previous study (Yazdani *et al.* 2014), we found that female *D. tasmanica* is able to parasitise the first three instars of LBAM so we focused on these instars. The larval instars were identified based on head capsule width (Yazdani *et al.* 2014) in which larvae with head capsule widths of 0.225, 0.325 and 0.500 mm were identified as first, second and third instars of LBAM, respectively. The experiment was conducted in a wind tunnel (for details see Keller 1990) at a wind speed of 20 cm/s at 23 ± 2 °C. Behaviour was recorded with event-recorder software (The Observer, version 3.0, Noldus 1991). Newly emerged larval LBAM were transferred to plantain leaves 16-18 h before recording an observation to allow them to produce feeding damage and deposit silk. Each leaf was infested with four larvae of a selected instar. The infested leaf was hung from a stable bar 25 cm above the floor of the wind tunnel.

Each female was exposed to a plantain leaf with wounds made with a pin for 30 s immediately before each trial to overcome its tendency to take flight when handled. The release vial was placed open end up, 25 cm downwind from an infested leaf. Observation time per individual was limited to a maximum of 10 min after release in the wind tunnel. The experiment was replicated 25 times.

Preliminary observations permitted us to develop a catalogue of searching behaviours of female *D. tasmanica*. Responses of the wasps to the different larval stages were defined using this classification of behaviours (Table 1). Three different behavioural phases, pre-flight, first flight and after first landing, were analysed for each instar exposed. The time from introduction into the wind tunnel until initiation of flight from vial (Pre-flight), and time from initiation of flight until first landing on the infested leaf (Flight time), were recorded for each female. Wasp behaviour was recorded continuously. Frequencies and mean durations of occurrence for each type of behaviour were calculated from the time wasps first landed on the infested leaf.

A separate experiment was carried out to determine which factors might affect stinging duration for the three instars. These factors were investigated among female wasps, instars and order of stinging of different instars. The experiment was repeated six times and, for each replication, six inexperienced 1-day old mated females were exposed to first, second and third larval instars in random order. One larva feeding on a plantain leaf was exposed to a 1-2 day-old female *D. tasmanica* in a 9 cm Petri dish. The stinging time was considered to be from when the female first stung the host until she departed. The interval time among the expositions was 1 min.

Statistical analyses

The elapsed time before and after taking flight from the vial until first landing on the infested leaf and the mean duration, frequency and proportion of time devoted to each type of behaviour on the leaf, were calculated for each instar. The differences in frequency and duration of behaviours were analysed with one way ANOVA. To visualize the time until specific events were observed, Kaplan - Meier survival curves were drawn and differences among these curves were tested using a one way Kruskal - Wallis Test (n = 25). Differences were considered significant at $P < 0.05$.

The temporal patterns of behaviour after first landing were analysed by comparing behavioural flow diagrams (Field and Keller 1993). The aim of this analysis was to gain insight into how behaviour was organised rather than to develop a precise model of behaviour. First-order behavioural transition matrices were constructed for each individual, with rows corresponding to preceding behaviours and columns to following behaviours. Data from all individuals for each treatment were pooled in the analysis. By pooling, it was assumed that there were no significant differences among individuals. Some further pooling of behaviour in the composite Matrices was necessary so that no expected values were less than 1 and no more than 20% of the expected values were less than 5. In our case the bahaviors *still*, *fly*, *groom*, *rest*, *drop* and *walk* were pooled and presented together as "Other" in the analysis. The principle diagonal elements of these matrices were logical zeros since behaviours could not follow themselves in our records. The expected values of the matrix cells were determined using the iterative proportional fitting method of Goodman (1968). The significance of this analysis was adjusted to a table-wide level of 0.05 using the Sequential Bonferoni Method (Rice 1989). The results of analyses of behavioural transition matrices are presented graphically in kingetograms (Field and Keller 1993; Wang and Keller 2002). Finally, the factors that might affect the stinging duration were analysed by univariate analysis of variances.

Results

Pre-flight behaviour: There was a period of pre-flight orientation in the presence of all larval instars that was characterised by walking while antennating, stationary and pointing behaviours. The time to initiate the first flight differed among host instars. The results of a Kruskal-Wallis test were significant between the susceptible instars (H (2) = 8.065; P = 0.018), with a mean rank of 47.90, 34.80 and 31.30 for first, second and third instars respectively. The time to initiate the first flight toward first instars was significantly longer than toward second or third instars (*P* = 0.014), but there was no significant difference in this response between second and third instars (*P* = 0.0691) (Fig. 1a).

Flight behaviour: After taking flight from the release vial, females that flew toward a leaf infested with first instars most often hovered in front of it at a distance of a few centimetres, which did not happen for second and third instars. The results of a Kruskal-Wallis test were significant between the susceptible instars (H (2) = 6.975; *P* = 0.031), with a mean rank of 44.28, 40.90 and 28.82 for first, second and third instars respectively.

So, the time after taking flight until the first landing on the plant was significantly longer for first instars (*P* = 0.033) (Fig. 1b).

After first landing: Upon arrival at an infested leaf, parasitoids responded to feeding damage and faeces by antennating and probing the leaf surface with their ovipositor. This was typically followed by stinging (Fig. 2). Sometimes a larva dropped from the leaf and hung on silk, when they were approached by *D. tasmanica*. When the first and second instars dropped from the leaf, the wasp reacted with dropping behaviour, which was sometimes characterised by walking down the silk strand and dropping to the floor if the silk was broken. After dropping to the floor, the wasp quickly walked and antennated the area near the host, which often led to it being stung. Third instars displayed the most vigorous defensive behaviours. When wasps touched them with their antennae, larvae moved rapidly and dropped from the leaf more frequently, sometimes without hanging on silk. Females typically reacted by pursuing them, sometimes stinging them and then flying after stinging. After stinging first instars, females tended to resume searching but engaged in other behaviour after stinging second instars. In contrast, post stinging behaviour had no detectable non-random pattern after a third instar was stung (Fig. 2).

For all stages, females spent a large fraction of their time antennating, but females spent more time probing the plant material with their ovipositors when third instars were present. In comparison to first and second instars, wasps searching in the presence of third instars flew away and landed again more often, but these flights were typically brief. Also, after females encountered a third instar, they spent a considerable amount of time either standing still on the substrate without moving their antennae or grooming, which we interpret as recovery time after stinging (Table 2).

Stinging behaviour: Data indicated that the susceptibility of LBAM to parasitism by *D. tasmanica* varies among instars. Statistical analysis showed that the stinging duration for different instars was significantly different (Table 2). There was a statistically significant difference between the susceptible instars (H (2) = 12.86, $P = 0.002$), with a mean rank of 28.86, 36.40 and 48.74 for first, second and third instars respectively.

The cumulative survival function shows that the rate of stinging first instars was greatest (Fig. 4). Observations suggested that the rate of host encounter was influenced by host defensive behaviour and wasp responses to it.

In the separate experiment on stinging behaviour, the stinging duration was not significantly different among females ($df_1 = 35$; $df_2 = 72$; $F = 0.954$; $P = 0.55$). Similarly, the order of stinging also had no significant effect on stinging duration ($df_1 = 2$; $df_2 = 68$; $F = 2.168$; $P = 0.122$). However, there were significant differences in stinging duration among larval instars ($df_1 = 2$; $df_2 = 68$; $F = 26.296$; $P \leq 0.000$). In the post-hoc analysis, the stinging duration on third instars was significantly shortest ($P=0.002$) and the first instar was longest ($P = 0.002$) (Fig. 5), which was consistent between experiments.

Discussion

We found that female *D. tasmanica* responds differently to distant cues associated with the susceptible stages of larval LBAM. The tendency to take flight toward a leaf infested with first instars was less than when second and third instars were present (Fig. 1a) and wasps more quickly landed on plants infested with second and third instars (Fig. 1b). First instars cause the least damage and produce the least faeces and silk, so they are likely to be associated with the least volatile emissions. The evidence indicates that female *D. tasmanica* do not detect them as quickly as they detect plants infested with later instars. It has been shown that female *D. tasmanica* fly exclusively upwind to damaged apple seedlings in a wind tunnel even after the removal of host larvae (Suckling *et al.* 2012). So it is known that attractive volatile chemicals are released from feeding sites. Similar observations in which wasps perceived lepidopteran host odours and initiated flight toward sources of host-associated odour have been reported in others studies (Turlings and Wäckers 2004), which are consistent our results. Thus plant responses to different herbivores lead to the release of volatile blends that may provide natural enemies with specific information on the identity of the herbivore (Turlings and Wäckers 2004; McCormick *et al.* 2012). It has been shown that the behaviour of parasitoids is influenced by quantitative changes in volatiles associated with varying host stages (McCormick *et al.* 2012), whereas qualitative changes in volatile profiles can also be caused by different growth stages of herbivores (Takabayashi *et al.* 1995; Turlings *et al.* 2000). Thus *D. tasmanica* responds to the susceptible instars of LBAM differently even before contacting them, either as a result of qualitative and/or quantitative differences in HIPVs. Further study is required to determine whether the wasp's behaviour is specifically influenced by qualitative changes in volatile profiles or simply through the quantitative changes caused by various instars.

Analysis of the behaviour of female *D. tasmanica* searching on plants infested with LBAM indicates that it differs among susceptible host instars (Table 2; Fig. 2 and 3). The amount of feeding damage caused by different larval stages was clearly different, and wasps allocated their times differently on leaves infested with different larval stages (Table 2 and Fig. 3). For instance due to the presence of more feeding damage and faeces on infested plant with third instars, wasps spend more time for probing while they spend considerable amount of time for antennating the leaf surface to search for any symptom of presence of first instars. So our results indicate that cues associated with different instars play a role in the behavioural sequences of this parasitoid (Fig. 2). *D. tasmanica* actively responds to the presence of some host-associated cues associated with feeding damage and faeces produced by different instars, by antennating and probing behaviours that slow the pace of their movement. This reduces the potential searching area and is likely to increase the probability of detecting hosts. Similar behaviour was described for *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) a parasitoid of *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae) (Wang and Keller 2002), and *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae) a parasitoid of *Heliothis zea* Boddie (Lepidoptera: Noctuidae) (Hérard *et al.* 1988). Females typically reacted by pursuing larvae which were scaping, sometimes stinging them and then flying after stinging. Similar behaviour was described for *Cotesia plutellae* Kurdjumov, which is another braconid wasp (Wang and Keller 2002). Wasps searched more frequently immediately after stinging the first instars, whereas for second and third instars a repeated cycle of searching and stinging was not so clearly detected (Fig. 2).

Host searching behaviour of *D. tasmanica* differed substantially (Fig. 2 and 3) not only in response to damage and cues, but also to host defensive behaviour. A change in defensive behaviours is common in caterpillars as they grow, which influences the searching success of their parasitoids and the resulting levels of parasitism (Waage 1983; Gross 1993; Weseloh 1993). Although here we focused specifically on parasitoid behaviour, host behaviour should also be considered because host behavioural defences may act after host location by reducing the probability of parasitoid oviposition (Bugila *et al.* 2014). We assume that the defensive behaviour of third instar LBAM and also their ability to escape from the site where they were contacted by wasps caused them to be less susceptible to being parasitised by *D. tasmanica* (Fig. 4). The results of our last experiment (Fig. 5) also showed that first instars are handled longer during stinging, which may be due to their less vigorous behaviour. A similar observation was made with *Cotesia glomerata* Linnaeus (Hymenoptera: Braconidae), and it was concluded that the mean duration of oviposition was significantly reduced by the defensive reactions of *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae), which increased with the host age (Brodeur 1996). Future study of host defences should contribute to a deeper understanding of parasitoid oviposition behaviour, as well as to understanding why some insect species are less susceptible to parasitism than others (Gross 1993). As *D. tasmanica* parasitises different species of leafrollers and LBAM is known to feed on more than 500 species of plants (Suckling and Brockerhoff 2010), further study is required to determine which other factors are involved in its interactions with host insects and their host plants. Furthermore, it may help to explain why *D. tasmanica* is the most common LBAM parasitoid in vineyards, and to assess if other species can be utilised though increasing vineyard populations. This knowledge is of practical importance because it will provide the deep understanding that is necessary to select the best parasitoid species to be managed using conservation biological control against light brown apple moth (Bugila *et al.* 2014).

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Table 1 A catalogue of behavioural acts of *D. tasmanica* searching for LBAM.

Events	**Descriptions**
Antennating (An)	Walking while drumming the substrate with antennae
Dropping (Dr)	Wasps either walked on to silk and then dropped to the floor or dropped to the floor directly from the leaf
Flying (Fl)	Flying
Grooming (Gr)	Preening antennae, legs, or both sides of wings
Pointing (Po)	Facing toward the target and continuously moving the raised antennae
Probing (Pr)	Walking while drumming the substrate with antennae and jobbing with ovipositor
Still (Stil)	Standing still on the substrate without moving antennae
Stationary (Stat)	Standing still on the substrate with moving antennae
Stinging (Stin)	Piercing host with ovipositor
Walking (Wa)	Walking while antennae don’t touch the substrate

Table 2 Characterization of *D. tasmanica* female behaviours in response to different larval stages of LBAM after landing on infested leaves.

Behaviours	**Mean frequency/min ± SE**		
	L1	**L2**	**L3**
Antennating	1.88± 0.19	1.90 ± 0.20	1.80 ± 0.17
Dropping	0.24 ± 0.16	0.08 ± 0.05	0.40 ± 0.24
Flying	0.04 ± 0.01^{b}	0.07 ± 0.02^{b}	0.21 ± 0.06^{a}
Grooming	0.23 ± 0.04^{b}	0.21 ± 0.04^{b}	0.33 ± 0.05^{a}
Probing	1.30 ± 0.18	1.41 ± 0.18	1.33 ± 0.15
Still	0.20 ± 0.03^{b}	0.20 ± 0.03^{b}	0.30 ± 0.04^{a}
Stationary	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Stinging	0.55 ± 0.05^{a}	0.40 ± 0.04^{b}	0.25 ± 0.02^{c}
Walking	0.05 ± 0.03^{b}	0.01 ± 0.01^{b}	0.13 ± 0.04^{a}
	Mean duration ± SE		
Antennating	14.26 ± 1.4^{a}	11.95 ± 1.13^{ab}	9.34 ± 1.02^{b}
Dropping	-	-	-
Flying	17.71 ± 8.84	14.89 ± 6.01	14.56 ± 4.42
Grooming	13.32 ± 2.65	10.37 ± 1.41	14.69 ± 1.82
Probing	5.92 ± 0.77^{a}	5.79 ± 0.63^{a}	9.77 ± 1.29^{b}
Still	83.07 ± 25.1^{a}	133.7 ± 29.56^{b}	94.56 ± 22.94^{a}
Stationary	11.21 ± 7.11	4.84 ± 1.04	5.75 ± 1.63
Stinging	24.59 ± 3.87^{a}	18.98 ± 3.1^{b}	7.55 ± 1.8^{c}
Walking	10.53 ± 6.19	2.85 ± 1.68	4.62 ± 0.83

Different letters within a row indicate significant differences between LBAM larval instars. ($P < 0.05$).

Figure legends

Fig. 1. Cumulative survival curves for (a) time to initiate first flight and (b) duration of first flight, for *D. tasmanica*. Different letters indicate significant differences between susceptible instars of LBAM ($P < 0.05$).

Fig. 2. Kinetograms of female *D. tasmanica* searching on a plantain leaf infested with first, second and third instar *E. postvittana.* The areas of circles are proportional to overall frequency of each behavioural event. Groom, walk, still, rest, drop and fly were pooled in the analysis and appear as "Other". Connecting lines show the non-random patterns of behaviour, as (➡) indicates behavioural transitions that are likely to happen and (– –➔) indicates those that are unlikely to happen ($P < 0.05$). The widths of connecting lines are proportional to the standardised residual of residuals of deviations from expected values:

$$\text{Standardized residual} = \frac{\text{observed} - \text{expected}}{\sqrt{\text{expected}}}$$

Fig. 3. Proportion of total time for each behavioural response to susceptible instars of LBAM. See Table 1 for definition of behaviours and associated abbreviations.

Fig. 4. Cumulative survival functions for time to sting four larvae of first, second and third instar LBAM. Different letters indicate significant differences among instars of LBAM ($P < 0.05$).

Fig. 5. The stinging duration of *D. tasmanica* when attacking susceptible instars of LBAM.

Fig. 1a

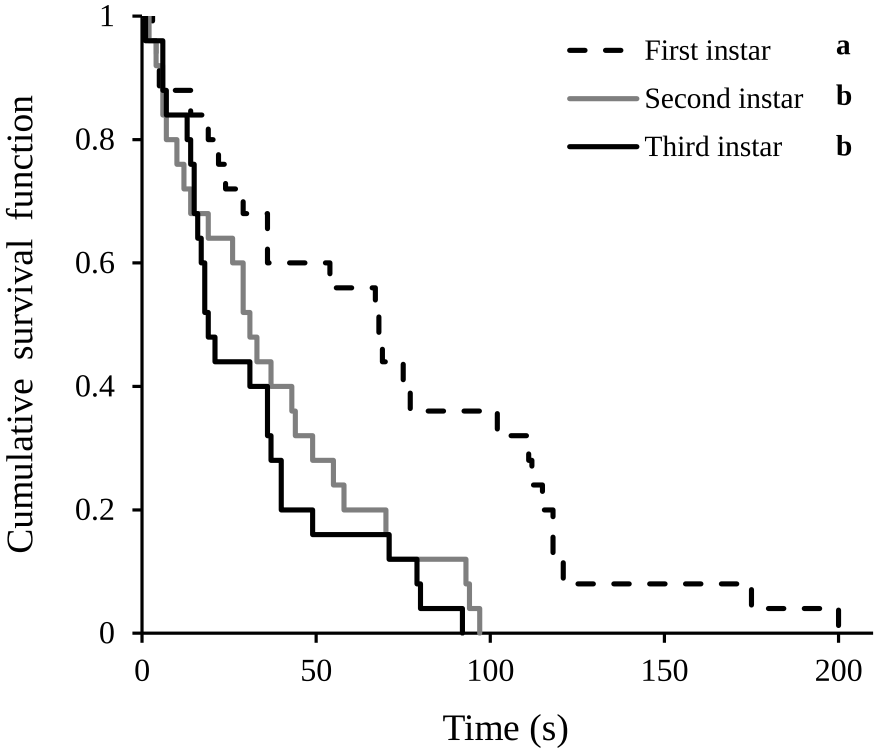

First instar a
Second instar b
Third instar b
Cumulative survival function
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
Time (s)

Fig. 1b

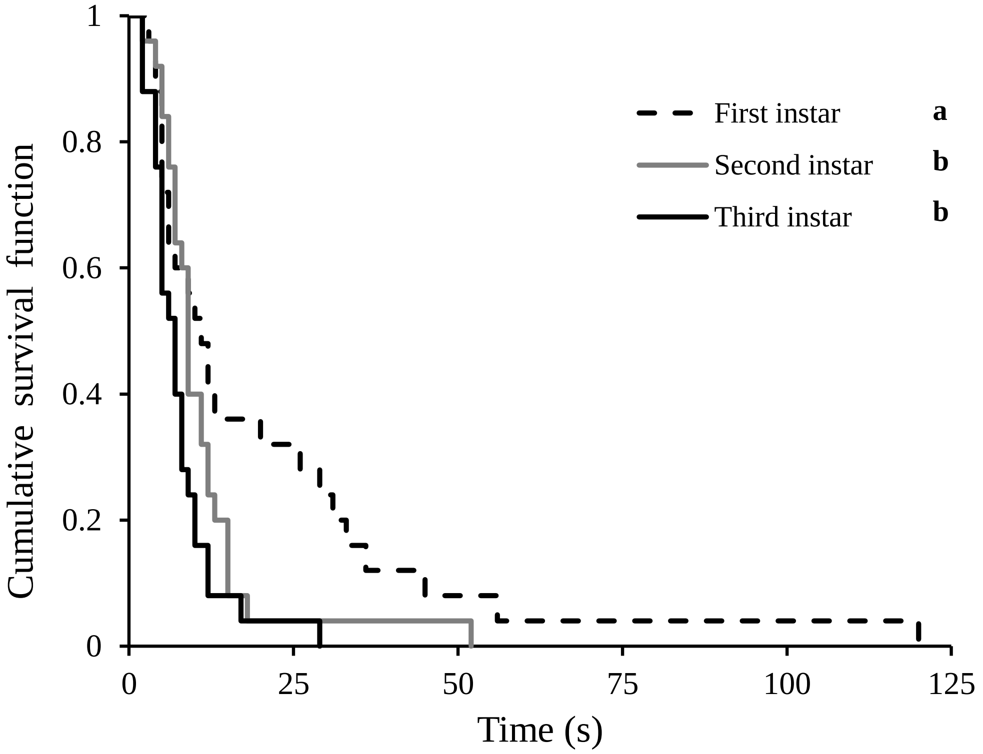
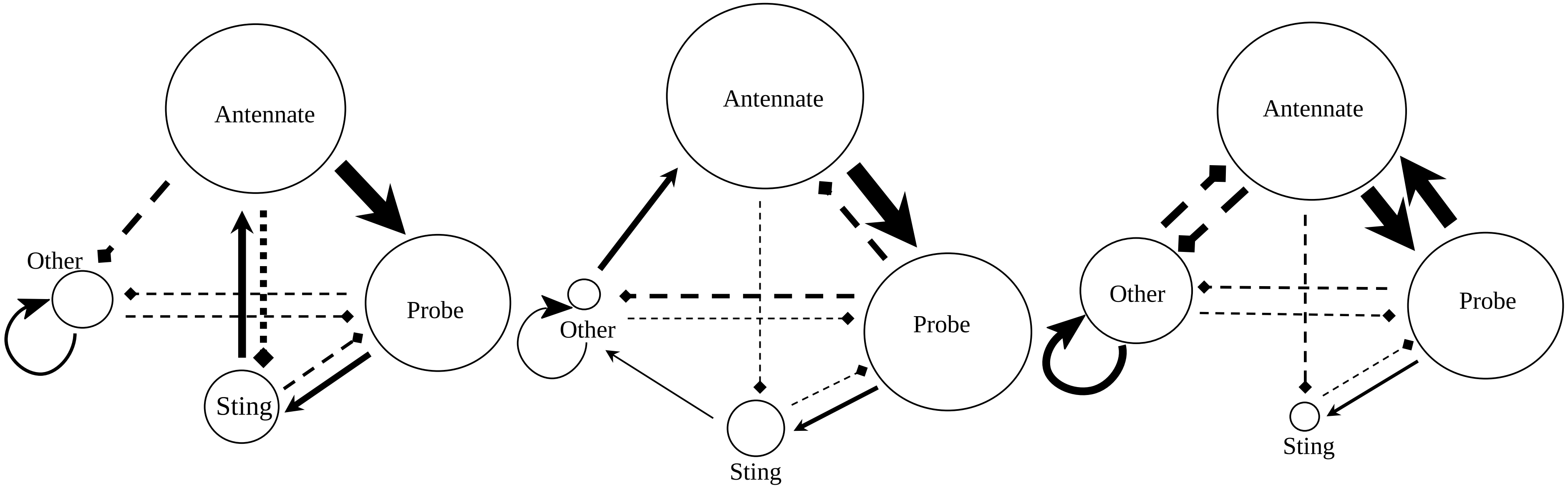

First instar a
Second instar b
Third instar b
Cumulative survival function
1
0.8
0.6
0.4
0.2
0
0
25
50
75
100
125
Time (s)

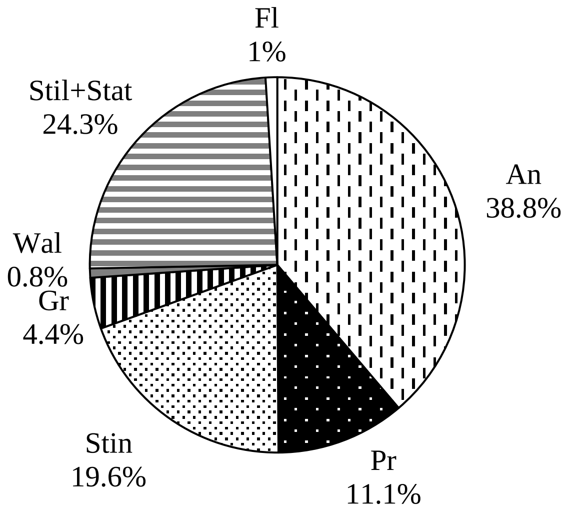
Fig. 2



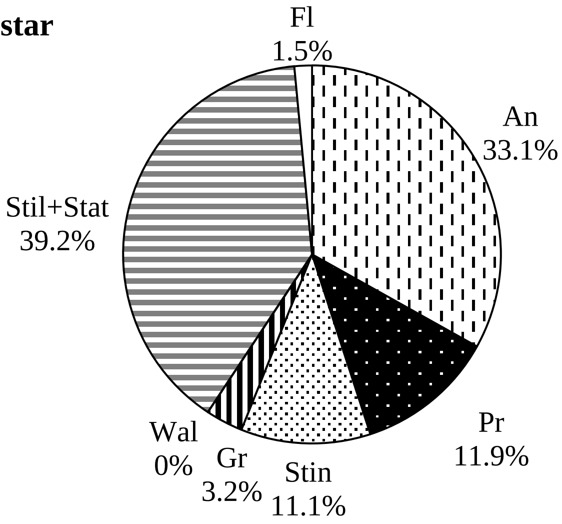
a) First instar **b) Second instar** **c) Third instar**

Fig. 3

a) First instar



b) Second instar



c) Third instar

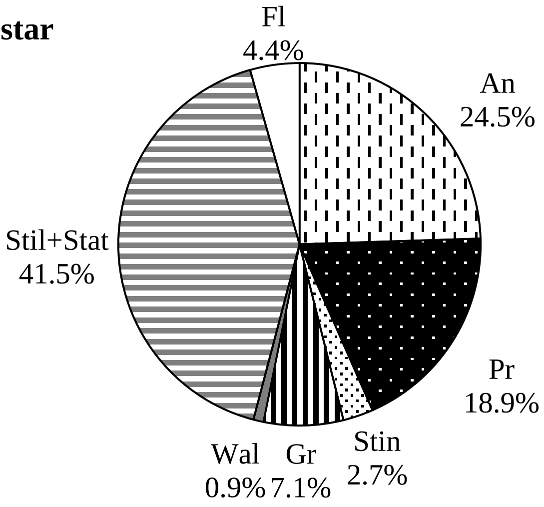
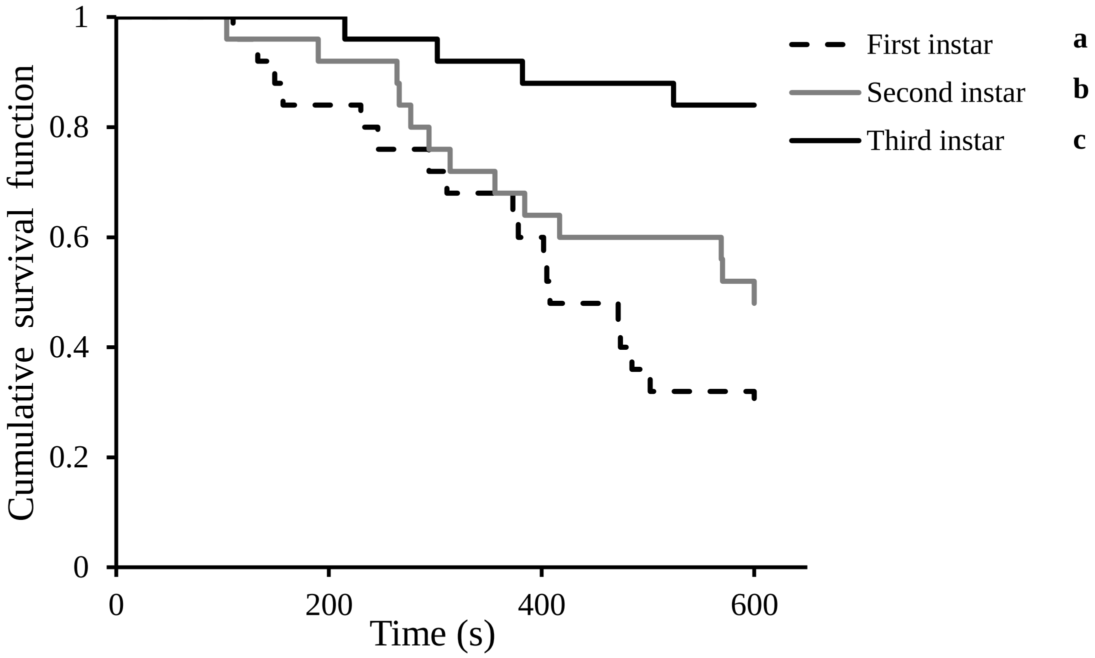
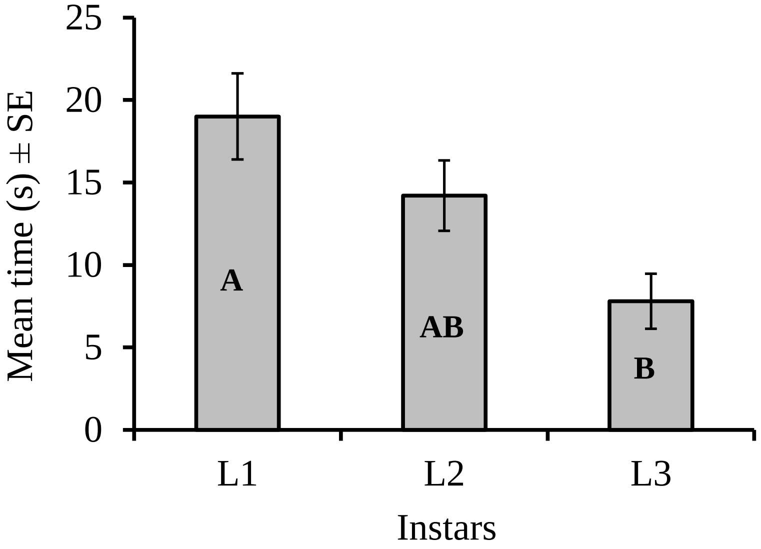


Fig. 4



Cumulative survival function
1
0.8
0.6
0.4
0.2
0
0
200
400
600
Time (s)
First instar
a
Second instar
b
Third instar
c

Fig. 5



Mean time (s) ± SE
25
20
15
10
5
0
A
AB
B
L1
L2
L3
Instars

CHAPTER 4

HOST DISCRIMINATION BY THE SOLITARY ENDOPARASITOID *DOLICHOGENIDEA TASMANICA* (HYMENOPOTERA: BRACONIDAE)

Statement of Authorship

Host discrimination by the solitary endoparasitoid *Dolichogenidea tasmanica*
(Hymenopotera: Braconidae)

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MY designed and performed the experiments, interpreted data and wrote the manuscrip; RG contributed to manuscript production; other conceptual input; MK provided guidance throughout and assistd with statistical analysis.

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Abstract

Successful parasitism of a host partly depends on a female's assessment of its quality, including whether the host has already been parasitised or not. We conducted experiments to elucidate host discrimination by *Dolichogenidea tasmanica* (Hymenoptera: Braconidae). It is the most commonly collected parasitoid of light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae). To assess the rate of superparasitism avoidance by *D. tasmanica,* female wasps were given choices between (1) unparasitised hosts versus freshly self parasitised hosts, (2) unparasitised hosts versus hosts at 24 h post-self parasitisation, and (3) freshly self-parasitised hosts versus hosts freshly parasitised by a conspecific female. Results confirm that host discrimination occurs in *D. tasmanica*. Females avoid laying eggs in hosts that have been parasitised by themselves or conspecifics, even though the frequency of first encounter with either an unparasitised or a parasitised host was the same for all choices. Thus, it appears that females are not able to discriminate the host parasitisation status prior to contacting a host, but host acceptance is not random. Host discrimination is time-dependent, with greater avoidance of superparasitism after 24 h. The ability of female *D. tasmanica* to distinguish healthy from parasitised hosts suggests that it could be an effective biological control agent in regulation of host populations. It should also ensure production efficiency in parasitoid mass-rearing.

Key words*:* Superparasitism, self-parasitised, conspecific parasitised, *Epiphyas postvittana*, light brown apple moth, biological control

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1. Introduction

Successful parasitism of a host encountered by a female parasitoid depends largely on host quality. One feature of host quality is whether the host has previously been parasitised or not. Females of many parasitoid species are able to distinguish between parasitised and unparasitised hosts (Salt, 1961), an ability termed host discrimination (van Lenteren, Bakker, &Van Alphen, 1978; Visser, Jacques, van Alphen, & Henk, 1990). Although many solitary parasitoids are known to be able to distinguish between unparasitised and parasitised hosts (Salt, 1961; van Lenteren et al., 1978 & 1981), superparasitism is common in nature (Salt, 1934 & 1961; Schroder, 1971), even in solitary parasitoids (Visser, 1993). Under specific conditions, superparasitism may be an adaptive strategy, which results from a balance between the benefits and the costs of laying an egg in an already parasitised host (van Alphen & Visser, 1990; Rosenheim & Hongkham, 1996; White & Andow, 2008; González, Montoya, Pérez-Lachaud, Cancino, & Liedo, 2010). It has been suggested that superparasitism by solitary parasitoids should occur based on their decision to oviposit rather than by imperfect discrimination between healthy and parasitised hosts as first suggested (van Lenteren, 1981; van Dijken, van Stratum, & van Alphen, 1992; Ueno, 1999; Zhang, Gu, & Wang, 2010).

van Lenteren et al. (1978) suggested that laboratory studies can remove environmental complexity and variability to give a quick and accurate answer to the question of whether parasitic wasps are able to discriminate or not. They argued that most field data are insufficient to allow any conclusions about host discriminative ability. Laboratory studies also provide insights into the circumstances under which superparasitism occurs. Furthermore, superparasitism studies should be considered in parasitoid mass-rearing protocols and augmentative field-release biocontrol programs to ensure production efficiency, prevent waste of parasitoid reproductive potential and to infer the performance of the parasitoids in the field (González et al., 2010). So, host discrimination has been the subject of considerable research.

Dolichogenidea tasmanica (Cameron) (Hymenoptera: Braconidae) is a solitary endoparasitoid that is native to Australia. We examined its response to a key larval host and well known pest, the light brown apple moth (LBAM) *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae). LBAM is a native species in south-eastern Australia, and it has been introduced to Western Australia, New Zealand, Hawaii, much of England, and California (Suckling & Brockerhoff, 2010). LBAM is the most damaging insect pest of wine grapes in Australia (Scholefield & Morison, 2010). *D. tasmanica* has been reported as parasitising the first three instars of LBAM (Yazdani, Feng, Glatz, & Keller, 2014 in press), however there are no reports regarding its superparasitism choices. Our observations of the searching behaviour of *D. tasmanica* indicate that hosts are frequently re-encountered by the same individual wasp. Therefore this study was conducted to determine whether *D. tasmanica* avoids superparasitism. The experiments described here deal with both self-superparasitism, i.e., laying eggs into a host that a female has herself previously parasitised, and conspecific superparasitism, i.e., superparasitising a host containing a conspecific’s egg.

2. Materials and Methods

2.1 Insect Culture A laboratory culture of *E. postvittana* was maintained at 22 ± 2 ºC and a 12 L: 12 D photoperiod, on an artificial diet mainly consisting of lima bean, agar and brewer’s yeast. The culture of *D. tasmanica* originated from specimens collected in South Australian vineyards and was maintained on plantain, *Plantago lanceolata* L., infested with larval LBAM at 23 ± 2 ºC and a 14 L: 10 D photoperiod, for several generations in the laboratory (for details see Yazdani et al., 2014).

2.2 Response to self-parasitised hosts We conducted two choice experiments to assess the rate of self-superparasitism avoidance by *D. tasmanica* and to examine the effect of the time elapsed since the first parasitisation on superparasitism. In the first experiment, wasps were presented with unparasitised hosts versus hosts that were freshly parasitised after 1 hour. In the second experiment they were presented with unparasitised hosts versus hosts at 24 h post-parasitisation. A pair of healthy early second instar LBAM of similar size was chosen for each replicate test. One larva feeding on a plantain leaf was exposed to a 1 to2 day-old female *D. tasmanica* in a 9 cm Petri dish. When the parasitoid female had stung it and departed, the parasitoid was enclosed in an 18 mm diam. × 50 mm glass vial containing a drop of honey and kept for either 1 or 24 hours, depending on the treatment to which it was assigned. After the relevant period, the parasitised larva along with an unparasitised larva were placed in a Petri dish with the same female parasitoid. After the first of these hosts was stung, the wasp was removed along with the two hosts. To facilitate handling of larval LBAM in the choice tests, one larva was randomly marked with a permanent marker (Artline® EK-700, Shachihata, Japan) on the dorsum. To ensure that this did not influence host selection, wasps were then exposed to a second pair of unparasitised second instar LBAM larva, one marked and one unmarked as a control. A neutral result, confirmed that the mark did not influence the host selection of *D. tasmanica*, and also these larvae were dissected to check that female wasps were fertile. After they were stung, the larvae were placed in separate 100 ml plastic cups with a plantain leaf as food, and kept at room temperature for three days. On the third day, the larvae were dissected with fine forceps under water to determine the numbers of eggs or larvae of *D. tasmanica* in each host larva. Forty and 36 replicates (different female *D. tasmanica*) were conducted for each interval.

2.3 Response to conspecifically-parasitised hosts The aim of this experiment was to assess the rate of superparasitism by *D. tasmanica* of both self-parasitised and conspecifically-parasitised hosts. A pair of healthy early second instar LBAM feeding on a plantain leaf was exposed to a female *D. tasmanica* in a Petri dish. Female wasps were paired to produce a replicate where an individual could assess a single self-parasitised and single conspecifically-parasitised larva. Each host was allowed to be stung once and subsequently isolated in a Petri dish on a plantain leaf for 1 hour. Both of the paired parasitoid females were exposed to another second instar LBAM as controls to confirm fertility. Then one of the parasitoid females was held in a 18 mm diam. × 50 mm glass vial containing a drop of honey for 1 hour. One of the previously attacked host larvae was randomly marked with permanent marker. After one hour, both of the previously attacked hosts were exposed to the isolated parasitoid female, in a Petri dish. This female was again allowed to sting once. All attacked larvae were kept at room temperature and three days later were dissected in water under a dissecting microscope to determine the numbers of eggs or larvae of *D. tasmanica* in each. The experiment was replicated 37 times.

3. Statistical analysis

The numbers of attacks for each pair of options in the choice tests were compared by Binomial tests and differences were considered significant at $P < 0.05$. The proportions of numbers of attacks that led to ovipositioning, and occurrence of superparasitism were compared using Chi-squared tests with Cochran's continuity correction (Cochran, 1942).

4. Results

4.1 Rate of attack Control tests show that marker had no effect on host selection by *D. tasmanica*, either in the frequency of marked vs. unmarked hosts that were attacked (P

= 1.0) or in the frequency of eggs laid in hosts that were stung (P = 0.909). A wasp egg was found in 109 out of 112 dissected control larvae (97.3%), which indicates that eggs could be found with a high level of confidence. There was no evidence that hosts were attacked discriminately in any of the experiments ($P \geq 0.35$) (Figure 1).

4.2 Frequency of oviposition When wasps stung host larvae, regardless of which type of larva was marked, the rate of parasitism for unparasitised hosts was significantly higher than previously self-parasitised hosts (Figure 2). So, female *D. tasmanica* showed a significant preference to lay eggs in unparasitised larval LBAM compared with hosts that were parasitised either 1 or 24 h previously (*P* = 7.34 E -06 and *P* = 1.05 E -08, respectively). The rate of superparasitism decreased from 26% in hosts that were self-parasitised 1 h earlier to nil after 24 h. *D. tasmanica* oviposited more frequently in conspecifically-parasitised larval LBAM than freshly self-parasitised hosts (*P* = 0.03). Although not evaluated experimentally, the rate of parasitism of hosts parasitised by a conspecific was less than that for unparasitised hosts.

5. Discussion

The observed host discrimination behaviour of *D. tasmanica* is consistent with many other species of parasitoids (van Lenteren, 1981; van Alphen & Visser, 1990). We found no evidence that females can perceive the parasitism status of a host before it is contacted. It seems unlikely that *D. tasmanica* detects any marking pheromone or other indication of parasitism of a previously parasitised host before contacting it. This is consistent with other species, such as *Collyria calcitrator* (Salt, 1932), *Anagrus delicatus* (Rosenheim, & Hongkham, 1994) and *Diadegma semiclausum* (Wang, 2002).

Females responded differently to parasitised hosts once they were contacted. Experiments demonstrated that *D. tasmanica* avoids laying eggs in hosts that have previously been parasitised by themselves or to a lesser extent by conspecifics (Figure

2). Our results correspond with the findings of other studies of solitary parasitoids (van Lenteren, 1981; van Dijken et al., 1992; Ueno, 1999; Zhang, Gu, & Wang, 2010). *D. tasmanica* probably deposits a mark when parasitising a host larva (van Lenteren, 1981), which may be deposited internally (Guillot & Vinson, 1972). The tendency to avoid superparasitism increased 24 h after oviposition. In this species, host discrimination expression was therefore time-dependent, similar to *Aphidius nigriceps* (Cloutier, Dohse, & Bauduin, 1984), *Ephedrus californicus* (Chow & Mackauer, 1986), *Ephedrus cerasicola* (Hofsvang, 1988), *Nemeritis canescens* (Hubbard, Marris, & Rowe, 1987) and *Dinarmus basalis* (Cloutier et al., 1996). This suggests that the larval LBAM response to parasitism, as a result of the ovipositing female's injection of the egg, venom and polydna-viruses (Pennacchio & Strand, 2006), produced cues that deterred oviposition. The apparent decrease in superparasitism at 24 h (compared to 1h) indicates that the relevant cues are unlikely to be residual from the ovipositioning event but rather are produced by the egg, hatching parasitoid larva, or are related to a systemic change in the host deriving from the injected compounds. Similar increases in the tendency to avoid superparasitism are commonly observed in other parasitoids (Klomp, Teerink, & Ma, 1980; van Lenteren 1976, 1981; Pak, Buis, Heck, & Hermans, 1986). For example in *Venturia canescens* the rate of avoidance of superparasitism has been shown to rise over the first 20 min from the deposition of the first egg, possibly because of a constraint in the detectability of the marker used to label parasitised hosts (Rogers, 1972).

The data presented here show female *D. tasmanica* are significantly more likely to avoid parasitising hosts containing their own eggs than they are to avoid hosts containing the eggs of other females (Figure 2). Any mechanism to avoid self-superparasitism is expected to be evolutionarily selected (Hubbard et al., 1987;

Rosenheim & Mangel, 1994, Zhang, Gu, & Wang, 2010). Therefore it is not surprising that many parasitoids can discriminate self-parasitised hosts from conspecifically-parasitised hosts (Hubbard et al., 1987; Voelkl & Mackauer, 1990; Baaren, Boivin, & Ne´non, 1994; Ueno, 1994). The ability to recognise self-parasitised hosts implies that female *D. tasmanica* deposits an individually distinguishable mark in or on their host (van Alphen & Visser, 1990). Although we did not test responses to unparasitised versus conspecific parasitised hosts, results suggest that avoidance of conspecific superparasitism is not very substantial soon after oviposition (oviposition in 85% of conspecific eggs vs 95-100% oviposition in unparasitised eggs; Figure 2).

Parasitoids used as biological control agents are expected to be highly efficient in finding hosts and able to discriminate between parasitised and unparasitised hosts (van Lenteren et al., 1978), to avoid superparasitism, and to minimise the time and energy associated with searching behaviour (Godfray, 1994; Mackauer, 1990; González et al., 2010). The ability of *D. tasmanica* to discriminate previously parasitised hosts is important because this should lead to females distributing their limited eggs into a greater number of suitable hosts, and therefore cause greater host mortality and increase parasitoid fitness and population abundance. Avoidance of superparasitism leads to mutual interference (Visser et al., 1990). Therefore the results of this study suggest that mutual interference is potentially a significant factor that influences interactions between populations of *D. tasmanica*, LBAM and perhaps other lepidopteran hosts.

Ultimately, host discrimination by female *D. tasmanica* is expected to play a role in interactions with its hosts and the extent to which hosts are controlled. If host densities are low, then within a search patch, females are likely to re-encounter hosts they have previously parasitised and it is unlikely an advantage to superparasitise such hosts. If densities are higher, then avoidance of oviposition in hosts that have been parasitised by conspecific females should lead to greater levels of parasitism. Some evidence suggests that emigration increases when some species of parasitoids encounter parasitised hosts (Bakker, Eijsackers, Lenteren, van, & Meelis, 1972; Rogers, 1970). If this is so for *D. tasmanica*, the emigration would likely lead spatially to more consistent levels of parasitism by *D. tasmanica* and thus, superparasitism could change its reproductive numerical response. Therefore, the host discrimination we have identified should enhance the capacity of *D. tasmanica* to efficiently parasitise host LBAM and contribute to biological control of the light brown apple moth as part of IPM (Integrated Pest Management) programs.

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Figure legends

Figure 1. Comparing the frequency of attacks within each treatment groups. ns = no significant difference.

Figure 2. Comparing the percentages of actual oviposition for each choice of host of varying prior parasitisation status. *** significant difference at $P < 0.0005$.

Figure 1

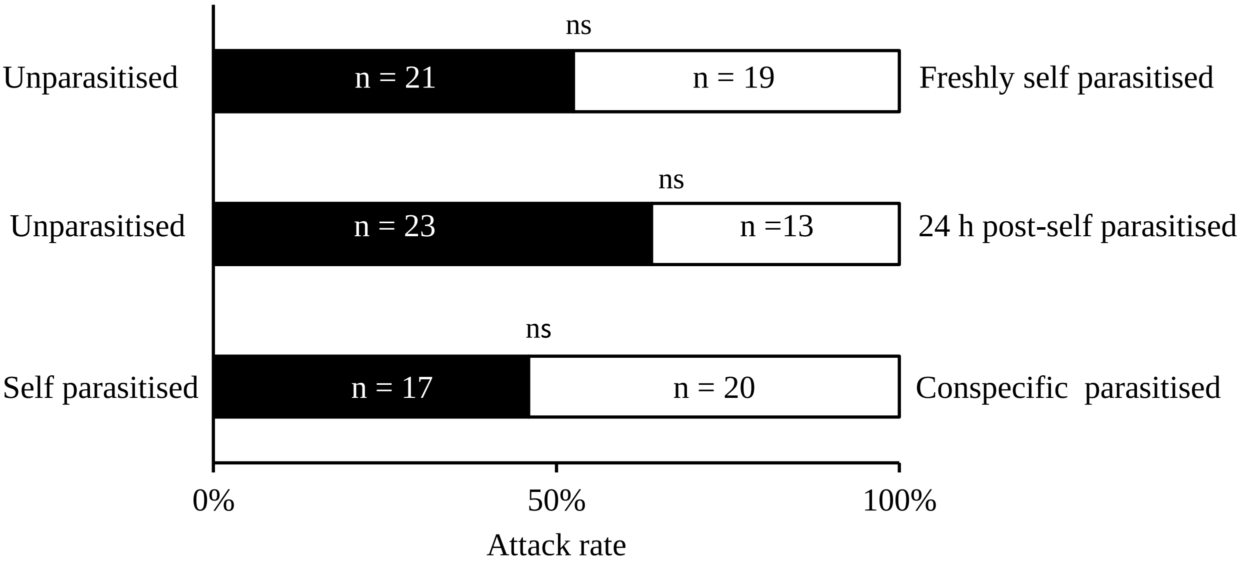
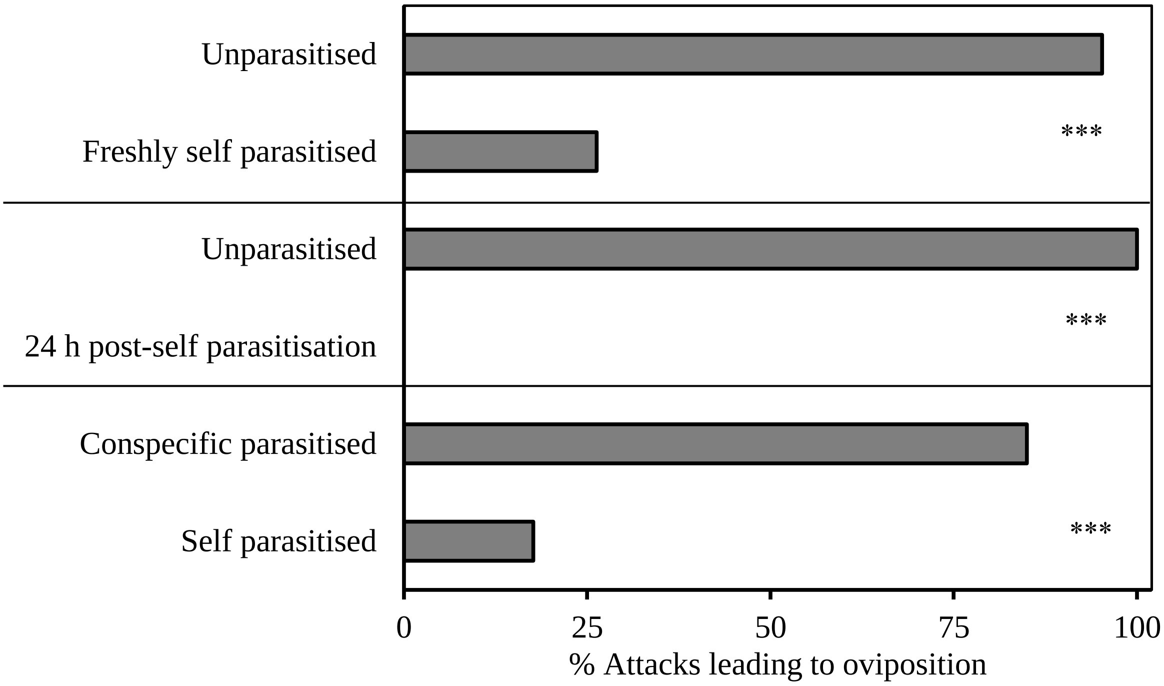

ns
Unparasitised
n = 21
n = 19
Freshly self parasitised
ns
Unparasitised
n = 23
n =13
24 h post-self parasitised
ns
Self parasitised
n = 17
n = 20
Conspecific parasitised
0%
50%
100%
Attack rate

Figure 2


Unparasitised
Freshly self parasitised
***
Unparasitised
24 h post-self parasitisation
***
Conspecific parasitised
Self parasitised
***
0
25
50
75
100
% Attacks leading to oviposition

CHAPTER 5

THE SIGMOID FUNCTIONAL RESPONSE OF *DOLICHOGENIDEA TASMANICA* (HYMENOPTERA: BRACONIDAE) IS AFFECTED BY RECENT EXPERIENCE

Statement of Authorship

The sigmoid functional response of *Dolichogenidea tasmanica* (Hymenoptera: Braconidae) is affected by recent experience

Maryam Yazdani and Michael Keller

Submitted to *Oecologia*

MY and MK designed the experiments; MY performed the experiments and wrote the manuscript; MK provided technical advice throughout.

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Abstract

Dolichogenidea tasmanica (Hymenoptera: Braconidae) is the most commonly collected parasitoid of light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae; LBAM) in Australia. We studied the functional response of *D. tasmanica*, and the effect of recent experience on this behaviour. The functional response was evaluated in wind tunnels and enclosed cages, to determine if wasps would behave the same in both arenas. In both arenas, *D. tasmanica* exhibited a sigmoid functional response, but there was no clear tendency for a deceleration in the functional response curve at high host densities as would be expected with a type III functional response. Parasitism rates were lower in the cages, possibly due to the lack of moving air which provides directional cues to foraging wasps. Recent experience with high host densities increases the searching rate of *D. tasmanica*, which explains much of the difference between the observed functional response curve and a typical type III curve. In general the searching behaviour of *D. tasmanica* varies in response to host density in a manner that directly affects its searching rate. At lower host densities that are characteristic of wild populations, *D. tasmanica* responded in a density-dependent manner that should contribute to suppression of pest populations before they reach economically damage levels.

Key words: density dependence, searching behaviour, vineyard, braconid wasp, parasitoid-host

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Introduction

The functional response of a parasitoid to changing host density provides important information on mechanisms underlying parasitoid-host dynamics (Lipcius and Hines 1986) and is an essential component of parasitoid-host models (Jeschke et al. 2002). The nature of the functional response determines whether a parasitoid is able to regulate the density of its prey (Murdoch and Oaten 1975). Usually, it is classified into one of three general types (Holling 1959) named I, II and III, which respectively describe curves that are linear, concave increasing to an asymptote, and sigmoid when numbers of parasitised hosts per female are plotted against host density. However, theoretically there are other possible forms, such as Type IV, Type V and a functional response with predator interference (Hassell 1978; Abrams 1982; Taylor 1984; Turchin 2001). The population consequences of each type of response are different. Whereas a Type I response implies a density-independent predator attack rate, a Type II response leads to inverse density-dependent predation or parasitism. The Type III functional response is the only response which may lead to direct density dependence when prey densities are low, and thus can potentially stabilize predator-prey interactions (Hassell et al. 1977; Hassell 1978; Collins et al. 1981; Chesson and Rosenzweig 1991; Berryman 1999; Bernstein 2000; Fernández-arhex and Corley 2003). Holling (1959) suggested that the Type II response may be typical of invertebrate predators, including parasitoids, whereas Type III responses are characteristic of vertebrate predators where switching and learning are more common. However, later work suggested that parasitoids may well display Type III curves (Fernández-arhex and Corley 2003). van Lenteren and Bakker (1976) and Hassell et al. (1977), argued that the reason why Type III responses are rarely observed in insect predators and parasitoids was the lack of proper studies at the time (see also Hassell 2000; Fernández-arhex and Corley 2003). In unstructured models, sigmoid (Type III) functional responses have the potential to stabilize predator-prey dynamics due to density-dependent mortality at low host densities. In contrast, a Type II functional response destabilizes the dynamics because the predators cause an inverse density-dependent mortality of the prey (e.g. Murdoch and Oaten 1975; Hassell 1978). So, distinguishing between Type II and Type III functional responses is critical in understanding the predator-prey dynamics. Due to the importance of functional responses in ecological processes, numerous empirical studies have characterized functional responses in a variety of predator-prey systems (Okuyama 2013).

van Lenteren and Bakker (1976) suggested that the apparent absence of a stabilizing density dependence functional response in invertebrate predators or parasitoids may be caused by experimental procedures in which the numbers of prey or hosts at low densities are higher than what can be expected in the field. Also they concluded that for a proper analysis of the functional response, it is essential to carry out behavioural observations (Fernández-arhex and Corley 2003). Hassell et al. (1977) in turn, argued that the practice of doing experiments in a relatively small, simple laboratory universe using large, preferred prey may ignore the full range of behaviours which invertebrate predators are capable of showing. Since Holling’s (1959) seminal work, a number of experiments in a variety of species, as well as theoretical studies have been carried out that draw attention to problems involved in measuring the functional response. On the one hand, it has been debated whether the design of some controlled experiments are representative of the behaviour that leads to the true shape of the functional response curve and how these should be carried out. On the other hand, the statistical analyses of the data and the mathematical models used in analyses have been widely discussed (Livdahl and Stiven 1983; Houck and Strauss 1985; Williams and Juliano 1985; Juliano and Williams 1987; Trexler et al. 1988; Casas and Hulliger 1994; Manly and Jamienson 1999; Juliano 2001; Fernández-arhex and Corley 2003).

Insect parasitoids are important subjects of behavioural and population studies because they are remarkably common in nature, are typically easy to rear and handle and, more importantly, are key species for the biological control of many insect pests (Waage and Hassell 1982; Godfray 1994; Fernández-arhex and Corley 2003). It is for this reason that functional responses have been investigated in many insect parasitoids (Fernández-arhex and Corley 2003). Understanding of the nature of the functional response should indicate likely patterns of parasitism, which is important in evaluating population dynamics and the capacity of a parasitoid to contribute to biological control.

In this paper we report the results of a series of experiments designed to investigate the functional response of a parasitic wasp, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae). It parasitises the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) and other tortricids. LBAM is a polyphagous native species in South-eastern Australia, where it is a key pest in vineyards. It has been introduced to Western Australia, New Zealand, Hawaii, England, and California (Suckling and Brockerhoff 2010). *D. tasmanica* is a commonly collected parasitoid of *E. postvittana* (Paull and Austin 2006). It is an arrhenotokous, solitary, koinobiont endoparasitoid of the first three instars of LBAM (Yazdani et al. 2014 in press), however no previous study of its functional response has been reported. Our goals were (1) to characterise the functional response of female *D. tasmanica* to changing densities of second instar LBAM, (2) to elucidate some of the key factors that affect the shape of functional response curve. A series of experiments was carried out in small wind tunnels to present conditions in which the parasitoid could detect and respond to host cues more naturally because of air flow.

Materials and methods

Rearing parasitoid and host

A laboratory colony of *E. postvittana* was reared at 22 ± 2º C and a photoperiod of 12 L: 12 D on an artificial diet. A culture of *D. tasmanica* was established from individuals collected from South Australian vineyards. The wasps were reared on larval LBAM infesting plantain, *Plantago lanceolata* (L.), at 23 ± 2 º C, 14 L: 10 D (for details see Yazdani et al. 2014 in press).

Functional response in wind tunnels

The functional response of *D. tasmanica* was investigated in four identical wind tunnels (for details see Yazdani et al. 2014 in press). The wind tunnels had inside dimensions of 35 cm (H)× 50 cm (L)× 30 cm (W). The mean wind speed was 29 ± 0.67 cm/s (mean ± SD). Each wind tunnel contained 20 small grape leaves (variety Chardonnay; 3.5 - 4.5 cm L and 4 - 4.5 cm W). Each leaf was placed in a 10 mm diam. × 50 mm glass vial filled with water, and vials were placed 5 cm apart in four rows in the wind tunnel. Six densities of second instar LBAM were tested independently 1, 2, 4, 8, 16 and 32, with 27, 17, 8, 9, 8 and 4 replications respectively. Four experiments were run concurrently, with densities chosen at random. For each density, leaves were randomly infested with larvae 24 h before the experiment. Every morning newly emerged females were collected and caged overnight with 5 males to ensure mating. Naïve 1-2 old mated females were used in the experiments. In order to stimulate the naïve wasps before starting the experiment, each wasp was exposed to a second instar host and allowed to sting it once. The wasp was then released in the wind tunnel 10 cm downwind from the first row of leaves. After 2 h, the wasps were removed, and the leaves were collected and placed in 100 ml plastic cups that contained a grape leaf for food. They were kept at room temperature for 4 days and then dissected to determine the frequency of parasitism of larvae by *D. tasmanica*.

Functional response in cages

In order to determine if the experimental arena affects the shape of the functional response curve, a second experiment was conducted in cages. In this experiment, six densities of second instar LBAM were presented to wasps, 1, 2, 4, 8, 16 and 32, with 26, 14, 6, 6, 10 and 4 replications, respectively. The experiments were conducted in plastic containers with inside dimensions of 17 cm (H) × 20 cm (L) × 13 cm (W). The container was modified by removing one side and replacing it with nylon mesh of the same dimension to allow for aeration. For each density, larval LBAM were placed randomly on 6 grape leaves. Each leaf was placed in a 10 mm diam. × 50 mm glass vial filled with water, and vials were placed 5 cm apart in three rows in the cage. The larval LBAM were exposed to a naïve 1-2 old mated female for 2 hours. Parasitism data were recorded as described in the previous experiment.

Analysis of functional response curves

The data from both functional response experiments were analysed using the approach described by Juliano (2001). First, the fraction of hosts parasitised vs. number present was subjected to logistic regression with linear, quadratic and cubic terms using the *glm* function (generalised linear model; family = binomial) in the statistical package *R* (version 3.1.0 (2014-04-10), "Spring Dance"). In both cases the coefficient of the linear term was found to be positive, which indicates a Type III functional response. Therefore the data were then fitted to a Type III functional response curve using the *nls* function (nonlinear least squares) of *R*, using the model suggested by Hassell et al. (1977) and elaborated by Juliano (2001):

$$N_{par} = N\left(1 - e^{-(d+bN)T_t \big/ \left(1+cN+dhN+bhN^2\right)}\right)$$

The mean fraction of parasitised hosts was used in these analyses, since the raw data had high levels of inherent variation, particularly at low host densities. Nevertheless, the nonlinear regressions did not converge to stable solutions for either data set. Further nonlinear regression analyses were performed with *Statistix* (version 10, Analytical Software, Tallahassee, Florida, USA), which provided approximate parameter estimates to visualise notional curves for the purpose of later discussion.

Effect of experience on Type of functional response

In the third experiment, we sought to determine if a rewarding experience of foraging for hosts would lead to greater subsequent success in locating hosts compared to wasps that searched in such an arena where no host were present, and hence they had a non-rewarding experience. In each replicate of this experiment, a pair of 1-2 day-old mated females was selected. One of them was designated as having a rewarding experience. It was released for 1 h in a wind tunnel containing 10 grape leaves, each infested with one second instar LBAM. The other wasp was designated as having a non-rewarding experience. It was released into a wind tunnel containing 10 uninfested grape leaves. The leaves were spaced in two rows across the width of the wind tunnel, and separated by a distance of 5 cm. Immediately after capturing them, the wasps with two types of experience were released separately in two wind tunnels which contained two second instar LBAM that were randomly placed on 20 grape leaves. Host larval positions were the same in both wind tunnels within a replicate. The leaves were arranged in four rows of five leaves and separated by a distance of 5 cm. After 2 h exposure to wasps, the infested leaves were collected, placed in plastic cups with a grape leaf and kept at room temperature for 4 days. They were dissected to determine the frequency of parasitism.

This experiment was replicated for 10 times. The differences in proportions of larvae parasitism between the two types of experience were analysed using Fishers's two-tailed exact test (Zar 1984) on pooled numbers.

In the fourth experiment, we sought to determine if rewarding and non-rewarding searching experiences have a longer term effect on searching behaviour. This experiment compared the behaviour of rewarding and non-rewarding experience wasps when foraging at three host densities after an interval of 8 h had elapsed following the first bout of searching. In all trials, wasps were released into wind tunnels containing 20 grape leaves using the same methods and arrangement as were used in the functional response experiment to allow the results to be compared. Naïve 1-2 day-old mated female parasitoids were used. Females considered to have a rewarding experience were released for 1 h in a wind tunnel in which each leaf was infested with one second instar LBAM. Females with a non-rewarding experience were released for 1 h in a wind tunnel with uninfested grape leaves. After 1 h, the wasps were transferred to an 18 mm diam. × 50 mm glass vials with a drop of honey and sealed with damp cotton. After 8 h, wasps were released again into wind tunnels that contained 4, 8 or 16 second instar LBAM that were randomly placed on 20 grape leaves. Pairs of wasps with rewarding and non-rewarding experience were released into separate wind tunnels with the same density of larval LBAM. After 1 h, wasps were removed and the larvae were placed in plastic cups, held for 4 days with grape leaves and dissected as described previously. This experiment was replicated 8 times for each density and the order of treatments was randomised. The data were analysed with Logistic regression, with linear and quadratic terms for host number using the statistical package R.

Results

Functional responses in wind tunnels and cages

Logistic regression indicated that the data conform best to the Type III functional response in both the wind tunnel and cage arenas (Tables 1 and 2; Fig. 1 and 2). Parasitism levels were higher at the lower host densities in the cages compared to the wind tunnels, but this trend was reversed at the two highest densities. In neither case could the data be fitted to the Type III functional response model, as the nonlinear least squares analyses did not converge on parameter values that were statistically significant.

Effect of experience on searching behaviour

Wasp searching behaviour was affected by experience. Females that had a rewarding experience parasitised more larvae at low density than those that had a non-rewarding experience (Table 5). When the interval between initial searching was extended to 8 h, females that had a rewarding experience consistently parasitised more hosts than those that had a non-rewarding experience (Z = -7.715, $P < 10^{-13}$; Fig. 3). Host density also affected the fraction of hosts that were parasitised in a non-linear manner (quadratic term for host number from Logistic regression: Z = -2.185, P = 0.0289), which is consistent with a Type III functional response.

Discussion

Invertebrate functional responses are normally measured in small and simple arenas. But Hassell et al. (1977) argued that sigmoid responses are more likely to be found for predatory and parasitic insects by employing larger and more complex arenas. In order to diminish the influence of artificial laboratory conditions on behavior, we carried out a series of experiments in wind tunnels to present conditions in which the parasitoid could detect host cues more naturally because of air flow. Also, in our experiments host larvae were distributed randomly among host plant leaves to mimic heterogeneity a wasp would encounter in the field. Thus, the parasitoids could move freely from leaf to leaf and express their full set of host finding behaviours. We also conducted experiments in a simple cage to allow us to determine if the type of arena would lead to a substantial change in the functional response. The cages were smaller than the wind tunnels and the range of densities tested was the same in both arenas, so host density should be perceived to be relatively higher in the cages. Overall the shape of the functional response curve was similar in both arenas (Fig. 1 and 2). However, parasitism was higher at the lowest host numbers tested in the cages, while it was higher in the wind tunnels at relatively higher host numbers. These results suggest that wasps could find hosts more easily when the density was low in the smaller area. But in the wind tunnel, the ability to track odour plumes using anemotaxis is likely to have led to greater searching efficiency at higher host densities.

Our results in both wind tunnel and cage experiments clearly showed the characteristics of a Type III functional response for *D. tasmanica* at low host densities (Fig. 4a, b). Hassell et al. (1977) argued against the notion that Type II functional responses are typical of parasitoids, and suggested that sigmoid Type III responses may be much more common than previously supposed. In subsequent research, other species of Hymenoptera, such as *Venturia canescens* (Grav.) and *Campoletis chlorideae* (Uchida) (Ichneumonidae), *Aphidius uzbekistanicus* (Luzhetzki), *Diaeretiella rapae* (M'Intosh) and *Aphidius salicis* (Haliday) (Aphidiidae), *Aphidius colemani* (Viereck) (Braconidae), *Ibalia leucospoides* (Hochenwarth) (Ibaliidae), have been shown to exhibit sigmoid functional responses (Fernández-arhex and Corley 2003). Fernández-arhex and Corley (2003) suggested that, the results of some functional response experiments may be overestimating type II curves. For instance, it has been suggested that time-limited experiments may force a type II curve on the insect’s behaviour (van Lenteren and Bakker 1976; Walde and Murdoch 1988; Ives et al. 1999). Furthermore, type II models may have been used to fit data that could be better served by type III models, especially in older work (Fernández-arhex and Corley 2003).

It is clear that even the best fitting, but statistically non-significant, Type III functional curve does not fit the data (Fig. 1 and 2). We could not statistically fit a Type III model to the data, even though a Logistic regression analysis clearly showed it is Type III in nature (Tables 3 and 4). This indicates that the searching rate varied in a complex manner. On the one hand, the results indicate the searching rate varied in a manner consistent with a hyperbolic relationship with host density at low densities (Juliano 2001). But at densities of 10 or higher per 20 leaves, the searching rate must have increased with increasing host density, which lead to the characteristics of Type I functional response (Fig. 4a). If the searching rate varied in a purely hyperbolic manner with increasing host density, then the searching rate would asymptotically approach a fixed maximum and the relationship between number parasitised and host density would produce a typical sigmoid curve (Fig. 4b). Thus it seems that a more detailed understanding of the factors that influence searching rate is needed in order to develop models of functional response.

We suspected that the lack of fit of the data to a Type III model was not simply a statistical problem, so we investigated whether the wasp would change its searching behaviour, and hence the estimated searching rate, at higher densities within the timeframe of a two hour experiment. There was a highly significant difference between the parasitism rates associated with wasps with rewarding v.s non-rewarding experience (Fig. 3). It seems that the searching behaviour of *D. tasmanica* varies in response to varying host density in a manner that directly affects its searching rate, even over short periods of time. The results of the experiments reported here elucidate two aspects of the behaviour of *D. tasmanica.* When wasps search in a non-rewarding area, they subsequently reduce the intensity of their searching activity. But if they search where host densities are high and have a rewarding experience, they subsequently search more intensively which leads to increasing the rates of parasitism.

It is noteworthy that the effects of rewarding and non-rewarding experience are observed after the relatively short time of one hour (Table 5), and that these effects persist for at least 8 hours (Fig. 3). This suggests that the wasp assesses host density and learns the characteristics of a rewarding environment. It implies that *D. tasmanica* uses information from previously visited patches to adjust its subsequent searching decisions. Learning is regarded as an important factor that leads to the expression of a Type III functional response (Real 1979). Parasitoids can change their behaviour in a repeatable way and learn through experience (Vet and Groenewold 1990, Turlings et al. 1993). Here we conclude that the shape of the functional response curve is determined in large part by both host density and the effects of recent experience.

Hassell et al. (1977) discussed the implications of Type III functional responses for species that search when prey densities are low. A reduction in searching effort in a non-rewarding environment which yields a very low fitness return may be an advantage, particularly if it is not possible to leave that environment. By reducing searching effort, a predator or parasitoid may reduce energetic costs until conditions improve. A similar argument may apply to *D. tasmanica* searching in a large relatively homogeneous vineyard. Although costs and gains are less easily defined for parasitoids, they may involve, for example, the costs that arise from metabolism of carbohydrates (Rivero and Casas 1999) or exposure of the parasitoid to its own natural enemies, both of which must be balanced against the gains that accrue from the number of hosts successfully parasitised. The sigmoid functional response curve of *D. tasmanica* at low host densities may reflect a strategy that balances the cost of foraging against expected oviposition success.

It is important to put the densities used in our experiments into the context of densities that occur in the field. No systematic study of the population dynamics of larval LBAM on grapevines has been published. However, treatment thresholds that guide grape growers in decision-making on the application of insecticides to control damaging infestations of LBAM have been published. On table grapes, the number of larvae per 100 shoots at which insecticidal control is recommended is 10 before flowering and five or less thereafter (Department of Primary Industries Victoria 2010). The treatment threshold for LBAM on wine grapes is reported to be 20 larvae on foliage per 100 shoots (CCW Cooperative 2008). Grapes have an indeterminate pattern of annual growth, but over the growing season, between 8 and 30 leaves per shoot are commonly present when LBAM is most likely to reach damaging levels (Lebon et al. 2004). Thus the treatment thresholds for LBAM, which indicate relatively high and damaging populations, are in the order of 0.002 to 0.025 larvae/ leaf when between 8 and 30 leaves are present on shoots. These densities are far below those used in the present study. There are two important implications that follow from this. First, the highest densities used in our experiments are arguably extremely high relative to those found in natural populations. Therefore it is not ecologically important that the upper asymptote of the functional response curve was not estimated in this study. The asymptotic maximum percentage parasitism is likely to be ecologically significant only in those species where it is approached at commonly observed high densities. Second, the sigmoid shape of the functional response curve at low host densities has the greatest relevance for natural populations (Hassell 1978; Fernández-arhex and Corley 2003). This sigmoid shape can lead to direct density-dependent parasitism. Arguably, even lower densities should be used in experiments like ours. But this poses significant practical challenges because larger arenas are needed to determine rates of parasitism at such lower densities, and very high levels of replication are needed to precisely estimate mean parasitism at very low densities. We conclude that experiments on functional response should focus on densities that start at the lowest practical number that can be investigated. Such experiments should be conducted in either outdoor arenas or laboratory arenas like wind tunnels that are conducive to the natural expression of searching behaviour.

The importance of distinguishing between Type II and Type III functional responses rests on their very different contributions to stability (Holling 1959; Murdoch and Oaten 1975). Only sigmoid functional responses are density-dependent up to some threshold prey density. This contributes to stability if average host densities fall below the threshold. Natural enemies that respond to prey in density-dependent manner may be able to quickly suppress pest population before they reach economically damage levels (Cappuccino 1995; Price 1997). However, Fernandez-Arhex and Corley (2003) examined the functional responses of parasitoids that have been used in classical biological control programs and found no correlation between the type of response and parasitoid success.

Paull, Schellhorn and Austin (2014) conducted large-scale field experiments to quantify and characterize the population response of *D. tasmanica* to different densities of LBAM in the field. In an apparent contradiction to our results, they concluded that the population response of *D. tasmanica* to varying host density was inversely density-dependent, which implies the species exhibits a Type II functional response. However, they did not investigate the components of functional and numerical responses that underlie the pattern of parasitism at the population level. They argued that an inversely density-dependent response may be due to inadequate resources such as access to carbohydrates, specific nutrients, shelter or alternative hosts, which are not available or are in short supply in vineyards. This is because parasitoids are likely to expend more energy and time searching for these resources when they are limiting and, as a result, the time available to maximize their response to increasing host density is reduced (Desouhant et al. 2005; Paull, Schellhorn and Austin 2014). The realised lifetime fecundity of *D. tasmanica* is also significantly increased in the presence of flowers, although this is a consequence of the increase in longevity, rather than an increase in daily fecundity (Berndt and Wratten 2005). And without flowers, offspring sex ratios are strongly male biased, but when females have access to flowers an approximately equal sex ratio is produced. Wasps in our experiment were well-fed, so their behaviour should not have been affected by hunger. We conclude that the functional response must be considered in conjunction with other aspects of biology and behaviour when developing models of parasitoid-host population dynamics.

D. tasmanica parasitises LBAM in a density-dependent manner at low host densities, which is important in regulation of host populations. This suggests that *D. tasmanica* contributes in a desirable manner to biological control of LBAM. Additional studies are needed, however, to investigate the role that experience and learning play in shaping the functional response over the lifetime of a wasp. It is known that experience over time can influence the searching behaviour of the parasitoid *V. canescens* (Froissart et al. 2012). It is likely that generalist species like *D. tasmanica* similarly responds to experience with a range of host-related cues over the span of its adult life.

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Figure legends

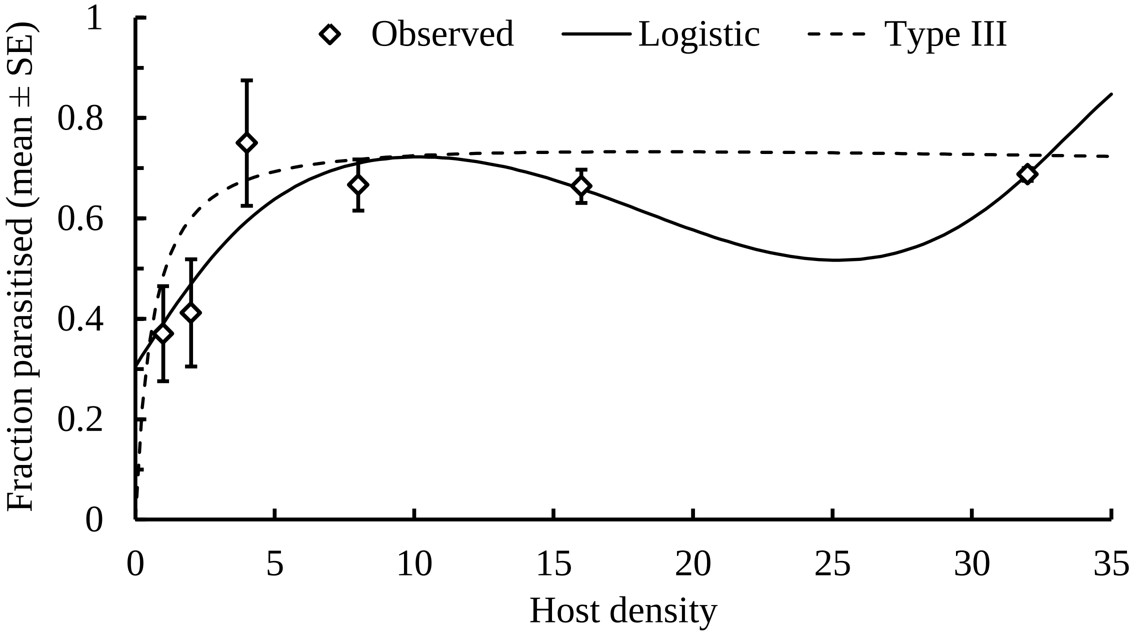
Figure1. Mean fraction of larvae parasitised (± standard error) by *D. tasmanica* for the 6 densities (1, 2, 4, 6, 8, 16 and 32) of second instar LBAM in the wind tunnels. The dotted line depicts functional response Type III fits to groups of data and black line shows Logistic regression analysis of the fraction of hosts parasitised by *D. tasmanica* vs. host number in wind tunnels.

Figure 2. Mean fraction of larvae parasitised (± standard error) by *D. tasmanica* for the 6 density treatments of second larval LBAM (1, 2, 4, 6, 8, 16 and 32) in the small cages. Dotted line depicts functional response Type III fits to groups of data and black line shows Logistic regression analysis of the fraction of hosts parasitised by *D. tasmanica* vs. host number in small cages.

Figure 3. The effect of previous rewarding or non-rewarding foraging experience by *D. tasmanica* on the mean fraction of larvae parasitised (± standard error) when foraging 8 h later at densities of 4, 8 and 16 larvae per 20 grape leaves.

Figure 4. Type III functional response curves (solid lines; $b = 0.005$, $c = 0.04$, $d = 0.000$, $h = 8$, $T = 120$) and the effect of a switch to a Type I response (dashed lines). a. Relationship between host number and fraction parasitised. b. Relationship between host number and number parasitised.

Figure 1



Observed
Logistic
Type III
Fraction parasitised (mean ± SE)
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
Host density

Figure 2

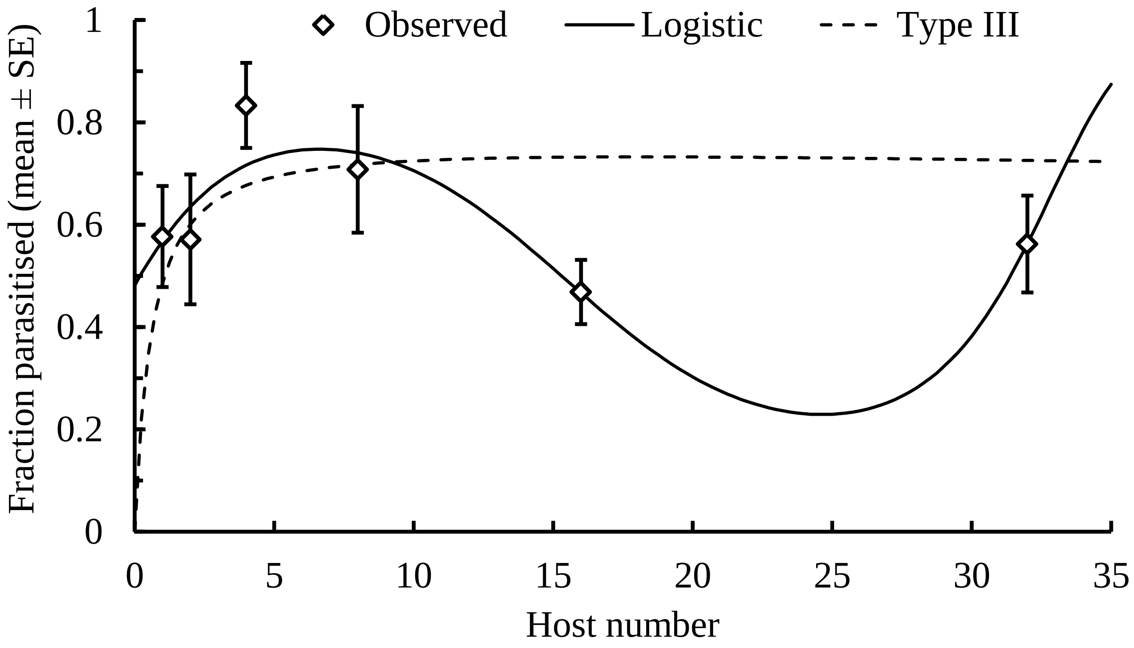

Observed
Logistic
Type III
Fraction parasitised (mean ± SE)
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
Host number

Figure 3.

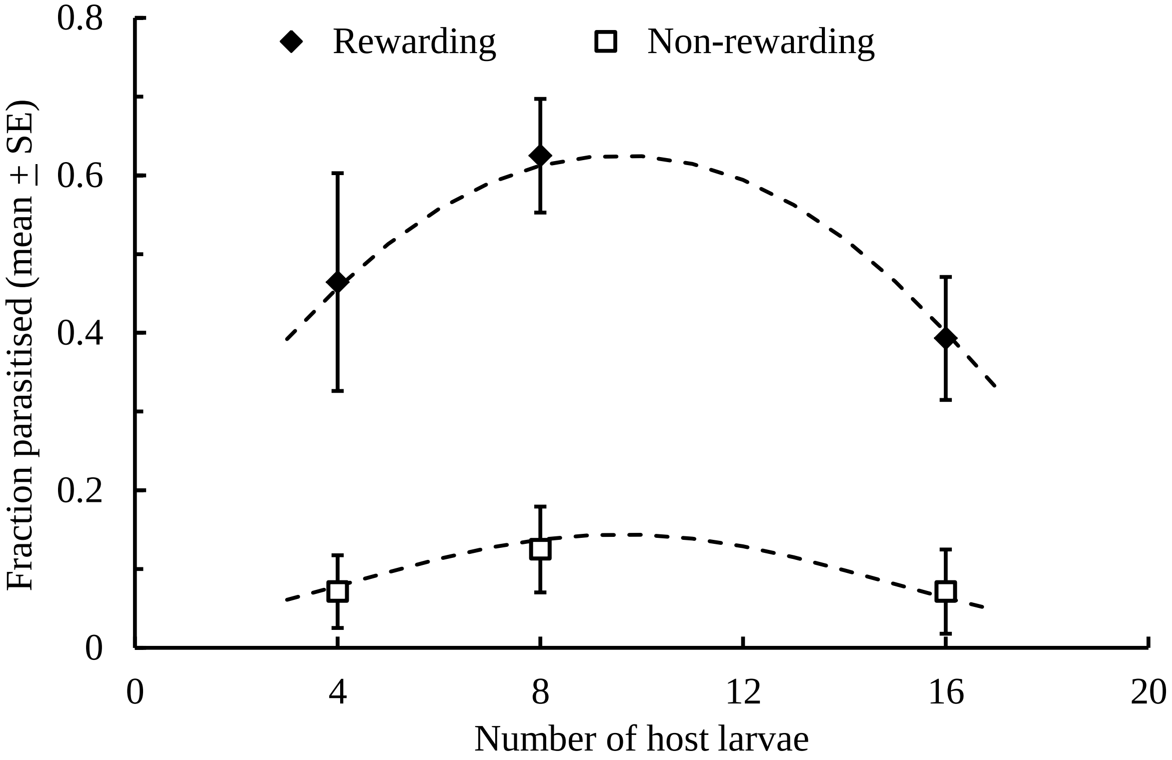
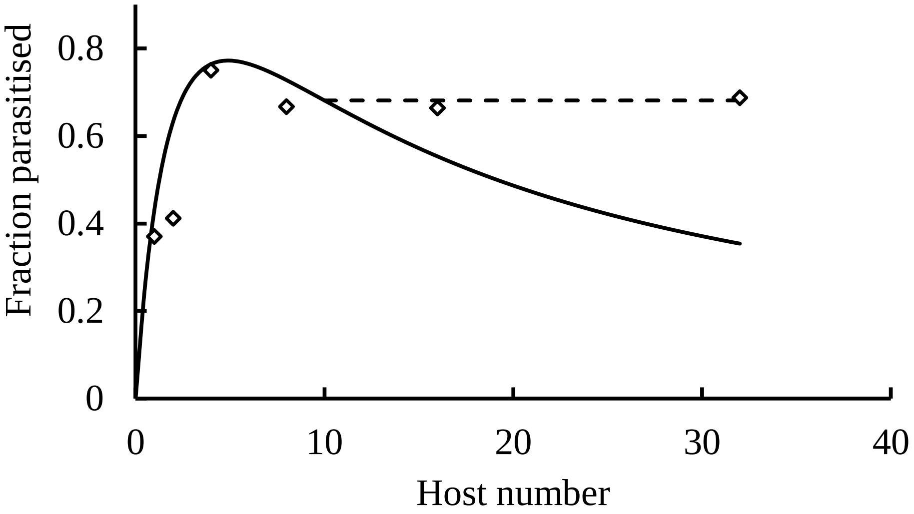

◆ Rewarding
☐ Non-rewarding
Fraction parasitised (mean + SE)
0.8
0.6
0.4
0.2
0
0
4
8
12
16
20
Number of host larvae

Figure 4

a.


Fraction parasitised
0.8
0.6
0.4
0.2
0
0
10
20
30
40
Host number

b.

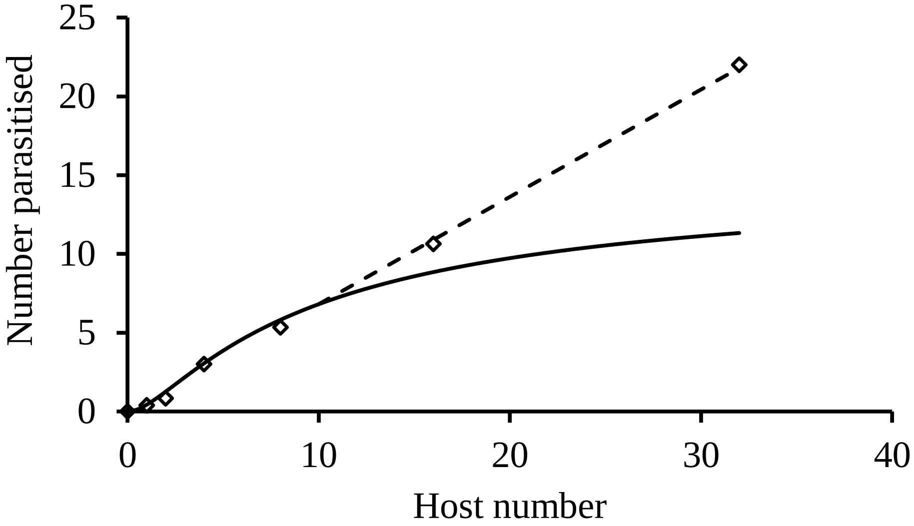

Number parasitised
25
20
15
10
5
0
0
10
20
30
40
Host number

Table 1. Results of logistic regression analysis of the fraction of hosts parasitised by *D. tasmanica* vs. host number in small wind tunnels.

Coefficient	**Estimate**	**Std. Error**	**z value**	**Prob.**
Intercept	-0.8238131	0.4015574	-2.052	0.04021
Host number	0.4047707	0.1447773	2.796	0.00518
(Host number)^2	-0.0279509	0.0116589	-2.397	0.01651
(Host number)^3	0.0005274	0.0002362	2.233	0.02553

Null deviance: 122.98 on 72 degrees of freedom

Residual deviance: 108.59 on 69 degrees of freedom

Table 2. Results of logistic regression analysis of the fraction of hosts parasitised by *D. tasmanica* vs. host number in cages.

Coefficient	**Estimate**	**Std. Error**	**z value**	**Prob.**
Intercept	-0.0741800	0.4347062	-0.171	0.86450
Host number	0.3851388	0.1675998	2.298	0.02156
(Host number)^2	-0.0368871	0.0134945	-2.733	0.00627
(Host number)^3	0.0007866	0.0002712	2.900	0.00373

Null deviance: 151.49 on 65 degrees of freedom

Residual deviance: 136.03 on 62 degrees of freedom

Table 3. Results of nonlinear least squares analysis of the fraction of hosts parasitised by *D. tasmanica* vs. host number in small wind tunnels using the model of Hassell (1975) as modified by Juliano (2001). See model equation in Materials and Methods.

Parameter	**Estimate**	**Std. Error**	**Prob.**
b	9.99892E-03	0.077997	> 0.05
c	0.799908	7.144216	> 0.05
d	6.42099E-23	0.040905	> 0.05
h	0.300152	3.564111	> 0.05

Residual SS (SSE): 0.0632

Residual MS (MSE): 0.0316

Standard Deviation: 0.1778

Degrees of Freedom: 2

Table 4. Results of nonlinear least squares analysis of the fraction of hosts parasitised by *D. tasmanica* vs. host number in cages using the model of Hassell (1975) as modified by Juliano (2001). See model equation in Materials and Methods.

Parameter	**Estimate**	**Std. Error**	**Prob.**
b	0.010003	0.111827	> 0.05
c	0.800233	10.24253	> 0.05
d	3.19499E-23	0.058635	> 0.05
h	0.300232	5.106190	> 0.05

Residual SS (SSE): 0.1298

Residual MS (MSE): 0.0649

Standard Deviation: 0.2548

Degrees of Freedom: 2

Table 5. The effect of experience on the frequency of parasitism of second instar LBAM by *D. tasmanica* when presented with two hosts in a wind tunnel.

	Previous experience	
Fate of larva	**Rewarding**	**Non-rewarding**
Unparasitised	5	16
Parasitised	15	4
Total No. wasps	10	10

Fisher's two-tailed exact test, *P = 0.0012*

CHAPTER 6

MUTUAL INTERFERENCE IN *DOLICHOGENIDEA TASMANICA* (HYMENOPTERA: BRACONIDAE) WHEN FORAGING FOR PATCHILY-DISTRIBUTED LIGHT BROWN APPLE MOTH

Statement of Authorship

Mutual interference in *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) when foraging for patchily-distributed light brown apple moth

Maryam Yazdani, Michael Keller

Submitted for publication in *Biological Control*

MY designed and performed the experiments, interpreted data and wrote the manuscrip; MK provided guidance throughout and assistd with statistical analysis.

Maryam Yazdani [1], Michael Keller[1*]

ABSTRACT

The solitary endoparasitoid *Dolichogenidea tasmanica* (Hymenoptera: Braconidae) has been reported as the most commonly collected parasitoid of light brown apple moth (LBAM). The foraging behavior of single and multiple female *D. tasmanica* in the presence of patchily-distributed hosts was observed in wind tunnels. Results show that *D. tasmanica* was able to choose those patches which initially offer the highest oviposition rate. Single foragers spent the longest times on patches with higher host densities, but they visited patches with lower host densities sooner in presence of other foragers. The overall rate of parasitism was equalized across patches at the end of the experiment, which indicates that hosts were equally susceptible to attack by *D. tasmanica*, irrespective of their local density. A decrease in the searching efficiency of individual parasitoids with increasing parasitoid density was evidence of interference among female *D. tasmanica*. Nonlinear regression indicated that there was a consistent pattern of mutual interference as wasp density increased and the area of discovery and mutual interference coefficients of Hassell and Varley's (1969) model of parasitoid foraging were estimated as Q = 0.4493 and m = 0.5257, respectively. Females chose to visit patches that were not previously visited, which suggests that repellent chemicals are produced by attacked hosts or marking pheromones are deposited by searching

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females on patches they have visited. This is one factor that can reduce searching efficiency and cause mutual interference among competing *D. tasmanica*.

Key words: Dolichogenidea tasmanica, mutual interference, optimal foraging, patch quality, ideal free distribution, stopping rule

1. Introduction

There are three basic responses that largely determine the outcome of an insect parasitoid's interaction with its host population. They are the response to host density (the functional response), the response to host distribution (the aggregative response) and the response to parasite density (the interference effect) (Hassell and Rogers, 1972; Rogers and Hassell, 1974; Hassell, Lawton and Beddington, 1976; Cook and Hubbard, 1977). In the field, parasitoids forage in the presence of other conspecifics and several parasitoid females are frequently observed exploiting the same patch of hosts simultaneously (Godfray, 1994). In such cases, the competitive interactions among the foraging parasitoids may reduce per capita search activity and attack efficiency at a given host density (Hassell and Varley, 1969; Waage, 1983). Surprisingly, few studies have investigated foraging strategies under competition (Cook and Hubbard, 1977; Bernstein et al., 1991; Sjerps and Haccou, 1994; Giraldeau and Caraco, 2000; Goubault et al., 2005).

Mutual interference is the combination of behavioral interactions among simultaneously searching parasitoids that causes a reduction in their searching efficiency (Free et al., 1977). The phenomenon of mutual interference was first modelled by Hassell and Varley (1969), who found an inverse relationship between the individual parasitoid searching efficiency and the density of parasitoids. Their analysis showed that mutual interference can play a role in stabilising parasite-host interactions.

Female parasitoids can interfere with each other either (1) directly by fighting, displaying, or hindering competitors (Field et al., 1998; Godfray, 1994; Hardy and Blackburn, 1991) or (2) indirectly by modifying their host exploitation strategies (Visser et al., 1990, 1992; Goubault et al., 2005). Two other forms of interference have been recognized: pseudo-interference and indirect mutual interference. Pseudo-interference (Free et al., 1977) occurs when parasitoids have a non-uniform distribution over patches, which causes variation in the risk of being attacked between individual hosts (Chesson and Murdoch, 1986, Pacala et al., 1991, Hassell, et al. 1991). Visser and Driessen (1991) considered changes in sex allocation, clutch size and superparasitm decisions to be indirect mutual interference. All three forms of interference cause a decline in measured parasitoid searching efficiency.

Mutual interference can be represented by a simple mathematical model. It is based on the model of a randomly searching parasitoid (Hassell, 1978):

$$N_a = N_t(1 - e^{aP_t t}) \qquad (1)$$

where N_t is the total number of hosts present, N_a is the number of hosts attacked, P_t is the number of searching parasitoids, a is the "area of discovery" which is a measure of searching activity, and t is elapsed time. If mutual interference is assumed to act in a linear manner, then the term aP_t in the random search equation can be replaced by $QP_t^{(1-m)}$ (Hassell and Varley, 1969):

$$N_a = N_t\left(1 - e^{QP_t^{(1-m)}}\right). \qquad (2)$$

The coefficient $Q = a$ when $P_t = 1$. This equation can be used to estimate the magnitude of the per-capita effects of mutual interference (m) among searching parasitoids.

In a natural environment it is likely that the spatial distribution of a host population will be patchy and resources will be used by many foragers. This means that foragers are faced with the problem of how to apportion their time among the different parts of the host habitat in order to parasitize the maximum number of hosts in the time available (MacArthur and Pianka, 1966; Cook and Hubbard, 1977; Wajnberg, 2006). Patch time allocation by insect parasitoids has been considered an important behavioural component of host-parasitoid interactions that ultimately influence population dynamics (Hassell and Southwood, 1978; van Alphen, 1988; Basset et al., 2002). Hence, an understanding of patch time allocation is also potentially important in determining the efficacy of parasitoids used in biological control. In other words, an accurate understanding of the mechanisms involved in patch time allocation by parasitoids should lead to a refined ability to select and use effective parasitoid species for pest control (Waage, 1990; Wajnberg, 2006).

Dolichogenidea tasmanica (Cameron) (Hymenoptera: Braconidae) is an endoparasitoid of tortricid species. It is a key biological control agent for the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Paull et al., 2014). Previously we found that an individual female *D. tasmanica* display a Type III functional response (Yazdani et al. 2014, unpublished results). In this study we observed and analysed the foraging behaviour of single and multiple female *D. tasmanica* in the presence of patchily-distributed hosts. We sought to determine 1) if *D. tasmanica* selectively forages on grape leaves that are more heavily infested by larval LBAM; 2) if their behavior is affected by the presence of competing conspecific females; and 3) the magnitude of any mutual interference among searching individuals that would cause a reduction in the parasitoid's searching efficiency.

2. Materials and methods

2.1 Insects and plants

A laboratory colony of *E. postvittana* was cultured at 22 ± 2 º C and a 12 L: 12 D photoperiod on an artificial diet mainly consisting of lima bean, agar and brewer's yeast. The *D. tasmanica* colony was originally collected from South Australian vineyards and was maintained on infested plantain, *Plantago lanceolata* (L.), with larval LBAM at 23 ± 2 º C and a 14 L: 10 D photoperiod for several generations in the laboratory (for details see Yazdani et al., in press).

2.2 Patch exploitation strategy and mutual interference

An experiment was conducted to determine if the searching behavior of individual wasps is affected by the presence of other foragers, and how competitors utilize patches infested with varying host densities. The experiment was conducted in four identical wind tunnels with inside dimensions of 35 cm (H)× 50 cm (L)× 30 cm (W). The mean wind speed was 29 ± 0.67 cm/s (mean ± SD) (Yazdani et al. in press). Each wind tunnel contained four "patches" that consisted of three grape leaves (var Chardonnay*;* 3.5 - 4.5 cm L. and 4 - 4.5 cm W.). The leaves were placed in a 10 mm diam. × 50 mm glass vial filled with water and the petioles were held together with a piece of clear tape. Each patch of leaves was infested with 0, 2, 4 or 8 second instar LBAM 16-18 h before each experiment. The four different patches were placed randomly in a wind tunnel, 10 cm apart in a square arrangement with in the same order in all wind tunnels of a replicate. 1-2 day old females *D. tasmanica* were released into the wind tunnels, where they were observed for 60 min. In order to stimulate naive wasps to search for hosts, the wasps for each density were exposed to a grape leaf infested with five second instar LBAM larvae for 5 min and allowed to search together and sting larvae. Each wasp was then collected into an 18 mm diam. × 50 mm glass vial and released in the appropriate wind tunnel 10 cm downwind from the first row of patches. After releasing them in all wind tunnels, the locations of wasps were recorded with event-recorder software (The Observer XT, version 11, Noldus, 2012) over 15 minute periods that were rotated among wind tunnels. After 60 min the wasps were removed. Leaves from each patch were collected in separate 50 ml plastic cups. On the fourth day after the experiment, the larvae were dissected to determine the frequency of parasitism. This experiment was replicated 16 times, and parasitoid behavior during each time interval was observed 4 times for each wasp density.

We found that wasps stopped searching on patches and moved away from them over time. The experiment was extended to determine if wasps avoid previously visited patches. Such avoidance of previously visited patches could be one cause of mutual interference among searching wasps. To test if such avoidance occurs, experienced wasps that searched alone in the previous experiment were released into a wind tunnel that contained the previously visited patch with eight larval LBAM and a fresh patch that was also infested with eight larvae. Fresh and previously visited patches were placed 10 cm apart in the wind tunnel and the wasp was released centrally 10 cm downwind from them. The wasp was observed until it visited one of the patches. Females from two replicates could not be included in this extension of the experiment, so an additional four experimental trials with single wasps were conducted. Thus this experiment was replicated 18 times.

3. Statistical Analysis

The fraction of time that wasps spent on each patch and elsewhere in the wind tunnels was subjected to analysis of variance to determine differences between means where appropriate (IBM SPSS Statistics v.20). An arcsin transformation was applied to the data before analysis. Where significant differences occurred, the LSD test was applied for mean separation.

The influence of host number in patches and wasp numbers on the fraction of hosts parasitized was analysed with a linear model, with replicates treated as blocks. When it was found that wasp number, but not host number, influenced the final level of parasitism, we estimated the mutual interference coefficient (*m*). First, the overall fraction of hosts that were parasitized in each wind tunnel within each replicate was calculated by pooling the data. Then non-linear regression was used to estimate the values parameters of Q and m in equation (2) (*nlreg* function of R version 3.1.0 (2014-04-10)).

The numbers of visits in the choice test was compared by a two-tailed Binomial test and differences were considered significant at $P < 0.05$.

4. Results

When a single wasp was released in the wind tunnel, in 75% and 25% cases it landed first on a high and medium density patch, respectively, and it was never observed to first land on a low density or empty patch. Single foragers spent significantly longer times on a patch with 8 hosts ($df_1 = 3$; $df_2 = 8$; $F = 12.91$; $P = 0.002$) in the first 15 min and then spent the greatest fraction of time on patches with 4 hosts in the second 15 min period ($df_1 = 3$; $df_2 = 16$; $F = 5.71$; $P = 0.007$; Figure 1a). After 15 minutes had elapsed, single foragers spent most of their time away from the grape leaf patches (“Elsewhere”). In presence of another wasp, females spent relatively more time on patches with fewer hosts. They were also more likely to spend time elsewhere in the wind tunnels (Figure 1b). When three wasps were searching together they tended to spend the most time on patches with the highest host densities, but quickly moved away from the grape leaves (Figure 1c). For all wasp densities, there was a general trend to spend the greatest fraction of their time in patches at each host density during the first 15 minute period, and this fraction declined over time. The time spent elsewhere increased over time, a trend that was most pronounced at the highest wasp density.

Experienced female *D. tasmanica* showed a significant preference to visit fresh patches of hosts (14) in comparison with patches they had previously visited (4) (P = 0.031).

***Parasitism and Mutual interference*:** Regression analysis indicated that the fraction of hosts parasitized on patches was affected by wasp density (F = 7.1728, df = 1,126, P < 0.01), but not host density in patches (F = 0.008, df = 1,126, P > 0.9) (Figure 2). There was also some day to day variation in the results as there was a significant effect of replicate (F = 2.0805, df = 15,126, P < 0.015). When overall parasitism was pooled within each wind tunnel, nonlinear regression indicated that there was a consistent pattern of mutual interference (Q = 0.4493, m = 0.5257; Figure 3).

5. Discussion

The distribution of female *D. tasmanica* in the presence of patchily distributed larval LBAM showed two opposing responses. First, patches were more attractive at high host densities, so single parasitoids arrived at patches with higher host densities first and spent more time there (Figure 1). This is consistent with adaptive foraging and the rewarding payoff expected from foraging where host are most abundant (Charnov, 1976; Cook and Hubbard, 1977, Wajnberg 2006). Second, interference appeared to be greater at high host densities so competing parasitoids were more likely to move to less rewarding patches (Zwarts, 1976; Goss-Custard, 1977a, b). Our results are broadly consistent with the predictions of the Ideal Free Distribution, which describes the equilibrium distribution of a population of females *D. tasmanica* among several habitat patches (Fretwell and Lucas, 1970; Sutherland, 1983; Krebs, 1978; Kacelnik et al., 1992; Figure 1b and c; 2). Resources, in this case host larvae, are shared equally by the competitors (Figure 2).

As the numbers of foragers increased, they spent less time searching on host infested patches in comparison with individual foragers, and they stopped searching more often and earlier (Figure 1). In all cases the female *D. tasmanica* virtually stopped searching in the wind tunnels after 45 min, a trend that was more pronounced when wasp numbers rose. These observations could be due to release of repellent chemicals by hosts after attack (Witz, 1990) or deposition of a marking pheromone by the parasitoids during host searching (Bernstein and Driessen, 1996). Some species of parasitic wasps avoid the sites on which they themselves previously deposited a marking pheromone (Price, 1970; Galis and van Alphen, 1981; Sugimoto et al., 1990). Such site discrimination by parasitoids may be closely related to the stopping rule for deciding when to leave the patch (Hassell and Southwood, 1978; Sugimoto et al., 1986, Wajnberg 2006).

There was a negative relationship between search-efficiency and parasitoid density in the experimental arena, which indicated mutual interference among female *D. tasmanica* (Figure 3). Our previous research showed that host discrimination occurs in *D. tasmanica* (Yazdani et al. 2014, unpublished results), and dissection did not reveal any instances of superparasitism in this experiment. So, we conclude that it is unlikely that indirect interference affects the searching efficiency of *D. tasmanica*. Also, we did not observe females interacting with each other directly, which further suggests that females deposit marks on patches that they visit.

In the vineyards and orchards, the variation in host density among patches is much more variable than in laboratory arenas. This is because natural systems include more patches, hosts are present at various developmental stages, alternative host species may be present, and the profitability of patches varies both in space and in time. Nevertheless we expect some interference in the field due to the tendency of females to avoid previously visited patches. However in a vineyard, foragers would have the chance to both immigrate into rewarding patches and disperse among other patches. They did not have this opportunity in the wind tunnel, so we suspect that interference would not have pronounced effects on the efficiency of wild *D. tasmanica*. Patch exploitation and the related spatial distribution of foragers in such a complex situation have been addressed theoretical models (Goubault et al., 2005; Wajnberg, 2006), but to our knowledge, not by experimental approaches. So, further field experimentation is essential to investigate the magnitude of mutual interference of *D. tasmanica* in vineyards and orchards.

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Figure legends

Figure 1. The mean fraction of time (± SE) that wasps spent in different locations in wind tunnels during 1 hour. Means labelled with the same letter do not differ statistically among locations within a time period ($P \geq 0.05$).

Figure 2. The effect of host number per patch and number of foraging female *D. tasmanica* on the mean fraction of parasitism of second instar LBAM

Figure 3. The effect of wasp number on the mean fraction of hosts parasitized (± 95% C.I.). Fitted curves show parasitism with mutual interference (Q = 0.4493 , m = 0.5257) and the expected pattern of parasitism if there was no interference.

Figure 1 abc

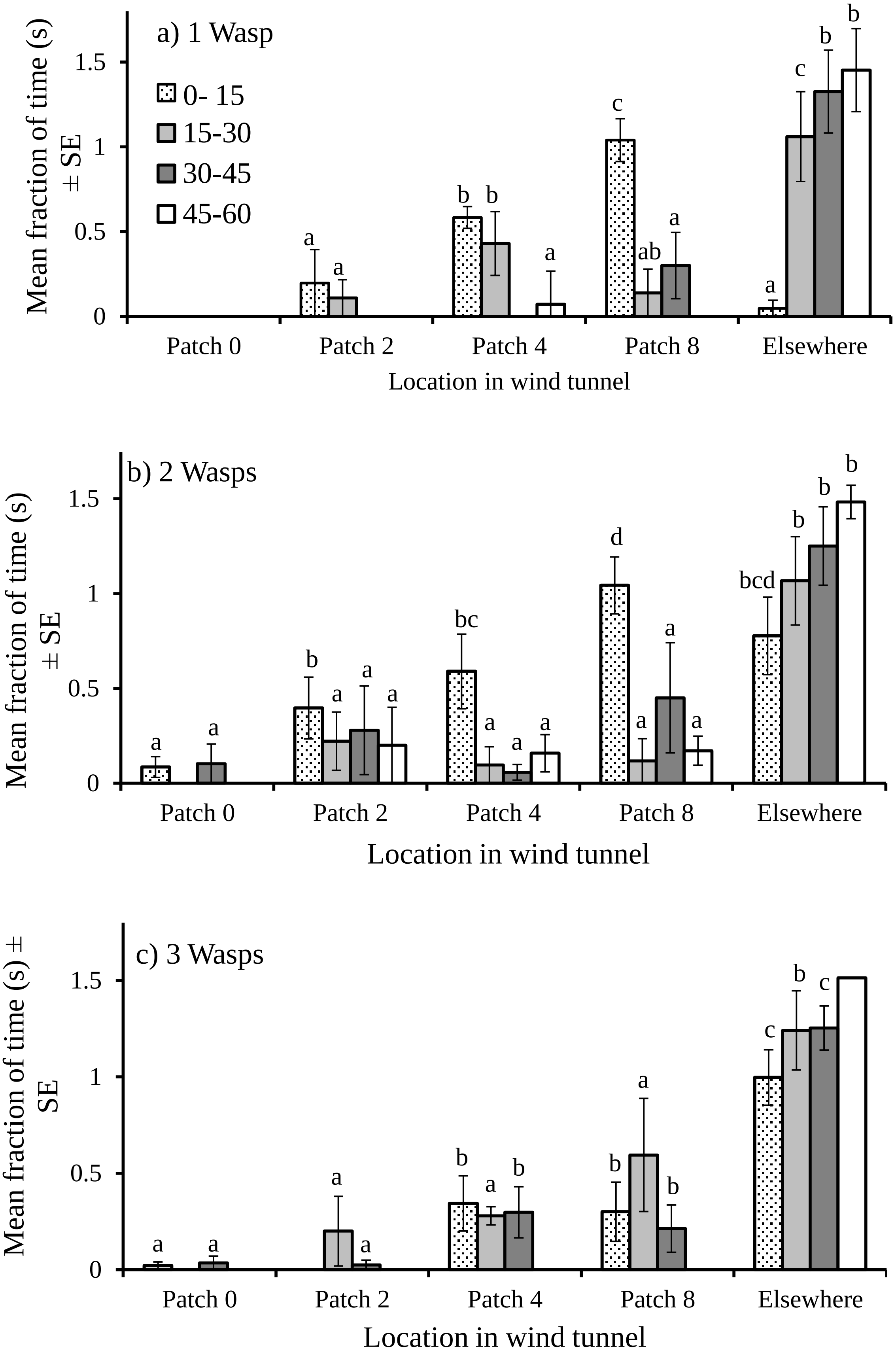

a) 1 Wasp
▨ 0- 15
▨ 15-30
■ 30-45
☐ 45-60
Mean fraction of time (s) ± SE
0
0.5
1
1.5
a
a
b b
a
c
ab
a
a
c
b
b
Patch 0
Patch 2
Patch 4
Patch 8
Elsewhere
Location in wind tunnel
b) 2 Wasps
Mean fraction of time (s) ± SE
0
0.5
1
1.5
a
a
b
a
a
a
bc
a
a
a
d
a
a
a
bcd
b
b
b
Patch 0
Patch 2
Patch 4
Patch 8
Elsewhere
Location in wind tunnel
c) 3 Wasps
Mean fraction of time (s) ± SE
0
0.5
1
1.5
a
a
a
a
b
a
b
b
a
b
c
b
c
Patch 0
Patch 2
Patch 4
Patch 8
Elsewhere
Location in wind tunnel

Figure 2

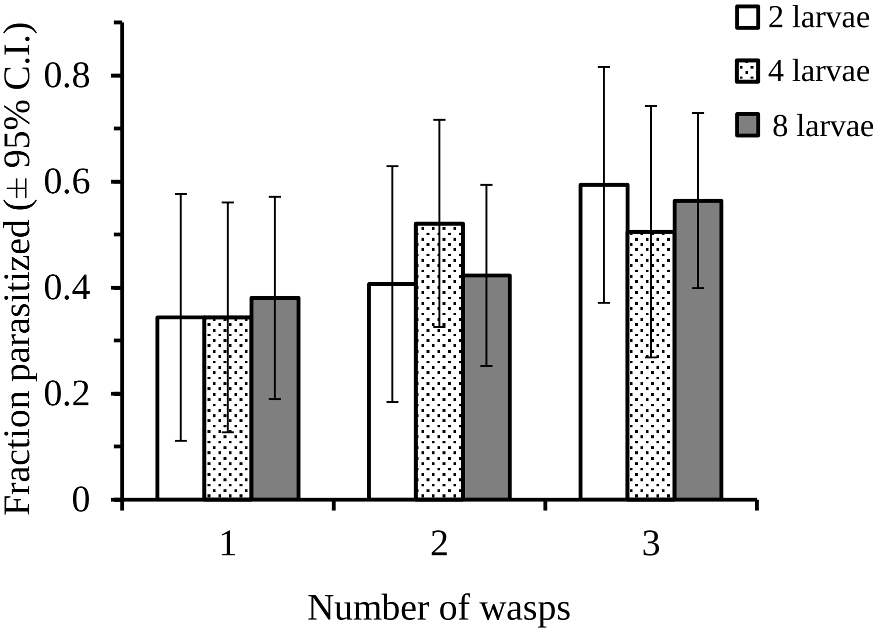
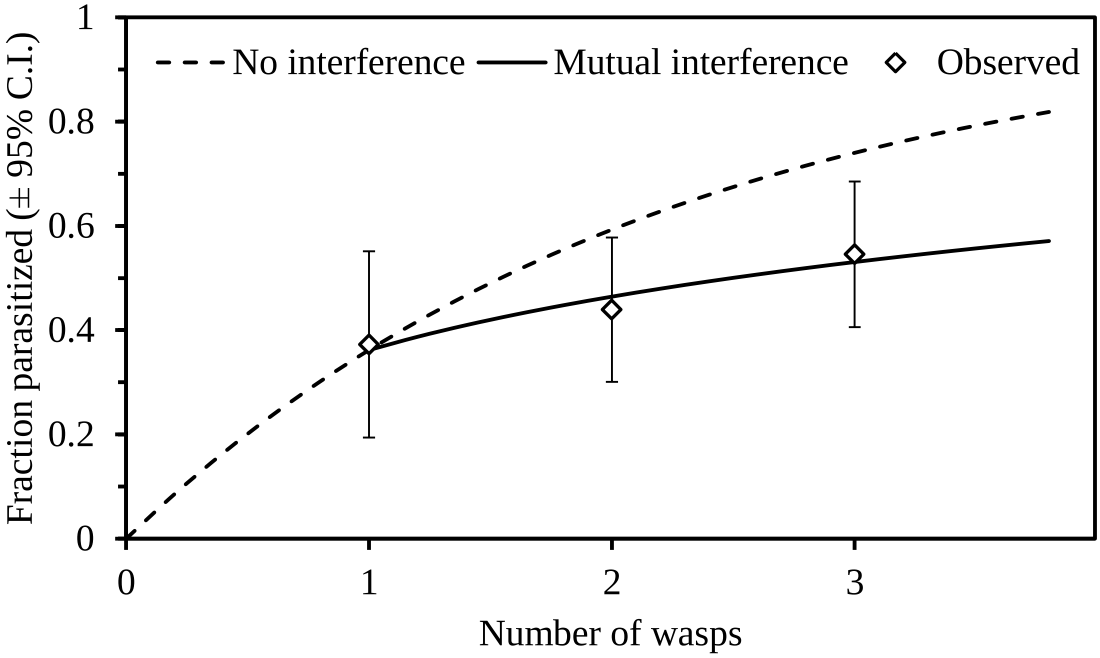

2 larvae
4 larvae
8 larvae
Fraction parasitized (± 95% C.I.)
0.8
0.6
0.4
0.2
0
1
2
3
Number of wasps

Figure 3


No interference
Mutual interference
Observed
Fraction parasitized (± 95% C.I.)
1
0.8
0.6
0.4
0.2
0
0
1
2
3
Number of wasps

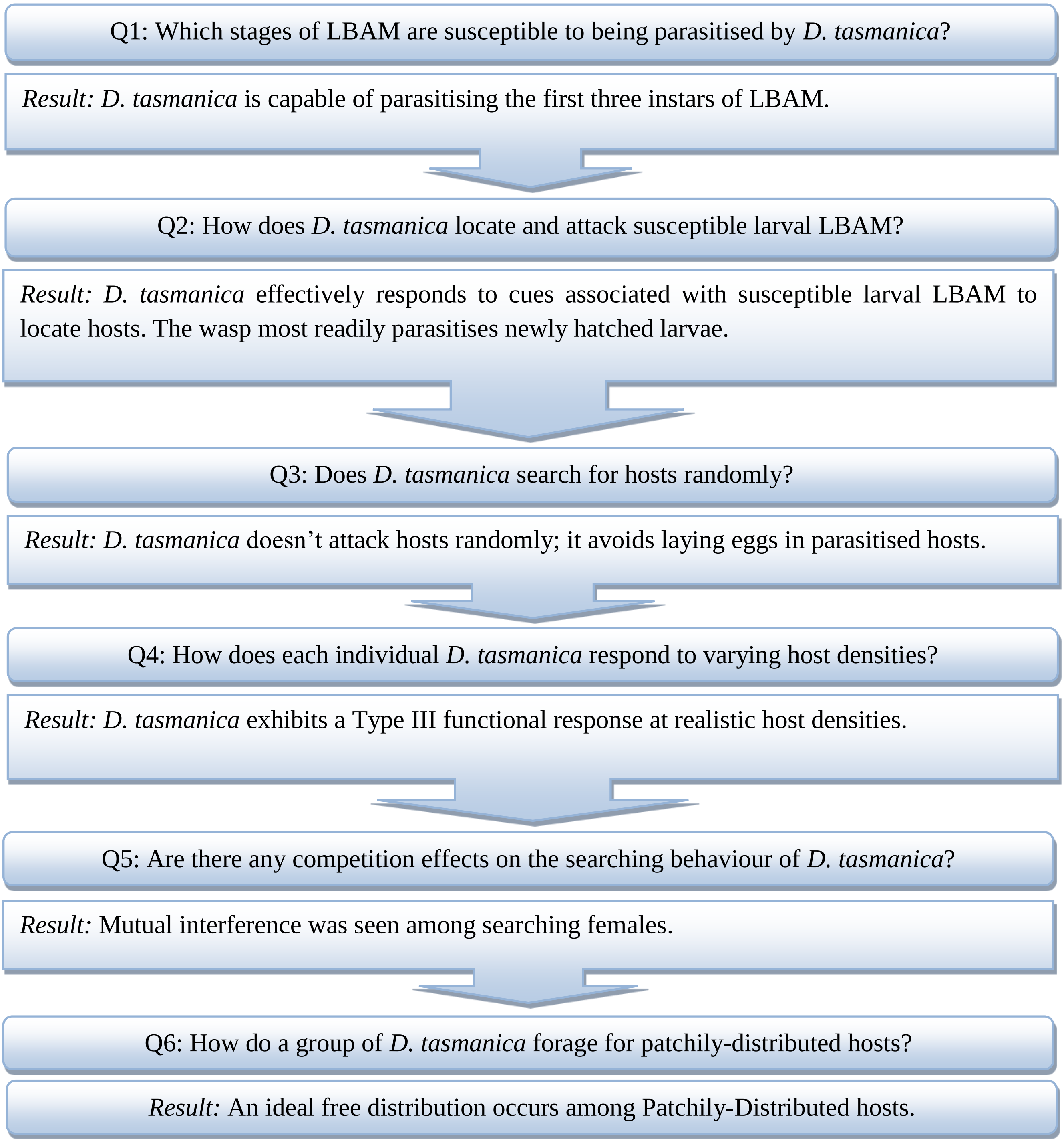
CHAPTER 7

GENERAL DISCUSSION

1. General discussion

Why some biological control programs succeed while others fail is a question that has received considerable attention from both biological practitioners and theoretical ecologists (Mills 1994; 2000). The foraging behaviour and resulting functional responses of natural enemies explain to some extent the reported failures and successes in biological control programmes, but they should not be considered in isolation (Fernández-arhex and Corley 2003). Theoretical ecologists have emphasized the importance of specific features of parasitoid behaviour such as searching efficiency (Hassell 1978) and heterogeneity of attack (e.g., Beddington et al. 1978; May and Hassell 1988; Murdoch 1990; Mills and Getz 1996) in determining the success of a parasitoid in reducing host populations. Theoretical models have repeatedly shown that the spatial distribution of parasitism between host patches is one of the major factors that can influence the persistence and success of biological control (e.g., Beddington et al. 1978; Chesson and Murdoch 1986; Hassell and Pacala 1990; Rohani et al. 1994).

In this study key aspects of the individual and population behaviour of *D. tasmanica* were investigated. The outcomes of my study should provide insights into parasitism of LBAM and other leafrollers that are susceptible to *D. tasmanica*. The results of this research provide a foundation for understanding the biological control potential of *D. tasmanica* against LBAM in vineyards and other agricultural ecosystems. Further research is needed to investigate how factors such as searching for food and mates, effects of host plant preference, alternative host species, host defensive behaviour and interspecific competition influence patterns of parasitism by this species under laboratory conditions and in the field (Figure 1).



Q1: Which stages of LBAM are susceptible to being parasitised by *D. tasmanica*?

Result: D. tasmanica is capable of parasitising the first three instars of LBAM.

Q2: How does *D. tasmanica* locate and attack susceptible larval LBAM?

Result: D. tasmanica effectively responds to cues associated with susceptible larval LBAM to locate hosts. The wasp most readily parasitises newly hatched larvae.

Q3: Does *D. tasmanica* search for hosts randomly?

Result: D. tasmanica doesn't attack hosts randomly; it avoids laying eggs in parasitised hosts.

Q4: How does each individual *D. tasmanica* respond to varying host densities?

Result: D. tasmanica exhibits a Type III functional response at realistic host densities.

Q5: Are there any competition effects on the searching behaviour of *D. tasmanica*?

Result: Mutual interference was seen among searching females.

Q6: How do a group of *D. tasmanica* forage for patchily-distributed hosts?

Result: An ideal free distribution occurs among Patchily-Distributed hosts.

Figure 1. Main objectives and outcomes of the thesis.

1.1 Practical prospective

From a practical perspective, this study has produced a better understanding of the efficiency of *D. tasmanica* as a key natural enemy of LBAM. The ability of *D. tasmanica* to parasitise the first three instars is important with respect to its potential to reduce LBAM feeding damage in vineyards (**Chapter 2**). Consequently, the searching behaviour of female *D. tasmanica* that leads it to locate plants infested with susceptible larval LBAM was studied next. I found that female *D. tasmanica* effectively responds to cues associated with all susceptible instars of LBAM when locating hosts. The pattern of host stage acceptance shows that first instar of LBAM is more susceptible to be parasitised by *D. tasmanica* (**Chapter 3**).

The ability of *D. tasmanica* to discriminate among parasitised hosts is another important attribute of a successful biological control agent (van Lenteren et al. 1978). This ability is necessary to avoid superparasitism and to minimize the waste of time and energy associated with this behaviour (Godfray 1994), and it should also lead to a more uniform distribution of parasitism among susceptible hosts (**Chapter 4**).

D. tasmanica responded in density-dependent manner to hosts when densities were low. It should be able to contribute to the suppression pest populations before they reach economically damage levels (**Chapter 5**). This suggests that *D. tasmanica* has potential to be an effective agent for biological control of LBAM in vineyards. In fact the key to understanding decision-making processes in individual parasitoids is to determine how they gather information about resource abundance and distribution over patches. I showed that *D. tasmanica* alters its behaviour after experience in rewarding and non-rewarding habitats, and subsequently adjusts its searching intensity. So females of *D. tasmanica* seem to assess patch quality which is likely to be influences by their ability to assess concentrations of kairomones in a given area. It is likely that there is a close relationship between kairomone concentration and the number of hosts available for *D. tasmanica* to parasitise (Vet and Dicke 1992). Therefore infochemical concentration should contribute to the foraging efficiency of *D. tasmanica*, and may explain this parasoitoid's patch selection, which was closely correlated with host density (Chapter 6).

Paull et al. (2014) found that there is a significant effect of variety on the level of parasitism of LBAM by *D. tasmanica*. I found that *D. tasmanica* effectively locates and parasitises larval LBAM feeding on Chardonnay leaves. However the degree of parasitism in the field is consistently and significantly higher on the grape variety Cabernet Sauvignon compared to Chardonnay (Paull, Schellhorn and Austin 2014). So, the efficiency of *D. tasmanica* is affected by host plants of its larval hosts. This could influence some of the behaviours reported in this thesis.

Parasitoids do not always forage alone, so the foraging behaviour of single and multiple females of *D. tasmanica* in the presence of patchily-distributed hosts was observed and analysed (**Chapter 6**). Although mutual interference among females significantly reduced their foraging efficiency, in the vineyards host patches are much more variable than in a laboratory arena like a wind tunnel. Also, in a vineyard foragers would have the opportunity to both migrate to rewarding patches and disperse among other patches if they don't readily locate hosts. They did not have this opportunity in the wind tunnel. So, I suspect that interference may not have such pronounced effects on the efficiency of wild *D. tasmanica*, but this needs further investigation in a more realistic setting. In addition results show that *D. tasmanica* was able to choose those patches which initially offer the highest oviposition rates, but that hosts were equally susceptible to attack by *D. tasmanica*, irrespective of their local density. Sirot and Bernstein (1996) argued that by finding the state-dependent ideal free distribution for a population of parasitoids, we should be able to predict their distribution between direct feeding areas and the host living areas at equilibrium. The proportion of parasitoids in each area is also altered by the number of competitors and the resulting interference. Sirot and Bernstein's (1996) model predicts that optimal time sharing between food searching and host searching may promote the stability of the host-parasitoid system.

If LBAM is efficiently parasitised, then the local populations of LBAM should be less likely to reach the economic threshold, or population increases may be delayed leading to reduced costs of control. From my results it seems that *D. tasmanica* should play a valuable role in the biocontrol of LBAM, but in fact parasitism levels by *D. tasmanica* in vineyards are usually low, with parasitism levels less than 20% commonly observed (Yi Feng 2014 unpublished results; Paull 2007). Also Paull, Schellhorn and Austin (2014) conducted large-scale field experiments to quantify and characterize the population response of *D. tasmanica* to different densities of LBAM in the field. In an apparent contradiction to my results, they concluded that the population response of *D. tasmanica* to varying host density was inversely density-dependent, which implies the species exhibits a Type II functional response. So I question why *D. tasmanica* exhibits behaviour that should effectively suppress the LBAM population under laboratory conditions, while is not an effective parasitoid in vineyards. This may be due to inadequate resources such as access to carbohydrates, specific nutrients, shelter or alternative hosts, which are not available or are in short supply in vineyards (Berndt and Wratten 2005). Parasitoids are likely to expend more energy and time searching for these resources when they are limiting and, as a result, the time available to maximize their response to increasing host density is reduced (Desouhant et al. 2005). When buckwheat flowers are planted in vineyards, parasitism rates by *D. tasmanica* can be raised substantially (Berndt et al, 2006). It has been shown that optimal individual behaviour of parasitoids when there is time sharing between searching for hosts and food may have a stabilizing effect on the host-parasitoid dynamics (Krivan and Sirot 1997). Therefore a broader understanding of tritrophic level interactions that encompasses parasitoid food considerations should enhance our ability to design effective biological control strategies for LBAM using species like *D. tasmanica* (Lewis et al. 1998). The realised lifetime fecundity of *D. tasmanica* is significantly increased in the presence of flowers, although this is a consequence of the increase in longevity, rather than an increase in daily fecundity (Berndt and Wratten 2005). Although, in my study key aspects of the individual and population behaviour of *D. tasmanica* were investigated, further understanding is required to determine why *D. tasmanica* doesn’t consistently perform better in vineyards.

Two other aspects of the biology of *D. tasmanica* warrant further investigation. It is known that *D. tasmanica* can locate and sting different species of leafrollers (Suckling and Brockerhoff 2010), and also its pest host LBAM has a wide plant host range (over 500 plant species) (Suckling et al. 2001). So there is variation in the volatile kairomones associated with different combinations of host larvae and host plants in nature. If the semio-chemichal cues from host insects and plants that enable *D. tasmanica* to locate hosts and assess their suitability are understood more deeply, this might provide an avenue to enhance biological control through habitat management involving attractive alternative host plants and non-pest host larvae. It is likely that the patterns of parasitism for other leafrollers by *D. tasmanica* are similar to those involving LBAM and, if this is so, then it should be possible to develop a model to explain when and how *D. tasmanica* could regulate its host populations. To support my assumption I imply the notion by Turlings et al. (1989) that the experience with an alternative host still causes a substantial increase in response which suggests that associative learning is not the only process involved but that sensitization takes place also. It is suspected that an associative learning process is involved, which is triggered when a parasitoid contacts one or more specific kairomones (unconditioned stimuli). The parasitoid then links the surrounding odours (conditioned stimuli) with the possible presence of host larvae. Subsequently, the wasps will use those odours as cues in the search for more hosts. (Lewis and Tumlinson 1988; Turlings et al. 1989). For example in vineyards LBAM attacks both grape and plantain which will be attractive for *D. tasmanica* and I assume that existence of an alternative host plants might be helpful to attract and maintain *D. tasmanica* as a generalist parasitoid in vineyards.

1.2 Theoretical perspective

The functional response curve for *D. tasmanica* showed the characteristics of a Type III response at lower densities, but at higher densities the trend switched to a Type I response (Figure 4 Chapter 5). But if densities between 4 and 16 larvae were tested, my results would show Type II functional response. So, my results suggests that the experimental design is important, which may explain why the type III functional response has been reported for parasitic insects in relatively few cases. Also, van Lenteren and Bakker (1976) suggested that the apparent absence of a stabilizing density dependent functional response in invertebrate predators or parasitoids may be caused by experimental procedures in which the numbers of prey or hosts at low densities is higher than what can be expected in the field. Indeed, the results of functional response experiments may be overestimating type II curves. For instance, it has been suggested that time-limited experiments may force a type II curve on the insects' behaviour (van Lenteren and Bakker, 1976; Walde and Murdoch, 1988; Ives et al., 1999). Furthermore, type II models may have been used to fit data that could be better served by type III models, especially in older work (Fernández-arhex and Corley 2003).

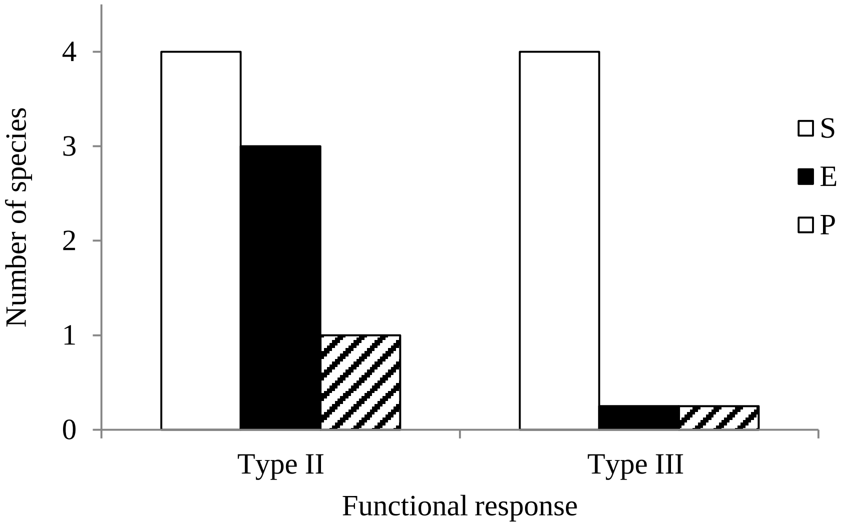


Figure 2. The results of biological control introductions of parasitoids for which the functional response is known. White bars shows successful cases (S), full bars established cases (E) and hatched bars show cases where partial control (P) has been reported (*from* Fernández-arhex and Corley 2003).

The functional response, has been considered in theory, to be an essential indicator in the selection of optimal biocontrol agents (see van Lenteren and Bakker, 1976; Hassell, 1978; Hughes et al. , 1992; Bernal et al. , 1994; Kumar et al. , 1994, van Steenis and El-Khawass, 1995; van Alebeek et al., 1996, Berryman 1999). Parasitoids may display Type III curves, which in turn may contribute to density dependent host population regulation (Fernández-arhex and Corley 2003). However, success does not appear to be directly related to the form of the functional response curve. Both Type II and III responses in parasitoids relate to some degree of success (including establishment and partial control) in a similar way (Fernández-arhex and Corley 2003). However, when excluding failures which may be more related to aspects other than host exploitation, it may be seen that several species with a Type II response have become established without achieving successful control (have become established or achieved partial control only). This fact, establishment without control, may relate to the parasitoid's inability to rapidly encounter hosts when these are in low densities. In contrast, among the few cases with Type III curves, the great majority of species that established controlled host populations (Figure 1) (Fernández-arhex and Corley 2003). This analysis suggests that *D. tasmanica* should be able to contribute to control of LBAM, but it is probably limited in some other way.

1.3 Further research

By studying individual and population behaviour, I have tried to build links between the theoretical and the practical, the potential and the realistic in developing an understanding of how *D. tasmanica* can contribute to the regulation of LBAM population in vineyards. But only with more experiments will it be possible to more fully use *D. tasmanica* in management of LBAM populations. And with a deeper understanding through further studies, we will gain greater capacity to utilise parasitoids in biocontrol of LBAM and other lepidopteran pests.

A parasitoid foraging in a vineyard is confronted by a wide range of cues. Kairomones are released by different hosts and other chemicals are releasd y host plants. Throughout the growing season, as the canopies of host plants develop, and host populations increase and the associated cues increase, adding complexity and noise to system. This increases the challenge a parasitoid faces and is likely to increase the time it takes a parasitoid to locate and recognise suitable hosts (Waage 1982; Casas 2000). So it is important to study how effectively *D. tasmanica* responds to combinations of cues to locate the most suitable host among others. Since our observations of parasitoid behaviour in a wind tunnel relate the behavioural responses to host-associated volatiles by *D. tasmanica* toward larval LBAM, I wonder if it is likely to respond similarly to other host species in a more complex system (Figure 3).

Adult parasitoids must not only find hosts for reproductive purposes. They must also locate food to meet their short-term nutritional needs. More knowledge of how female *D. tasmanica* deals with the often competing needs for these two vital resources is essential for understanding their foraging strategies. Another factor in determining variation in foraging behaviour is the parasitoid's physiological state relative to needs for mating. The respective roles of these variables and their interactive effects on foraging behaviour of *D. tasmanica* should be examined.

The Tortricidae is one of the largest families of Lepidoptera with over 10,000 described species. Many of these are economically important pests (Brown 2005). Most of the parasitoids that attack tortricids are generalists that parasitise diverse species of this family, which potentially leads to indirect interactions among hosts sharing these parasitoids. Therefore it is important to determine how *D. tasmanica* responds to varying local densities of multiple host species, which ultimately interlinks the population dynamics of the individual host species sharing this parasitoid. Generalist parasitoids like *D. tasmanica* may show distinct host insect and host plant preferences that influence where they forage. These preferences could lead to complex interactions that determine the capacity of this parasitoid to regulate populations of its hosts. Therefore, the aggregative responses of a female *D. tasmanica* to a range of common host species should be assessed.

In order to better understanding the link between individual behaviour and population dynamics, two types of research are needed. Firstly laboratory and simple patch-level experiments are needed to obtain a thorough understanding of individual decision-making of parasitoids on patch exploitation, which I did in my PhD project. Secondly, it is necessary to create stronger links between experiments, both in the laboratory and in the field, and theatrical population models. So, further field experiments are essential to investigate functional responses and inter- and intraspecific competition involving *D. tasmanica* in vineyards. Then host-parasitoid population dynamic models could be derived from theoretically optimal behavioural processes. It could be especially valuable to incorporate more biologically realistic behavioural attributes based on field-oriented studies into a theory-driven host-parasitoid interaction model in the future. Therefore, the results presented in this thesis could be used to inform future field experiments with the aim of developing a more comprehensive model based on the behavioural phenomena of the functional response, host discrimination and mutual interference in a practical setting.

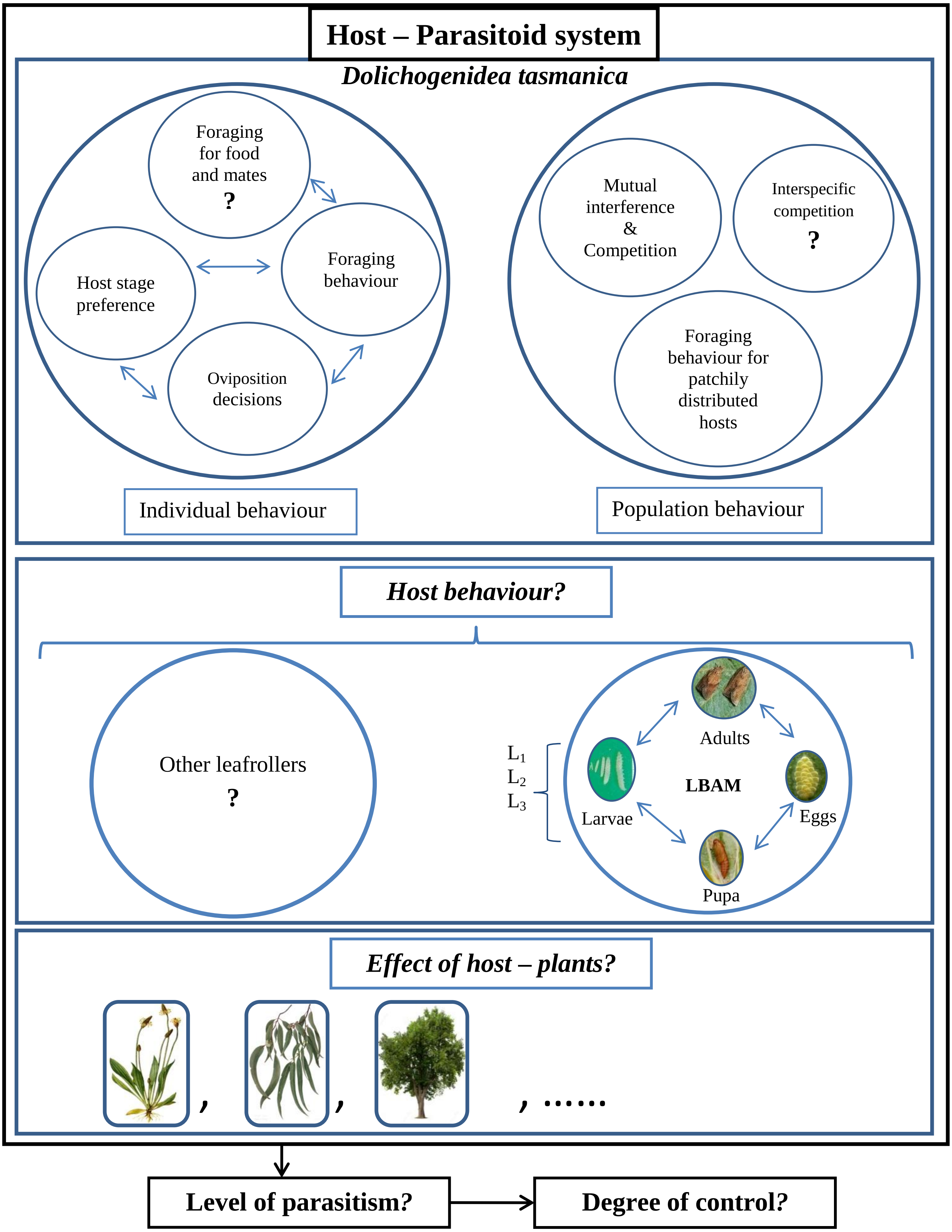


Figure 3. Outcomes and research gaps

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APPENDIX

1. Pre Experimental Methods

1.1 Growing Host Plants

Plantain (*Plantago lanceolata*) was chosen as the host plant for rearing LBAM because the body weight of females and the finite rate of increase are consistently higher on plantain in comparison to other host plants (Danthanarayana et al. 1983, 1995). Also fecundity is greater by a factor of 1.5–2.5 on plantain. The maximum fecundity (1492 eggs per female) was recorded from plantain (Suckling 2010). In addition, regularly growing plantain in a greenhouse as a host plant for experiments is easy, cheap and quick.

1.2 Rearing LBAM

A colony of LBAM, which was previously maintained by the South Australian Research and Development Institute, was used to produce hosts for experiments and parasitoid cultures. In this study we used the same methods as described by Cunningham (unpublished).

A laboratory colony of *E. postvittana* was reared at 22 ± 2ºC with a photoperiod of 12L: 12D on an artificial diet. The diet consisted of dried lima beans (250 g), which were soaked overnight in cold water, brewer's yeast (80 g), sorbic acid (2.5 g), methyl-p-hydroxy benzoate (5 g) and water (600 ml). The mixture was sterilised in an autoclave for 20 minutes at 120 ºC. When the bean mixture cooled to 70 ºC, ascorbic acid (8 g), formaldehyde (4 ml) and agar (32 g) dissolved in 1L water were added and mixed well in a blender. The hot diet was poured to depth of 1-2 cm into 100 ml plastic cups that served as rearing containers. Three to five individual egg masses were cut from the adult holding cups (Figure 1) and inserted into the diet layer in the rearing containers. Development from egg to pupa was completed in 30-35 days, when the pupae were harvested. Pupae were sexed by examining the anatomy of the abdominal segments, in which female has 3 while male has 4 abdominal segments (Figure 2). Female and male pupae were placed in separate emergence cages and supplied with 10% honey solution via a wick. Six newly emerged females and six males were placed in a 285 ml plastic cup with vertical ridges that contained a dental wick soaked in honey solution and was covered with a sheet of voile. Cups were left in natural light at room temperature for at least three days to allow adults to mate and lay eggs along the ridges of cups. Eggs are laid in flat overlapping masses (that resemble fish scales) containing 20-50 eggs.

a) Rearing cups

b) Harvested pupa from rearing cups

c) Female and male emergence cages

d) Adult cup

Figure 1. Rearing method for LBAM under laboratory conditions

Figure 2. Abdominal view of pupa female (left) and male (right) *Epiphyas postvittana*.

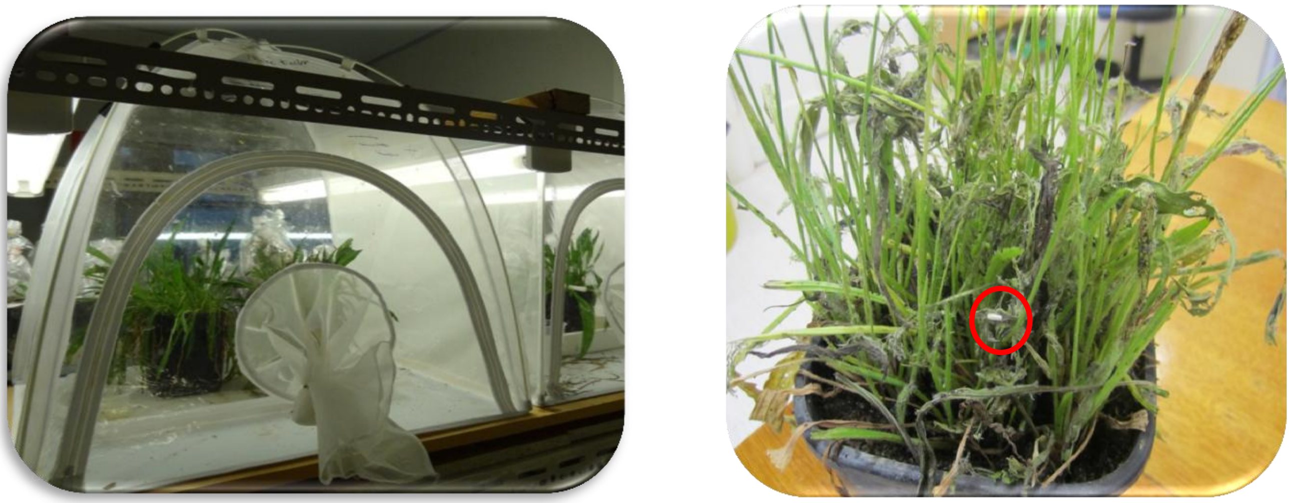
1.3 Rearing *D. tasmanica*

A culture of *D. tasmanica* was established from individuals collected in 2012 in South Australian vineyards. Larval LBAM were collected by searching for their conspicuous leaf rolls on plantain and grape leaves. All collected specimens were kept individually under insectary conditions at 24°C in rearing cups. They were provided with plantain foliage and development was recorded every day until any parasitoids emerged. Then, any newly emerged *D. tasmanica* were released into cages in which infested plantain with first instar LBAM were provided and maintained at 23 ± 2ºC, 14L: 10D.

Based on the work of Paull (2007), approximately 20-30 eggs of LBAM were placed in each pot of plants in order to infest plantain for the parasitoid culture. After 5-7 days, LBAM larvae hatched and could be kept for 3-5 more days to let the larvae settle. Then female and male *D. tasmanica* were added to the rearing cage (Figure 3). The interior of cages was sprayed with water once every day or second day. A cotton wick containing 1.7 ml of 50:50 honey/water solutions was placed into each cage as a food source for the adult parasitoids. When wasp cocoons formed, they were isolated in

100 ml containers together with a drop of honey. Every morning the newly emerged females were collected, caged overnight with 5 males for mating, and provided with water and honey. Females were subsequently re-isolated in 18mm diam. × 50 mm glass vials 1h before being used in experiments.

For general rearing, each pot of infested plantain was replaced with a new one every 2 weeks, and then the larvae kept in containers under insectary conditions until the emergence of new wasps. Field- collected *D. tasmanica* males were added to the culture periodically to maintain genetic diversity.



a) Rearing tent

b) Harvesting cocoon from infested pot

Figure 3. Rearing method for *D. tasmanica* under laboratory conditions