

Ecological interactions between two natural enemies of the Light Brown Apple Moth



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A thesis submitted for the Degree of Doctor of Philosophy

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29th April 2022



University of
Nottingham
UK | CHINA | MALAYSIA

Table of Contents

ABSTRACT	i
DECLARATION	iii
ACKNOWLEDGEMENTS	v
PREFACE	vii
CHAPTER ONE	1
General Introduction.....	
CHAPTER TWO	20
Walk this way, fly that way: <i>Goniozus jacintae</i> attunes flight and foraging behaviour to leafroller host instar	
CHAPTER THREE	63
Family planning for parasitoids: reproductive responses to leafroller host development in <i>Goniozus jacintae</i>	
CHAPTER FOUR	100
Don't judge a 'bug' by its cover: responses of the ectoparasitoid <i>Goniozus jacintae</i> to hosts containing the endoparasitoid <i>Dolichogenidea tasmanica</i>	
CHAPTER FIVE	136
General Discussion and Conclusions	
REFERENCES	149
APPENDICES	
Appendix I – Insect Rearing & Management.....	167
Appendix II – Career and Research Skills Training.....	171
Appendix III – University Achievements	173

ABSTRACT

The light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is the most damaging insect pest of grapevines in Australia, causing upwards of \$70 million AUD worth of damage to the Australian wine grape industry annually. The gregarious ectoparasitoid *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae) and the solitary endoparasitoid *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) are the two most common, native, natural enemies that parasitise LBAM. *Dolichogenidea tasmanica* parasitises the early larval instars of LBAM, whilst *G. jacintae* parasitises the later instars. Both parasitise the 3rd instar of LBAM, suggesting that direct interspecific competition may occur.

Biological control methods that suppress pest populations to below economically damaging thresholds are sought after, but require a sound understanding of the interactions between parasitoids and hosts prior to their application. There must also first be a foundation of knowledge for the biology of each species in the interaction. *Dolichogenidea tasmanica* has been well-studied, but little is known about the behavioural ecology of *G. jacintae*. Hence, this project began by studying the biology of *G. jacintae* before moving on to the evaluation of interactions between the two parasitoids. Specifically, this project aimed to investigate: Part One: (1) Foraging behaviour of *G. jacintae* towards different larval instars of LBAM; (2) Oviposition behaviour of *G. jacintae* towards different larval sizes and instars of LBAM; and Part Two: (3) Interspecific competitive interactions between *G. jacintae* and *D. tasmanica* when attacking the same LBAM host.

The key results were that: Part One: (1) *Goniozus jacintae* exhibits host-stage dependent foraging behaviour towards LBAM: different behaviours were shown at the pre- and post-flight stages and varied according to host instar, flight duration was shortest around 5th instar LBAM, *slow walking* behaviour was only seen in close proximity to potential hosts and was more common around larger hosts; (2) *Goniozus jacintae* females produced bigger broods on larger hosts, brood sex ratios were female biased with extremely low variance, and body size of offspring was positively correlated to the amount of host resource

available; and (3) *Goniozus jacintae* has some ability to discriminate between unparasitized and previously parasitised hosts. The probability of oviposition on the second host encountered was influenced by parasitism status of both the current and previous host, clutch size laid on the second host was influenced by parasitism status, but *G. jacintae* laid more eggs on larger hosts and laid more eggs on the first host encountered.

Collectively, these findings contribute towards determining the efficacy of *G. jacintae* as a potential biocontrol agent of LBAM and could ultimately lead to the improvement of LBAM biological control practices in the field. In addition, these findings contribute to the understanding of bethylid behavioural ecology and can be applied across a range of agro-ecosystems, promoting the long-term stability of managing lepidopteran pest species in the field.

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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I acknowledge the support I have received for my research through the provision of a scholarship from the University of Nottingham and University of Adelaide Joint PhD programme.

Emma Aspin

28/4/2022

This thesis is dedicated to my biggest supporters...

Dad

Mum

Amedeo

Ben



And to you, the reader.
For no knowledge should go unshared.

ACKNOWLEDGEMENTS

When I began this PhD in late 2017, I could never have predicted the magnitude of the challenges I was going to face along the way. The nature of my candidature already involved moving across the world, reaching beyond my comfort zone and pushing the boundaries of my personal and professional development. I set sail to an unknown land, excited for new horizons, unaware of what baggage I was bringing with me, or the baggage that was yet to come.

The completion of this thesis would not have been possible without the support of everyone listed below, and then some.

First of all, I'd like to thank Hui Trung Bui for showing me the ropes when I landed in Adelaide. Without your guidance, I wouldn't have been nearly as successful in rearing the insect cultures and sampling out in the field on my own. Your support was invaluable, and I thoroughly appreciate you taking the time to ensure my success whilst finalising your own thesis at the same time.

To the many occupiers of my supervisory panel throughout my candidature – Ian Hardy, Helen West, Mike Keller, Maryam Yazdani, Jerome Buhl and Tom Reader. You have all contributed important values to my research portfolio. Thanks especially to Ian and Mike; your joint wisdom and discerning eye for detail has made my work refined, kept my ideas grounded and reminded me to pursue the logic. A truly necessary duo to balance my creative mind! In addition, I thank Helen West for her support towards the end of my candidature; working in isolation was difficult for me, and I truly needed our weekly check-ins to stop me from going utterly bonkers.

To my now lifelong friends who happened to be workmates: Matteo R, Cate S, James C, Aaron P, Matthias S (+ Gabi!), Helen L, Amelie J, Mathieu B and Kala B. You all accompanied me on a journey of self-discovery... one which mostly boils down to the fact that I love camping so much now. But alongside that, you were there for me through thick and thin. You accepted me, laughed at my jokes and instilled a confidence in me that resides to this day. You each made me the best person I could possibly be, and I am forever grateful of that.

To the others who came and went along the way... no, I didn't forget you. I only refuse to acknowledge that our time together has passed. You may all be spread around the globe now, but I don't intend to let you go that easily!

Big shout out to my fellow Nottingham-Adelaide Joint PhD cohorts, especially Sally D, Olivia C, Rachna R and Cindy C for introducing me to the best Adelaide had to offer. On top of that, your hard work is inspiring and I hold each of you as role models in the highest regard. The world needs more Women in Science and I had the good fortune to meet four kick-ass examples of them. Keep doing what makes you awesome.

Thanks to the Austin lab for taking me under their wing, especially Andy, Erinn, Brock and Alana. You were the lab group I never knew I needed. Our chats fuelled my intellectual fire at the times it was dwindling low, and you acted as my own personal group of cheerleaders for every Sci-Comms related venture I made. I look forward to the next infamous lab retreat!

I must mention my beloved family and friends who supported me upon my return to the UK. That time was especially tough for me, and I wouldn't have made it through the darkest days without you. A PhD isn't a walk in the park at the best of times, never mind when the world as you know it is changing.

And yet, by some miracle, the worst year of my life simultaneously became the best year of my life when I met you, Amedeo. I can't wait to see what the rest of our lives brings us together; you have already given me more than I could ever imagine. *Ti amo, amore mio. La tua 'faccina', per sempre.*

Not forgetting my precious four legged friends, Nala, George and Juno. Thanks for the walkies. Your owners Bec, Jess and JC weren't too bad either!

Finally, thank you to Sarah at Yarrow Place and Andrea Z. I arrived in your offices a broken person and, with a lot of hard work, we put those pieces back together. Thank you for helping me realise my worth and having the strength to reach this seemingly impossible milestone. As one of you once said to me, "Emma, I think you can accomplish anything you put your mind to"...

And so, I did.

PREFACE

The research discussed in this thesis has led to the generation of three journal papers that have either been accepted for publication, or unpublished and written in manuscript style. In addition, E.A. performed multiple conference, symposium, and workshop presentations.

List of publications, published and proposed:

Aspin, E., Keller, M.A., Yazdani, M. and Hardy, I.C.W., 2021. Walk this way, fly that way: *Goniozus jacintae* attunes flight and foraging behaviour to leafroller host instar. *Entomologia Experimentalis et Applicata*, **169**, 350-361. DOI: [10.1111/eea.13028](https://doi.org/10.1111/eea.13028)

Aspin, E., Keller, M.A. and Hardy, I.C.W. Family planning for parasitoids: reproductive responses to leafroller host development in *Goniozus jacintae*.

Aspin, E., Keller, M.A. and Hardy, I.C.W. Don't judge a 'bug' by its cover: responses of the ectoparasitoid *Goniozus jacintae* to hosts containing the endoparasitoid *Dolichogenidea tasmanica*.

Oral conference and symposium presentations:

Aspin, E. (2022). Thesis findings: Ecological interactions between two natural enemies of the Light Brown Apple Moth. 26th International Conference of Entomology. 17 – 22 July 2022, Helsinki, Finland. [Abstract Accepted]

Aspin, E. (2019). Come fly with me: Searching behaviour of *Goniozus jacintae* in response to susceptible instars of *Epiphyas postvittana* (Light Brown Apple Moth). Australian Entomological Society Conference. 1 – 4 December 2019, Brisbane, Australia

Aspin, E. (2019). Come fly with me: Searching behaviour of *Goniozus jacintae* in response to susceptible instars of *Epiphyas postvittana* (Light Brown Apple Moth). Annual Postgraduate Symposium. 24 – 25 September, 2019, Waite Campus, Adelaide, Australia

Aspin, E. (2018). Ecological interactions between two natural enemies of the Light Brown Apple Moth (LBAM). 10 – 12 October 2018, University of Nottingham Malaysia Campus, Selangor, Malaysia

Oral workshop presentations:

Aspin, E. (2019). Parasitoid wasps for pest control on grapevines. EcoVineyards Workshop. 21 November 2019, Barossa Valley, Adelaide, Australia

Aspin, E. (2019). Parasitoid wasps for pest control on grapevines. National School Wine Show. 24 October 2019, Waite Campus, Adelaide, Australia

Aspin, E. (2018-2020). 'Insect Ninjas' Workshop. Multiple dates. Why Waite outreach labs, Waite Campus, Adelaide, Australia

CHAPTER ONE

General Introduction

Glossary

A list of some of the field-specific terms used in this thesis and their definitions.

Allospecific	An organism belonging to another species.
Arrhenotoky	A form of parthenogenesis in which unfertilised eggs develop into males (fertilized eggs develop into females).
Augmentative Biological Control	Release of additional numbers of a natural enemy when too few are present to control a pest effectively.
Biological Control	The use of living organisms, such as predators, parasitoids, and pathogens, to control pest insects, weeds, or diseases. Typically involves some human activity.
Classical Biological Control	Importation of exotic natural enemies to control previously introduced, or native, pests.
Conservation Biological Control	Promotion of the effectiveness of native natural enemies to control pests.
Conspecific	An organism belonging to the same species.
Ectoparasitoid	A parasitoid that feeds externally on the host as a juvenile.
Endoparasitoid	A parasitoid that feeds internally in the host as a juvenile.
Gregarious (parasitoid)	More than one offspring may develop to maturity from a single host.
Host	The organism in or on which a parasite or parasitoid lives; a plant on which an insect feeds.
Integrated Pest Management (IPM)	An approach to the management of pests in which all available control options, including physical, chemical, and biological controls, are evaluated and

	used in a unified program.
Interspecific	Existing or occurring between different species.
Intraspecific	Existing or occurring within a species or between individuals of the same species.
Koinobiont	A parasitoid whose host continues to feed and grow after parasitisation.
Mass-reared	Natural enemies produced in large numbers, usually for release programs as part of biological control.
Multiparasitism	A form of parasitism in which an individual host is attacked by two or more species of parasitoids.
Multivoltine	A species that produces two or more broods of offspring per year.
Natural Biological Control	Reduction of pest populations by resident natural enemies without any human intervention.
Natural Enemies	Organisms that kill, decrease the reproductive potential of, or otherwise reduce the numbers of another organism.
Parasitoid	An insect whose larvae feed and develop within or on the bodies of a single host, eventually killing the host. The adults are free living.
Pest	An organism that interferes with human activities, property, or health, or is objectionable.
Pesticide	A substance that is used to kill, debilitate, or repel a pest.
Polyphagous	An organism that is able to feed on a wide variety of different foods.
Solitary (parasitoid)	Only one parasitoid may develop to maturity from a single host.
Superparasitism	A form of parasitism in which an individual host is attacked more than once by a single species of parasitoid.

Project summary

This project aimed to untangle and elucidate interactions between two natural enemies of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae): *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) and *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae). The initial approach of this project was to observe *G. jacintae* host finding ability and reproductive biology to provide a foundation of knowledge for this relatively unstudied species. Furthermore, this study analysed ecological interactions between *D. tasmanica* and *G. jacintae* when attacking the same host; specifically the ability of *G. jacintae* to discriminate between unparasitised and parasitised hosts. This research provides a better understanding of how two parasitoid wasps interact with both their host and competitors, and how this affects their ability to effectively suppress host populations. Ultimately, this study will help to refine the understanding of biocontrol practices for LBAM and improve integrated pest management methods to reduce LBAM induced damage on the wine industry.

Introductory background

The mechanisms and composition of ecosystems are fundamentally dynamic, and can be driven, in part, by multi-species interactions. Parasitoid communities are often complex, intricate webs of potential host-parasitoid interactions, ranging from some parasitoid species having general associations across several host species, to specific adaptations which allow specialism on one host. However, there is also the overlap of interactions between species (such as feeding and competitive relationships) that vary in intensity and consequential outcomes. In turn, these outcomes can result in the structuring of ecological communities (Paine, 1966; Guimarães Jr., 2020).

Parasitoid species that utilise the same host species will have to face competitive interactions with one another in some, if not all, stages of their life histories if there is a degree of niche overlap (Hassell and Waage, 1984; Ode *et al.*, 2022). Competition can also arise between conspecifics if they are both foraging for a suitable host in the same patch (Godfray, 1994), meaning that

conflicts of interest are present across both intra and interspecies interactions. The relative strengths of these intra and interspecific competition can determine whether species are able to coexist in the long term on an ecological time scale (Adler *et al.*, 2018).

This study aims to clarify the outcomes of such interactions and the consequences these may have on ecological processes in the field. In this section, I will provide a brief overview of all elements that are discussed, and return to them in more detail later in the chapter.

The light brown apple moth, *Epiphyas postvittana*, is a prominent pest in Australian vineyards (Scholefield and Morison, 2010). The annual economic impact that LBAM induced damage has on the wine grape industry is estimated to be around \$70 million (AUD) per year (Scholefield and Morison, 2010). This cost comprises both direct damage, where early larval instars cause damage to leaves and fruit surfaces (Danthanarayana, 1975) (\$18 million per year), and indirect damage, such as the promotion of rot caused by *Botrytis spp.* (\$52 million per year) (Bailey *et al.*, 1997; Scholefield and Morison, 2010). As there are no records since 2010, it is likely that the actual cost of damage is even higher than the figures indicate. Despite some impacts resulting from the worldwide pandemic (2020 – present), Australian wine production and exports have increased year on year, and the average value of wine grapes has risen from approximately \$550 per tonne in 2010 to \$694 per tonne in 2020 (Grapegrower and Winemaker, 2018; Collins and Xia, 2021).

The light brown apple moth is a polyphagous, multivoltine leafroller, capable of feeding on a wide variety of plants (Suckling and Brockerhoff, 2010). The moth has been recorded feeding on 123 genera in 55 families within Australia, including 22 native genera, as well as over 500 plant species in 363 genera from other invaded countries (Suckling and Brockerhoff, 2010). The versatility of the insect and its wide range of host plants has had profound economic and ecological impacts on agriculture, horticulture, natural and urban ecosystems (Suckling and Brockerhoff, 2010).

Many invertebrate prey species experience a high level of mortality from invertebrate predators and parasitoids, which is often exploited for biological

pest control (Macfadyen *et al.*, 2015). Successful application of biological control can result in the long term, or possibly permanent, management of the target pest species, limit or remove necessity for chemical insecticides, and produce a favourable cost-benefit ratio with pest number reductions over a broad geographical range. However, the ecological processes underlying successful biocontrol practices are often complex and require in-depth understanding before they can be implemented in the field.

Classical biocontrol programmes are under scrutiny due to the devastating consequences that some early biocontrol programmes had on non-target organisms, deemed 'non-target effects' (Simberloff and Stiling, 1996; Hawkins and Marino, 1997; Follett and Duan, 2012; Zaller and Brühl, 2019). Examples such as these reiterate the need to develop an understanding of host-prey interactions, as well as interactions and competition between natural enemies in the same system.

Insect parasitoids, especially those from the orders Diptera and Hymenoptera, are one of the most commonly used organisms for the biological control of other arthropods. Hymenopterous parasitoids use a variety of visual (Segura *et al.*, 2007; Benelli and Canale, 2012; Kawamata *et al.*, 2018), vibrational (Meyhöfer and Casas, 1999; Laumann *et al.*, 2011) and olfactory stimuli (Wajnberg and Colazza, 2013; Wilson and Woods, 2016) to forage for hosts, food and mates in their environment. However, in the field, it is likely that a foraging female will have to overcome challenges to obtain resources from conspecifics as well as allospecifics (intra and interspecific competition) in order to successfully utilise a host and produce offspring (Frost *et al.*, 2016; Frago, 2016).

Interspecific competition is an important driver of niche differentiation and a key aspect in ecological and evolutionary theory. In nature, multiple species of parasitoid frequently attack the same species of host (Price, 1972; Polis and Strong, 1996; Heimpel *et al.*, 2021). The coexistence of competitors is mediated by specialisation of each parasitoid species to different life stages of the host (Harvey *et al.*, 2013), meaning that interspecific competition plays an important role in defining parasitoid community and niche structure.

Interspecific competition amongst parasitoids also has the potential to shape behavioural strategies with regards to host resource exploitation (Connell, 1980; Hawkins, 2000; Ortiz-Martínez *et al.*, 2019). Unlike prey that are immediately consumed by their predator, parasitised hosts remain *in situ* and are vulnerable to subsequent attack by other foraging parasitoids or natural enemies (Godfray, 1994; Wajnberg *et al.*, 2008).

Understanding intraspecific and interspecific competition in parasitoid communities is important for the screening of efficient parasitoid species and utilisation of the most appropriate parasitoid species combinations (Pérez-Lachaud *et al.*, 2004; Batchelor *et al.*, 2005; Batchelor *et al.*, 2006; Harvey *et al.*, 2013; Ode *et al.*, 2022). It is often the case that the parasitoid species most negatively influenced by competition is the most effective biocontrol agent against the host when alone (Leveque *et al.*, 1993; Carvalheiro *et al.*, 2008; Cebolla *et al.*, 2018). Therefore, consideration of competition between parasitoid species is necessary prior to their release in the field. Furthermore, the study of ecological interactions between two parasitoids can lead to the understanding of host-parasitoid and parasitoid-parasitoid dynamics that can be utilised across many parasitoid systems (Rodríguez *et al.*, 2017).

Biological control

Biological control, or biocontrol, is a valuable ecosystem service involving the management of invertebrate and vertebrate pests by natural enemies, resulting in a pest control service with diverse benefits to human stakeholders (DeBach and Rosen, 1991; Stenberg *et al.*, 2021). These natural enemies are not limited to predators and parasitoids but also include nematodes and pathogens such as fungi and viruses (Hajek and Eilenberg, 2018).

All biocontrol methods can be classified into four main categories depending on whether resident natural enemies are utilised, with or without human intervention (conservation biological control and natural biological control, respectively) or whether natural enemies are introduced for permanent or temporary establishment (classical biological control and augmentative biological control, respectively) (reviewed in Stenberg *et al.*, 2021).

In augmentative biological control, natural enemies (parasitoids, predators or microorganisms) are mass-reared for release in large numbers, with the aim of controlling a target pest on a temporary basis (Cock *et al.*, 2010; van Lenteren *et al.*, 2018). Augmentative biocontrol that uses predatory arthropods or parasitoid wasps has become a major factor in the reduction of insecticide use, particularly in protected horticulture (Pilkington *et al.*, 2010; van Lenteren *et al.*, 2018), as large scale releases and mass production of natural enemies are viable on a commercial level (van Lenteren, 2012). Hymenopteran species are commonly used in augmentative biocontrol as, in comparison with predators, hymenopteran parasitoids are more specific in regards to their host range, which prevents undesirable side effects such as the mortality of beneficial insects (Bigler *et al.*, 2006; van Lenteren 2012).

Research conducted on pest control in recent years has focussed on biological control as a component of Integrated Pest Management (IPM) (Giles *et al.*, 2017; Barratt *et al.*, 2018; Torres and Bueno, 2018). IPM programmes utilise biological knowledge of a pest species to assess which control methods have the potential to prevent pest-related damage or reduce current damage in a system to below economically damaging levels (Baker, 1999; Peshin and Pimentel, 2014; Johnson *et al.*, 2020). These methods include but are not limited to: mechanical weeding, pheromone traps, crop rotation and use of biological control agents (Barzman *et al.*, 2015). Monitoring, decision making and integration of a combination of these controls together in a unified program has led to some major successes in pest management (reviewed in Deguine *et al.*, 2021). Initially, IPM was designed to replace the heavy use of pesticides, but has come to rely on its involvement in small quantities when determined to do so during the monitoring of pest populations (Peshin and Pimentel, 2014). Broad-spectrum pesticides in high amounts raise the potential for devastating impacts to ecosystems, such as the killing of natural enemy populations alongside target pest populations (e.g. organophosphates; reviewed in Cloyd, 2012). This in turn releases pests from natural biocontrol services, thereby causing a resurgence in the pest species (Dutcher, 2007; Wu *et al.*, 2020).

Demand for food security due to human population growth and problems arising from climate change is as important as ever. Global food security

requires both the improved production and the improved protection of agricultural products (Dhankher and Foyer, 2018; Prosekov and Ivanova, 2018). IPM methods could result in the establishment, and persistence, of a natural enemy population to suppress pests in the natural environment (Torres and Bueno, 2018), bringing long-term stability to the system. However, these programmes require careful management in order to effectively regulate pest populations.

The Light Brown Apple Moth

The host species used in this study was *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), also known as the light brown apple moth (LBAM), and has been known as a pest for over a century. The tortricid leafroller is best known as a pest of tree fruits, including apples, pears, citrus, peaches, nectarines, apricots, vines, and to a lesser extent forestry, vegetable and flower crops (Wearing *et al.*, 1991; Suckling, 2021). In Australia, the economic damage is greatest on apples, pears and grapes (Suckling and Brockerhoff, 2010; Suckling, 2021). The eggs, larvae and pupae of the moth can be associated with plant material and readily transported (Suckling, 2021). A wide host plant range has facilitated the spread of LBAM beyond its native region in southeast Australia to countries such as New Zealand, UK, Ireland, California, Hawaii and Sweden (Suckling and Brockerhoff, 2010; Suckling 2021). This has led to LBAM becoming a well-studied target throughout the past few decades, with considerable research effort being put towards analysing its available insect control methodology, biology and ecology, as well as its use as a model organism (Danthanarayana, 1975; Geier and Briese, 1980; Danthanarayana, 1983; Wearing *et al.*, 1991; Suckling and Brockerhoff, 2010; Suckling *et al.*, 2012a; Suckling *et al.*, 2012b; Suckling *et al.*, 2014; Feng *et al.*, 2015; Yazdani *et al.*, 2015; Bui *et al.*, 2020; Roltsch *et al.*, 2021).

LBAM completes 2-4 generations per year, depending on climate and latitude (Danthanarayana, 1975; Brown *et al.*, 2010). At lower temperatures, the life cycle of LBAM from egg to adult takes longer to progress, whereas in warmer regions 3-4 overlapping generations can occur (Danthanarayana,

1975). In Australian vineyards, there are typically 3 generations of LBAM annually; a summer generation (January-April), an autumn-winter generation (May-September) and a spring generation (October-December) (Danthanarayana, 1975). In vineyards, only the spring and summer generations of LBAM affect wine grape production via pest-induced damage, as the winter generation migrates to and persists on other plants in and around vineyards when grapevines have little to no foliage (Danthanarayana, 1975; Braybrook, 2013).

Early LBAM larval instars feed on the underside of leaves within a silk chamber. Late instars may fold individual leaves, create a nest of multiple leaves webbed together, or web leaves to fruits, to feed on the surface of fruit. All LBAM larval feeding activities can cause damage (feeding on stems, buds, shoots, flowers, etc.) and reduce crop yield (Braybrook, 2013), but it is the damage to fruit that causes the largest economic impact (Wearing *et al.*, 1991), as blemishes to fruit surfaces can make them unsuitable for sale (Irvin, 2009). Furthermore, damage to fruits provide entry points and increased transmission for bunch-rotting fungi such as *Botrytis cinerea* (Buchanan and Amos, 1992; Ferguson, 1995). The act of webbing together fruits or leaves can also enhance conditions for rot expansion due to the trapping of debris inside bunches (Braybrook, 2013).

LBAM induced damage effects crop productivity on a global level. In New Zealand, several tortricids cost fruit export growers \$35 million NZD per year in control costs (Suckling, 2021). An economic risk analysis for LBAM in the United States in 2008 estimated that costs to four major fruit crops (apple, grape, orange, and pear) could surpass \$105 million USD, including costs of direct damage to crops (\$93 million), quarantine order implementation (\$7.5 million) and research (\$4.5 million) (Fowler *et al.*, 2009). The U.S. Department of Agriculture (USDA) allocated a \$74.5 million USD budget for eradication, research, monitoring and regulation of LBAM in 2008 (Fowler *et al.*, 2009).

Numerous methods have been employed to manage LBAM, such as the application of insecticides (Liu and Simmons, 2021), disruption of mating with sex pheromone traps (Suckling *et al.*, 2012a), and biological control practices

(Bui *et al.*, 2020; Roltsch *et al.*, 2021). Out of these, insecticide sprays are the most commonly used method (Suckling *et al.*, 2001; Suckling and Brockerhoff, 2010). DDT (dichloro-diphenyl-trichloroethane) was the first synthetic insecticide introduced into Australian orchards to control LBAM and codling moth (Thwaite *et al.*, 1993). It is estimated that without insecticide application, damage caused by larval LBAM to fruits in Australia could range from 5-20%, and may even exceed 30% (Wearing *et al.*, 1991). In New Zealand, unsprayed crops received damage levels of up to 70% (Wearing *et al.*, 1991).

Although effective at suppressing LBAM populations, insecticides have various limitations associated with their use. Insecticide use in Australian vineyards has been restricted to before berries reach “pea-size” in order to minimise the likelihood of toxic residues in wine (Essling and Lord, 2018), allowing some life stages and generations of LBAM to persist. In addition, the toxic nature of insecticides is relatively unappealing to the viticulture industry and consumers due to the undesired effects insecticides have on the environment, problems related to human health, and destruction of biodiversity; specifically the impact on non-target arthropods which may have useful applications for biocontrol (Smith and Riethmuller, 1999; Lockie *et al.*, 2002; Chang and Zepeda, 2005; Douglas *et al.*, 2015).

Another factor to consider is insecticide resistance. The light brown apple moth is already known to have developed resistance to multiple synthetic insecticides that were released throughout the 1950’s (dichloro-diphenyl-dichloroethane) and 1960’s (Azinphos-methyl) to combat the pest (Smith, 1961; Thwaite *et al.*, 1993; Suckling and Khoo, 1993; Suckling *et al.*, 1984). In Australia, the trend of insecticide resistance in crop pests is currently on the rise (Umina *et al.*, 2019) The limited range of suitable insecticides and the rising costs associated with registering them also highlight the need for other control methods (Wearing *et al.*, 1993; Huang *et al.*, 2018; Kranthi and Stone, 2020). These factors, combined with the consumers increasing demand for lower pesticide residues, illustrates that alternative management strategies that do not rely on chemical applications are necessary to provide sustainable management for LBAM populations on wine grapes.

Biocontrol of LBAM has been recognised as a viable solution for sustainable management of the pest. Between 1967 and 1972, 10 out of 19 considered parasitoids were eventually released in New Zealand following surveys conducted in Australia (Thomas, 1989). In 1969, nearly 250 specimens of *D. tasmanica* were released in New Zealand (Thomas, 1989).

Dolichogenidea tasmanica has become the most abundant parasitoid of LBAM in New Zealand, with parasitism rates of up to 50%, particularly in areas not using insecticides (Wearing *et al.*, 1991; Suckling *et al.*, 1998). An egg parasitoid, *Trichogramma carverae* (Hymenoptera: Trichogrammatidae), was also tested in Australian vineyards (Gurr *et al.*, 1998). However, the inadequate provision of a sugar source for females led to short-lived effectiveness for the control of LBAM.

Natural enemies of LBAM

A wide range of predators and parasitoids can attack LBAM: including earwigs, ladybird beetles, lacewings and spiders (Danthanarayana, 1975; Paull and Austin, 2006). Parasitism of LBAM in Australian vineyards and orchards is an important mortality factor; parasitism of final larval instars and pupae can reach 20% and 18% respectively (Danthanarayana, 1983). At least 25 species of parasitoids and hyperparasitoids are reported to be associated with LBAM in Australia (Paull and Austin, 2006). Among the larval and pupal parasitoids, the braconid *D. tasmanica* and the bethylid *G. jacintae* are reported to be the first and second most common that parasitise LBAM (Danthanarayana, 1983).

Dolichogenidea tasmanica

Dolichogenidea tasmanica is one of the two parasitoid species considered in this study. The braconid *D. tasmanica* has been recorded as the most abundant of LBAM, and comprises 66-97% of the parasitoids reared from LBAM in some field sites (Charles *et al.*, 1996; Paull and Austin, 2006). *Dolichogenidea tasmanica* is an arrhentokous, solitary, koinobiont endoparasitoid of LBAM (Dumbleton, 1935).

Koinobionts complete most or all of their larval development within the host, and require specialised manipulation and evasion of host defences in order to survive. Koinobionts will allow the host to continue development until the parasitoid larvae pupate and emerge, killing the host. Endoparasitoids oviposit directly into the host's bodily fluids, where the larvae will feed and develop. Female *D. tasmanica* wasps lay a single egg into the first three larval instars of LBAM with a preference for the 2nd instar (Yazdani *et al.*, 2015) and the larvae continue to develop until its 4th instar.

In recent years there have been numerous publications on *D. tasmanica* related to its efficacy as a parasitoid of LBAM. Yazdani *et al.* (2014) reported that *D. tasmanica* are capable of discriminating between unparasitised and parasitised hosts that have been parasitised by themselves or a conspecific female. Moreover, *D. tasmanica* have been shown to respond to low host densities in a density-dependent manner, which could contribute to the suppression of LBAM populations before they reach economically damaging levels (Yazdani and Keller, 2015). These findings suggest that *D. tasmanica* could be an effective candidate for the biological control of LBAM.

Goniozus jacintae

Goniozus jacintae is the main parasitoid species utilised in this study. This bethylid is an arrhentokous, gregarious, koinobiont ectoparasitoid of LBAM which parasitises the mid to late larval instars of LBAM (3rd-6th), with the 4th instar being predominantly parasitised (Danthanarayana, 1980). Ectoparasitoids lay their eggs on the external cuticle of the host, and the larvae will feed on host fluids slowly, without intimate interaction with the hosts internal environment (Godfray, 1994). A female wasp lays between one and seven eggs onto each host larva in the field, with a mean of 1.8 (Danthanarayana, 1980). The life cycle of *G. jacintae* is well synchronised to that of LBAM in the field, and host-parasitoid relationships show a delayed inverse density-dependent response (Danthanarayana, 1980). Despite this, the impact of *G. jacintae* as a mortality factor of LBAM was deemed insignificant in a field experiment by Danthanarayana (1980), as the degree of parasitism of host generations

ranged from only 0 to 8.3%. Moreover, the abundance of *G. jacintae* in the field is lower than that of *D. tasmanica* (M.A. Keller, E. Aspin, pers. obs.).

Little research has been conducted on the efficacy of *G. jacintae* as a potential biocontrol agent for LBAM since the singular paper by Danthanarayana in 1980. Although it was reported that *G. jacintae* was not a predominant parasitoid of LBAM in the field (Danthanarayana, 1980), it is nonetheless the second most abundant parasitoid of LBAM after *D. tasmanica*. Experiments conducted with *G. jacintae* have had relative success at maintaining parasitism rates in the laboratory (Hopper and Mills, 2015). Furthermore, other *Goniozus* species have been used in various biocontrol applications in other agro-ecosystems around the world, including sugarcane (Graham and Conlong, 1988), almond (Legner and Gordh, 1992), coconut (Shameer *et al.*, 2018) and date palm (Polaszek *et al.*, 2019) farming industries. The realised success of *Goniozus* as biocontrol agents has been variable but they have consistently been viewed as beneficial organisms.

The biology and efficacy of *G. jacintae* as a parasitoid of LBAM is, thus, relatively unstudied. What little evidence exists suggest that *G. jacintae* achieves less parasitism in the field compared to a laboratory setting. Hence, it can be considered that factors in the field may be influencing the effectiveness and success of LBAM parasitism by *G. jacintae*. These factors could include the presence of alternative host species, *G. jacintae* host finding abilities and/or competitive interactions between *G. jacintae* and other natural enemies.

Intraspecific competition

Individuals of the same species have similar needs concerning survival, growth and reproduction. Hence, the search and exploitation for similar resources may consequently lead to intraspecific competition (Harvey *et al.*, 2013). Parasitoid wasps are free-living as adults; females search for and attack hosts, laying a single egg or multiple eggs on or in these hosts, which then provide the only food resource for the developing parasitic offspring until the eventual death of the host (Godfray, 1994). Several parasitoid females foraging for hosts in the field will often simultaneously exploit the same patch of hosts (Godfray, 1994).

In this case, the females may interfere with each other through a variety of mechanisms. This could be directly, via fighting, displaying or hindering one another's searching efficiency (Hassell and Varley, 1969; Hardy and Blackburn, 1991; Godfray, 1994; Field *et al.*, 1998; Yazdani and Keller, 2015; Sreenivas and Hardy, 2016), as well as indirectly, by modifying individual host exploitation strategies (Visser *et al.*, 1990; Visser *et al.*, 1992). These actions include sex allocation choices, dispersal and superparasitism decisions (laying an egg onto a host which has already been parasitised by another female of the same species) (reviewed in Visser and Driessen, 1990).

Multiple conspecifics or a low host density in a patch could result in the presence of hosts that have already been parasitised. This confronts female wasps with choices regarding their egg allocation, particularly concerning the number of eggs laid (if a gregarious parasitoid) and sex ratio. In some cases, if the host is not deemed suitable, the female could reject the host for oviposition altogether. However, females are more inclined to superparasitise a host when the presence of unparasitised hosts is rare (Visser *et al.*, 1990; Visser *et al.*, 1992; van Alphen, 1988; Díaz-Fleischer *et al.*, 2015; Harba and Idris, 2018).

Ovipositing females tend to avoid competition and superparasitism by employing a variety of strategies. Some use marking pheromones or chemicals on the surface of the host to deter potential competitors (Salt, 1961; van Lenteren, 1981; Wajnberg and Colazza, 2013). Females of many parasitoid species are able to distinguish between self-parasitised or conspecifically parasitised hosts and unparasitised ones, termed host discrimination, possibly via these markings. This ability allows a female to avoid oviposition in a parasitised host that she herself has laid eggs on or into (self-superparasitism) and this minimises wastage of time and eggs (van Lenteren, 1981; Chen *et al.*, 2020). However, superparasitism is still common in nature, even in solitary parasitoids where only one offspring is capable of developing per host (Salt, 1961; Schröder, 1974; Díaz-Fleischer *et al.*, 2015; Chen *et al.*, 2020).

The reasons for superparasitism can in part be due to maternal investment. Factors such as host quality, previous oviposition experience and host prevalence could influence a female's decision to superparasitise. For

example, large hosts can provide more resources for parasitoid larvae, and can sometimes support multiple clutches, so are seen as more desirable by gregarious foundresses as they can enhance their chances of reproductive success (Godfray, 1994). Hence, although the behaviour can be costly, the benefits of superparasitism are variable depending on the circumstances.

Interspecific Competition

Understanding the dynamics of competition between parasitoids that could potentially have uses as biological control agents is important for evaluating their efficiency (Carvalho *et al.*, 2008; Harvey *et al.*, 2013; Orre-Gordon *et al.*, 2013; Cusumano *et al.*, 2016; Ode *et al.*, 2022). Unlike arthropod predators, which may require multiple prey to achieve maturity, the growth and development of parasitoids is dependent on the resources contained within a single host that is often similar in size to the adult parasitoid (Godfray, 1994). Hence, individual hosts are usually only capable of sustaining the development of one parasitoid species. When the host is attacked by multiple parasitoid species, one competitor will ultimately dominate the host resources and eliminate other competitors (Harvey, 2005; Harvey *et al.*, 2013). To avoid competitive exclusion and the monopolisation of the host as a resource by a single species, parasitoids that share the same host species will often be specialised to parasitise different stages of the hosts' life cycle (e.g. egg, larvae, or pupae). This often leads to interspecific competition between parasitoid species (Harvey *et al.*, 2013; Ode *et al.*, 2022).

As *D. tasmanica* is the most abundant parasitoid of LBAM in the field and attacks early life stages of LBAM, it is likely that in nature *G. jacintae* will encounter hosts that have already been parasitised by *D. tasmanica*. If individuals from both species attempt to parasitise the same individual host, multiparasitism may occur. Therefore, interspecific competition is likely to take place between the parasitoids (Godfray, 1994; Paull and Austin, 2006). It is important to note that this is a separate concept to two adult females of the same species competing directly to obtain a host in the form of intraspecific contest competition (Hardy and Briffa, 2013; Stockermans and Hardy, 2013);

this example involves the competitive interactions between the resulting larva/offspring of different species of foraging females once they have been oviposited on/in the host.

Whilst competing parasitoid species are able to co-exist in the same environment (van Nouhuys and Hanski, 2005; Aluja *et al.*, 2013), certain circumstances, such as the lack of an alternative host species, can lead to the domination of parasitism by one parasitoid species (Pijls and van Alphen, 1996; Liang and Liu, 2017). Introduction of more parasitoid species to a system may result in a decline in the efficiency of biological control (Collier and Hunter, 2001; Collier *et al.*, 2002; Xu *et al.*, 2016; Ode *et al.*, 2022), and the overlapping of host range raises concerns that interspecific competition may compromise the establishment of successful biocontrol management schemes. Therefore, it is important to understand how competing parasitoid species interact, and whether there is superiority of one species under certain circumstances.

Interspecific competition between *D. tasmanica* and another parasitoid of LBAM, *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae), was investigated by Feng *et al.* (2015). *Therophilus unimaculatus*, like *D. tasmanica*, is a solitary, generalist endoparasitoid that attacks the early larval instars of LBAM. Within the host, larvae of *D. tasmanica* out-competed those of *T. unimaculatus*, irrespective of order or time interval between host attacks by both species (Feng *et al.*, 2015). Extrinsically, the parasitoids did not show any difference in behavioural responses to unparasitised hosts and hosts parasitised by the other species (Feng *et al.*, 2015). However, in a separate study, it was shown that *D. tasmanica* is capable of discriminating between unparasitised hosts and hosts parasitised by conspecifics (Yazdani *et al.*, 2014).

Research Aim and Objectives

Given all the information above, there remained a challenge to untangle and elucidate the roles of two parasitoids within the context of both a host-parasitoid and parasitoid-parasitoid interaction system. Understanding the behavioural

ecology of these species could potentially lead to improvements in biological control programmes.

This study aimed to develop an understanding of how two parasitoid wasps interact with each other as well as their host, the light brown apple moth. To understand how two species interact together, there must first be a foundation of understanding for the biology of each species in the interaction. *Dolichogenidea tasmanica* is a relatively well-studied organism, although little was known about the behavioural ecology of *G. jacintae*. Hence, this study began by observing the biology of *G. jacintae* before progressing to the evaluation of multi-species interactions.

The first phase of my research focussed on deciphering the behavioural ecology of *G. jacintae* as a parasitoid of LBAM by addressing the following:

1. How do *G. jacintae* host finding strategies differ in response to different larval instars of LBAM?
2. How do *G. jacintae* oviposition behaviours vary in response to different larval sizes and instars of LBAM?

The second phase focussed on the interspecific interactions between *D. tasmanica* and *G. jacintae* when competing for the same host:

3. How does *G. jacintae* respond to hosts that have been previously parasitised by *D. tasmanica*?

An important goal of ecological studies of parasitoids, such as this one, is motivated by applied questions to determine what attributes would make a species a successful agent for biocontrol (e.g. Ridland *et al.*, 2020). Acquiring this knowledge may help to improve the selection of appropriate agents for classical and augmentative biological control and to increase the overall chances of success in biocontrol programmes in general. Information generated from this study identifies important characteristics of *G. jacintae* as a potential

biocontrol agent of the LBAM, and such information will help to inform practitioners of biological control when considering the application of this parasitoid in the field.

Limitations to the research programme

Due to the unforeseen effects of the worldwide pandemic in 2020, my list of realised research questions is shorter than the list I conceptualised at the beginning of my PhD studies.

The Joint PhD scheme I am a part of involves a collaborative approach, where students can perform research at both the University of Nottingham and the University of Adelaide. At the time the pandemic began (March, 2020), I was based in Adelaide. As I was working with an invasive species that was native to Australia, my experiments had been arranged to occur during my placement there.

However, due to restrictions to laboratory and field access, I was unable to conduct the remainder of the experiments that I had planned. For instance, I had limited ability to go into the field and collect samples for my insect cultures during a national lockdown, which resulted in the collapse of the *D. tasmanica* culture at one stage. In addition, I was facing personal upheaval at a very uncertain time. I had to emigrate back to the UK from Australia in December 2020, with the UK going into another national lockdown shortly after my arrival. On top of this, I was coming to terms with the fallout of a serious assault I experienced some years ago.

The stress and isolation of the pandemic has indeed had a severe impact on my personal and educational progress, but I can only hope that it has not confounded my efforts to formulate a robust thesis. Nonetheless, planned topics I was unable to cover in my research time frame are discussed in Chapter 5, as they can suggest how the current findings could be taken forward in the future.

CHAPTER TWO

Walk this way, fly that way: *Goniozus jacintae* attunes flight and foraging behaviour to leafroller host instar

Title:

Walk this way, fly that way: *Goniozus jacintae* attunes flight and foraging behaviour to leafroller host instar.

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Short Title:

Bethylid foraging behaviour

Key words:

Host location, host stage selection, light brown apple moth, *Epiphyas postvittana*, wind tunnel, flight duration, parasitoid, bethylid

Statement of Authorship

Title of Paper	Walk this way, fly that way: <i>Goniozus jacintae</i> attunes flight and foraging behaviour to leafroller host instar.
Publication Status	<input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Unpublished and Unsubmitted work written in manuscript style
Publication Details	Aspin, E., Keller, M.A., Yazdani, M. and Hardy, I.C.W., 2021. Walk this way, fly that way: <i>Goniozus jacintae</i> attunes flight and foraging behaviour to leafroller host instar. <i>Entomologia Experimentalis et Applicata</i> , 169(4) pp.350-361. DOI: 10.1111/eea.13028

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Name of Principal Author (Candidate)	Emma Aspin			
Contribution to the Paper	Conceived the study, designed and tested the method, conducted experiments, analysed data, wrote the manuscript.			
Overall percentage (%)	75%			
Certification:	This paper reports on original research I conducted during the period of my higher degree by research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.			
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	Date	18.04.2022		

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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	Date	19.04.2022		

Name of Co-Author	Michael A. Keller			
Contribution to the Paper	Assisted with design of the method, data analysis and manuscript preparation.			
Signature	<table border="1" style="width: 100%;"> <tr> <td style="width: 80%;"></td> <td style="width: 20%;">Date</td> <td>24 April 2022</td> </tr> </table>		Date	24 April 2022
	Date	24 April 2022		

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	Date	24/04/2022		

Abstract

Parasitoids exhibit distinct behaviours whilst foraging for their herbivorous hosts. Some are specialised with respect to the host stage they can successfully utilise and even the age of individuals within a single stage. Observing the behavioural response of parasitoids to hosts of varying age can provide more practical understanding of potential biocontrol agents. A wind tunnel experiment was conducted to test for host-stage dependent foraging behaviour in *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae), a common but understudied parasitoid of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) which is a pest of grapevine, *Vitis vinifera* L. (Vitaceae), and other crops in Australia. *Goniozus jacintae* was already known to parasitise the third to sixth larval instars of LBAM, but most readily parasitises the later instars. Later instars are larger and have a higher rate of feeding, producing more faeces and silk deposits than earlier instars, and are likely to also produce more volatile cues that may be detected by parasitoids. We found that the timing, frequency and duration of foraging behaviours of *G. jacintae* varied significantly among host instars. Flight duration was shortest when females were exposed to leaves infested by fifth instar larvae. The profile of behaviours exhibited was different during pre- and post-flight foraging, with the newly described *slow walking* behaviour only exhibited in close proximity to a potential host. These results contribute to the understanding of bethyrid foraging, a relatively unexplored aspect of bethyrid reproductive behaviour, and towards enhancing the efficacy of utilising *G. jacintae* in the control of leafroller pests.

Introduction

Parasitoids often mature in locations remote from suitable hosts (Tuda & Iwasa, 1998) and females must then disperse to forage for reproductive opportunities in complex environments. Many parasitoid species are specialised in regard to the range of species they can utilise as hosts (reviewed in Strand and Obrycki, 1996), the life-history stage of their target hosts (egg, larval stage, pupae or adult) and even the age of individuals within a given life-history stage (e.g. early or late larval instars) (Mattiacci & Dicke, 1995). Therefore, finding suitable hosts is, for many parasitoids, a substantial challenge. This challenge is important to practitioners of biological pest control because it influences the efficacy of a given parasitoid as a pest suppression agent. When designing biological control systems that deploy parasitoids, it is vital to establish which stages of the target species are susceptible to parasitism (Pandey & Singh, 1999; Canale & Loni, 2006) and to understand the abilities of parasitoids to locate such hosts at low densities (van Lenteren *et al.*, 1976; Drost *et al.*, 2000; Hudak *et al.*, 2003). We analysed the behaviour of a bethylid parasitoid, *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae), in response to cues from one of its pest hosts, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Danthanarayana, 1980) to understand how it locates hosts.

The sequential phases of host searching that can lead to reproduction have been classified as host habitat location, host location and host acceptance (Vinson *et al.*, 1975; Vinson, 1976). In each phase, foraging behaviour is characterised by responses to environmental stimuli, or cues, (Price *et al.*, 1980; Geervliet *et al.*, 1994) categorised into three groups: (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 (De Moraes & Lewis, 1999). Stimuli arising from an individual host (including deposited silk and faeces) are some of the most reliable cues for a foraging parasitoid,

especially when in close proximity to the host (Sternlicht, 1973; Vet *et al.*, 1990). However, natural selection does not favour hosts that are easily found by natural enemies and thus, host species have evolved to minimise the emission of cues that could be utilised by foraging parasitoids (reviewed in Vet & Dicke, 1992). In response, parasitoids have evolved to use indirect cues associated with the presence or activity of the host for long-range detection (Vet & Dicke, 1992; Cardé & Bell, 1995).

Chemical information from plant volatiles related to host feeding damage are often important in mediating long-distance searching (Nordlund *et al.*, 1988; Geervliet *et al.*, 1994). Damage from herbivores significantly increases the emission of plant volatiles (Dicke & Sabelis, 1989), information which foraging parasitoids can readily exploit (Mattiacci & Dicke, 1995). Some volatiles released by plants are indicators of herbivore identity (Dicke *et al.*, 1990a; Dicke *et al.*, 1990b; Turlings *et al.*, 1990), and can even vary between different herbivore life-stages (Takabayashi *et al.*, 1995; Gouinguéné *et al.*, 2003), providing parasitoids and predators with more specific cues. However, not all stimuli from a host's food source are reliable: the presence of a host's food plant does not guarantee presence of a suitable host (Zanen & Cardé, 1991), and plant volatile production can be highly variable, e.g. due to differences in growing conditions (Visser, 1986). This reliability *vs.* detectability foraging challenge selects for parasitoids that can exploit both direct and indirect cues (Vet & Dicke, 1992; Cardé & Bell, 1995).

Although foraging has been studied in many parasitoid species that belong to the monophyletic group of hymenopterans known as the Parasitica (Peters *et al.*, 2017), host finding by species in the hymenopteran family Bethyridae has had little evaluation. Bethyrids are members of the Aculeata ('stinging wasps') (Peters *et al.*, 2017) and most studies of their reproduction have focussed on decisions made by females only once a host has been located (e.g. Legner & Warkentin, 1988; Hardy *et al.*, 1992; Gao *et al.*,

2016; Abdi *et al.*, 2020a,b). As bethylids are encouraged and deployed as biocontrol agents against many coleopteran and lepidopteran pests of economic importance (Cock & Perera, 1987; Legner & Gordh, 1992; Batchelor *et al.*, 2006; Jaramillo *et al.*, 2009; Yang *et al.*, 2014; Polaszek *et al.*, 2019) the lack of information on their foraging behaviour is a potentially important knowledge gap.

Goniozus jacintae is a gregarious ectoparasitoid of tortricid moths. It is a commonly occurring parasitoid of the light brown apple moth (LBAM), *E. postvittana* (Danthanarayana, 1980), which is an invasive generalist herbivore and a pest in agro-ecosystems in Australia and elsewhere (Suckling & Brockerhoff, 2010). The light brown apple moth has particular prominence in, and economic impact on, the Australian wine grape industry (Scholefield & Morison, 2010). Despite its common occurrence as a beneficial insect, there is surprisingly little knowledge of the efficacy of *G. jacintae* as a biological control agent for LBAM (Danthanarayana, 1980; Paull & Austin 2006), and no prior information on its foraging behaviours or ability to locate hosts.

Here we evaluate the foraging behaviour of female *G. jacintae* when presented remotely, in a wind tunnel, to feeding LBAM. Previous studies using wind tunnels have demonstrated that oriented flight responses of parasitoids to airborne environmental cues can usefully be studied under laboratory conditions (Drost *et al.*, 1986; Keller, 1990; Guerrieri *et al.*, 1993). As *G. jacintae* is known to parasitise the third to sixth larval instar of LBAM (Danthanarayana, 1980), we investigated whether its foraging behaviour varies according to host instar, having first established that later instars generate more leaf damage and thus are likely more strongly associated with volatile cues. The ultimate aim was to use the understanding of foraging behaviour to enhance the potential of *G. jacintae* to control agricultural pests in the field. Furthermore, as this study is among the first to quantify the host location behaviour of a bethylid, the results may be applicable to further

agro-ecosystems in which species of *Goniozus* and/or other bethylids have been successfully deployed as, or considered as, agents of biological pest control (Legner & Gordh, 1992; Baker, 1999; Batchelor *et al.*, 2006; Shameer *et al.*, 2018; Polaszek *et al.*, 2019).

Materials and methods

Rearing *E. postvittana*

The culture of *E. postvittana* (LBAM) used in this experiment was established at the South Australian Research and Development Institute in 1994 and has since been maintained with annual additions of wild moths. LBAM was reared on an artificial diet at 22 ± 2 °C under 12L:12D photoperiod, following methods reported in Yazdani *et al.* (2014).

Rearing *G. jacintae*

A culture of *G. jacintae* was established from individuals reared from parasitised LBAM that were collected in vineyards at McLaren Vale, South Australia in 2017. The wasp culture was reared at 23 ± 2 °C, 14L:10D in cages on larval LBAM that infested plantain, *Plantago lanceolata* L. (Lamiales: Plantaginaceae). Adult wasps were provided with water and honey *ad libitum*. Wasp cocoons were isolated in 50 mm × 18 mm diam. glass vials containing a drop of honey and fitted with caps that had screens for ventilation. Upon emergence, females were caged serially, 2-5 at a time, with 5 males to allow mating, and then re-isolated and held in vials for at least 1 h before being used in experiments.

Experimental plant

Plantago lanceolata was selected as the experimental plant in this study. LBAM is a polyphagous, multivoltine leafroller that can feed on a wide diversity of plants (Suckling & Brockerhoff, 2010). As well as grapevine, *G. jacintae* has been associated with parasitising LBAM on many plant species, including *P. lanceolata* (Danthanarayana, 1980). *Plantago lanceolata* is commonly found in inter-rows and underneath the grapevine canopy in Australia, and supports LBAM populations throughout the year, including during grapevine dormancy. *Plantago lanceolata* has been used as a model species in numerous experiments on LBAM (Tomkins *et al.*, 1991; Yazdani *et al.*, 2015a) as well as other life history and population studies of host plant-herbivory interactions for decades (Bowers *et al.*, 1992; Gange & West, 1994). These observations make this plant a suitable candidate for this study.

Leaf damage by host instars

As leaf damage is a primary source of volatiles associated with the attraction of parasitoids to plant-feeding host insects (Whitman & Eller, 1990; Turlings *et al.*, 1990), we first assayed the amount of leaf damage caused by LBAM larvae in different larval instars. In each replicate ($n = 20$ per instar), a fresh plantain leaf of uniform size was presented to either a single third, fourth, fifth or sixth instar in a plastic container (8 cm \times 11.5 cm \times 11 cm). After 24 hours, the larva was removed, and the leaf was scanned into a digital image format (JPG). This allowed for leaf area damage and subsequent perimeter of leaf damage to be measured using *ImageJ* for Windows (64 bit version 1.52, Schindelin *et al.*, 2012).

Response of *G. jacintae* to host instar

In order to elucidate the sequence of behaviour that leads a parasitoid to a host, we observed responses of female *G. jacintae* to leaves infested with susceptible larval stages of LBAM (third to sixth larval instar: Danthanarayana, 1980). Instars were identified by measuring head capsule width (Yazdani *et al.*, 2014), with measurements of 0.545 (third instar), 0.875 (fourth instar), 1.248 (fifth instar) and 1.422 mm (sixth instar) used accordingly. Two larvae of a selected instar were transferred to a single plantain leaf and left for 16-18h to allow them to feed on the plant, produce frass and deposit silk. The leaf was then hung from a bar fixed 25 cm above the floor of a wind tunnel (see Keller 1990 for details). Wind speed was set at 20 cm s⁻¹ and temperature at 23 ± 2 °C. A single female wasp, which had been isolated in a glass vial (see above), was released 25 cm downwind from the infested leaf (Fig. 1).

Preliminary observations were used to distinguish and define the array of behaviours exhibited by females (Martin & Bateson, 1993) (Table 1). Observations were divided into three phases: (1) the time from introduction into the wind tunnel until initiation of first flight from the release vial was defined as ‘pre-flight’, (2) the time from initiation of flight until first landing on the infested leaf was defined as ‘flight time’ and (3) the time spent on the infested leaf was defined as ‘post-flight’.

Parasitoid behaviour was recorded continuously using *Noldus* event-recorder software, *The Observer XT* (Zimmerman *et al.*, 2009), with each observation lasting for either 10 minutes or until the wasp attacked a host larva. Observations did not continue past the host attack stage as the interactions between host and parasitoid are complex, involving multiple stinging events and ovipositional decisions, which require separate analysis (Aspin *et al.*, unpublished data). The mean duration and mean frequency (min⁻¹) of each behavioural category was calculated for pre-flight and post-flight phases within

each replicate. The experiment was replicated 20 times for each instar, using a different female *G. jacintae* in each replicate ($n = 80$).

Statistical analyses

Statistical analyses were conducted to formally test for effects of experimental treatments. Effects of host larval instar on the dimensions of leaf damage (leaf area removed and length of perimeter of leaf damage) were analysed using two-tailed one-way ANOVAs, followed by Tukey's HSD tests. Differences in the behavioural profiles exhibited by wasps exposed to different host instars were assessed using multivariate analysis of variance (MANOVAs). ANOVAs were conducted when significant differences were observed, followed by Tukey's HSD post-hoc tests, to explore the effects of host instar on the occurrence of each of the behavioural categories. The significance thresholds for these ANOVAs were adjusted for multiple comparisons to control Type I error rates via the false discovery rate (FDR) procedure, with the family-wide α -value set to 0.05 (Benjamini & Hochberg, 1995; McDonald, 2014). Data on the time to initiate first flights and the duration of first flights were analysed using Cox proportional hazards regression models with the 'coxph' function in package *Survival* (version 3.2-3, Therneau *et al.* 2019). Kaplan-Meier survival curves were constructed to illustrate how times were affected by host instar treatments. Chi-square tests of independence were used to analyse the frequencies of completed flights and host encounter occurrences, depending on which instar group the parasitoid was presented with. All statistical analyses were conducted using *R* (version 3.6.0, R Core Team, 2019) with *RStudio* (version 1.2.1335, RStudio Team, 2019).

Results

Leaf damage by host instars

Area of consumed leaf tissue and length of the perimeter of leaf damage differed significantly among larval instars (Area: $F_{3,72} = 27.93$, $P < 0.001$; Perimeter; $F_{3,72} = 22.81$, $P < 0.001$, Fig. 2) and, for both, values were significantly greater for fifth and sixth instars compared to third and fourth instars (HSD test, $P < 0.001$).

Pre-flight response to host instar

There was a period of pre-flight orientation exhibited by female *G. jacintae* on the rim of the release vial which was characterised by *walking* (Supplementary video 1), *grooming* and *pointing* behaviours. The overall behavioural profile of pre-flight behaviours exhibited differed among host larval instars (Table 2, MANOVAs), as did the mean duration and mean frequency of every individual behavioural category (Table 2, ANOVAs). Similarly, the proportions of time spent on each behaviour during the pre-flight phase differed among host instars (Fig. 3). The mean duration of *walking* was highest when wasps were exposed to third instars but decreased significantly when downwind of sixth instars (Table 2, HSD test, $P < 0.05$). *Pointing* was a behaviour that usually preceded flight (E. Aspin, pers. obs.), and both the mean duration and mean frequency of *pointing* was significantly higher when wasps were downwind of larger the instars, fifth and sixth, compared to the smaller instars, third and fourth (Table 2, HSD test, $P < 0.05$).

Time to initiate flight differed according to the host instar presented (Likelihood ratio test: $G = 93.14$, d.f. = 3, $P < 0.001$). Four females exposed to third instars and one female exposed to fourth instars did not take flight during the designated 10-minute trial time. All other females took flight. Pre-flight times were longer when larvae were third

instars than when they were fourth ($z = 5.07$, $P < 0.001$), fifth ($z = 8.84$, $P < 0.001$) or sixth instars ($z = 4.27$, $P < 0.001$). Time taken to initiate first flight when presented with fifth instar hosts was significantly shorter than when presented with third ($z = 8.84$, $P < 0.001$), fourth ($z = 6.20$, $P < 0.001$) and sixth instars ($z = 6.57$, $P < 0.001$). There was no significant difference in timing when fourth or sixth instars were presented ($z = 0.72$, $P = 0.471$, Fig. 4a).

Flight response to host instar

The duration of first flight differed among host instars (Likelihood ratio test: $G = 55.46$, d.f. = 3, $P < 0.001$), with flights to the infested leaf taking significantly longer for third instars compared to fourth ($z = 4.39$, $P < 0.001$), fifth ($z = 5.61$, $P < 0.001$) and sixth instars ($z = 4.91$, $P < 0.001$). Duration of first flight towards fifth instars was significantly shorter than fourth instars ($z = 2.52$, $P = 0.0119$), but there was no significant difference between fourth and sixth instars ($z = 1.03$, $P = 0.305$, Fig. 4b).

Post-flight response to host instar

The overall behavioural profile during the post-flight period differed among host instars (Table 3, MANOVAs) and mean duration and mean frequency of all behaviours differed significantly among instars (Table 3, ANOVAs). Again, the proportions of time spent on each behaviour during the post-flight phase differed among host instars (Fig. 3). The *grooming thorax* behaviour was significantly lower in mean duration and mean frequency when a wasp was on a leaf infested with fifth and sixth instars compared to third and fourth instars (Table 3, HSD test, $P < 0.001$). Mean duration and mean frequency of *slow walking* differed significantly among host instars. *Slow walking* was often seen when wasps were in close proximity to a host and preceded attacking (Supplementary video 2).

The mean duration and mean frequency of *slow walking* was the shortest for third instar compared to fourth, fifth and sixth instar (HSD test, $P < 0.05$). *Slow walking* mean frequency was longest for the sixth instar (HSD test, $P < 0.001$).

Occurrence of completed flight and host finding occurrences

As not all trials resulted in the initiation of flight or encounter of female *G. jacintae* with a larval LBAM (Table 4), a Chi-square test of independence was conducted to assess whether the instar presented influenced the frequency of parasitoid flight or LBAM encounter. The likelihood of a wasp taking flight towards an infested leaf differed significantly among instars ($\chi^2 = 9.17$, d.f. = 3, $P < 0.05$), and the likelihood of a wasp encountering and attacking a larva was also influenced by instar ($\chi^2 = 14.01$, d.f. = 3, $P < 0.01$). The frequency of wasps that encountered and attacked a host given that they took flight significantly differed among instars ($\chi^2 = 9.20$, d.f. = 3, $P < 0.05$). Removal of the third instar from the analysis consequently resulted in no significant difference among instars ($\chi^2 = 4.23$, d.f. = 2, $P = 0.120$), indicating that third instar hosts were attacked less frequently by *G. jacintae*.

Discussion

We believe that this is the first experimental study of the foraging-flight behaviour of a bethylid wasp. We found that time for *G. jacintae* to initiate flight towards fifth larval instars was less than when hosts were in other instars, whereas the duration of flight towards third instar hosts was greater than for other instars studied. This shows that female *G. jacintae* can detect and respond to host-associated cues remotely. It suggests the females do not detect the presence of leaves infested with third instar LBAM as

quickly as those infested with later instars, such as the fifth and sixth instar, and/or that they find cues emanating from third instar larvae less attractive.

It is known from studies of other parasitoid species that herbivore-damaged plants are a source of volatiles that attract females to the locality of hosts (Nordlund *et al.*, 1988; Turlings *et al.*, 1991; Turlings & Wäckers, 2004), that parasitoid behaviour can be influenced by host-stage specific volatile profiles (Takabayashi *et al.*, 1995; Turlings *et al.*, 2000; Gouinguéné *et al.*, 2003; McCormick *et al.*, 2012; Yazdani *et al.*, 2015b) and that larger lepidopteran larvae are more damaging to plants than smaller larvae (Mattiacci & Dicke, 1995; Yazdani *et al.*, 2015b). Given that we established that larger larval instars of LBAM inflict more leaf damage than smaller instars, a likely explanation for the observed time-to-flight differences is that the quantity of volatile cues is higher - and thus more readily detectable by female *G. jacintae* - when the feeding larvae are larger, although the qualitative composition of cues may also vary according to LBAM instar.

Our evidence that *G. jacintae* has a stronger response to larger hosts is also consistent with reports of *G. jacintae* and other *Goniozus* species having greater reproductive success when attacking larger hosts (Danthanarayana, 1980; Hardy *et al.*, 1992; Abdi *et al.*, 2020a; Aspin *et al.*, unpublished data). *Goniozus jacintae* parasitises the third to sixth larval instars of the LBAM while *Dolichogenidea tasmanica*, a common parasitoid, parasitises the first to third instars of the same host species and has the shortest flight duration when exposed to third instar larvae (Yazdani *et al.*, 2015b), indicating that flight behaviours are aligned with the range of host instars that parasitoids normally exploit. However, the observation that *G. jacintae* females would initiate flight towards fifth instar larvae significantly earlier than towards sixth instars is perplexing. Sixth instars are typically larger than fifth instars and, in this study, consumed an equal amount of leaf area, thereby exposing an equal perimeter of damaged leaf tissue as fifth instars.

Volatile semiochemicals are released from the larval damage site (Rose *et al.*, 1996), making the length of perimeter of leaf damage a suitable proxy for estimating degree of volatile emission per instar. This suggests that the quantities of volatiles emitted from fifth and sixth instars are similar. Therefore, as above, there could be qualitative differences that are influencing the response of *G. jacintae* to fifth and sixth instars.

A qualitative difference between fifth and sixth instar LBAM could be the likelihood of a sixth instar being close to pupation. The light brown apple moth pass through up to six moults prior to pupation (Danthanarayana, 1983) and a host that has pupated is unsuitable for *G. jacintae* to produce offspring as it attacks the larval stage (Danthanarayana, 1980), and any eggs laid prior to imminent pupation would be shed along with the moulted cuticle. Larvae produce hormones that trigger morphological changes during moult or pupation (Maróy & Tarnóy, 1978; Riddiford, 1996) that in turn influence the formation of larval or pupal cuticle (Riddiford *et al.*, 1999). Many hymenopterous parasitoids are known to inspect the surface of the host, usually as a means of discriminating between parasitised and unparasitised hosts (van Lenteren, 1981). Evidence of host discrimination mechanisms used by parasitoids include perceiving marking pheromones from other parasitoids (Vinson & Guillot, 1972) as well as physical changes in the host surface. For example, ovipositional punctures have been suggested to function as an external marker (Boldt & Ignoffo, 1972). Considering this information, parasitoids may be able to use surface markers or signals to identify when a potential host is close to pupation. Detecting such surface markers or pupation cues on a host at a distance seems unlikely. While it is impossible to reduce the amount of damage that is associated with growth and development, perhaps sixth instars have evolved the ability to reduce the quantity of attractive compounds associated with their feeding. Also, if the moulting or pupation process caused notable changes to the volatile profile

associated with different host stages, this could influence the behavioural response of *G. jacintae*. Hence, *G. jacintae* may have developed a mechanism to detect and avoid parasitising hosts that are close to moulting or pupation, thus limiting the costs of securing a host and laying eggs that will not develop. Further study is required to determine if *G. jacintae* females actively avoid hosts that are close to pupation.

In addition, *G. jacintae* may respond differently to sixth instars compared to fifth instars due to differing risks involved in host attack. Later host instars are often larger and better equipped with defensive tools and behaviours, including aggressive biting behaviour and regurgitation (Supplementary video 2) that can both result in irreversible damage to, or even death of, natural enemies (Greeney *et al.*, 2012; Abdi *et al.*, 2020b). Sixth instar LBAM are capable of these behaviours and have been observed to use them to escape from parasitism by *G. jacintae* (E. Aspin, pers. obs.). Hence, differences in flight response by *G. jacintae* between fifth and sixth instars may reflect both the risks involved in handling larger host and/or host adaptation in terms of cue production.

We found that female *G. jacintae* display different behavioural profiles when exposed to different host instars and also that the exhibited behaviours vary between pre-flight and post-flight stages of foraging. Information gained during the pre-flight and flight phases of foraging is most likely to be transmitted via volatile chemicals (Fatouros *et al.*, 2016). *Pointing*, for instance, involves standing still, facing upwind with raised head and continuously moving the antennae and is very likely associated with the detection of windborne chemical cues prior to making a decision to engage in flight (Beyaert and Hilker, 2014; Fürstenau *et al.*, 2016). While commonly observed during pre-flight, pointing was only once observed post-flight. Information acquired during the post-flight stage can also include contact and perhaps visual cues based on feeding damage, faeces and silk deposits associated with larger instars, and the proximity of the

host itself (de Bruijn *et al.*, 2021). *Slow walking* is a distinct behaviour that was exhibited only during post-flight and showed an inverse relationship to walking. The largest distinction between *slow walking* and *walking* is a considerably reduced pace and higher antennation of the substrate (Supplementary videos 1 & 2). Antennation is associated with orientation and searching in parasitoids (Olson *et al.*, 2003), and *slow walking* was usually the behaviour that occurred immediately before a female attacked the host. *Slow walking* was also the most common when hosts were large, particularly in the sixth instar; it seems likely that this behaviour reflects the higher physical risk to the parasitoid that is incurred when tackling larger hosts. A slower, more cautious pace may reduce the likelihood of detection by a host and thus elicit fewer defensive behaviours, which are often detrimental to the success of a parasitoid (Waage, 1983; Gross, 1993; Greeney *et al.*, 2012; Abdi *et al.*, 2020b). In addition, as LBAM are leafrollers, it would be beneficial for the parasitoid to exercise caution when entering a leaf-rolled enclosure; it is not uncommon for other natural enemies, such as spiders and earwigs, to occupy these shelters, and presence of LBAM cues (frass, faeces) does not guarantee presence of a suitable host.

It is important to note that LBAM is only one of many host species that *G. jacintae* is capable of parasitising (e.g. *Merophyas divulsana* Walker (Lepidoptera: Tortricidae), E. Aspin, pers. obs.), some of which may not be leafrollers. Thus, foraging behaviour in *G. jacintae* may not be uniform across host species, as non-leafroller hosts could require a different foraging approach than that is shown towards LBAM.

Flight, and subsequent, host finding success did not occur in all replicates of the wind tunnel experiment and the likelihood varied throughout each phase of foraging. The occurrence of flight towards an infested leaf and the overall frequency of attacking a larva differed among larval instars. Additionally, the likelihood of a wasp attacking a larva

given that it *had* taken flight was different among instars. These results indicate a sequential process of foraging: together with the flight time results, this shows that before flight, during flight, and after landing on the infested leaf, *G. jacintae* exhibited different responses to different susceptible instars. This wind tunnel study of foraging behaviour of a bethylid wasp provides new understanding of the behavioural repertoires exhibited and their likely relationships with different larval instars of host and different classes of cues. The results suggest that hosts close to pupation may be avoided, or at least less preferred than those that are not about to undergo changes to the integument on which parasitoid eggs are deposited and further that females may approach larger hosts with caution due to the physical risks involved in host attack and suppression. While these aspects require further investigation, we have demonstrated that (1) *G. jacintae* females can detect host-associated cues remotely and actively travel by a combination of flight, walking and slow walking, towards their origin, and (2) behaviours are attuned to the information received regarding the developmental stage of the host. Such knowledge is useful for designing and implementing effective programmes of biocontrol of LBAM in vineyard settings, for instance, when considering how best to release mass-reared parasitoids into the field.

Acknowledgements

We thank Dr Hieu Trung Bui for assistance with Observer and ImageJ software. The culture of LBAM was obtained from the South Australian Research and Development Institute (SARDI). E.A. was funded by the University of Nottingham - University of Adelaide joint PhD Programme.

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Figure legends

Figure 1: Diagram of the wind tunnel used in experiments.

Figure 2: Mean leaf area damaged (mm²) and mean perimeter of damaged leaf tissue (mm) caused by different larval instars of *E. postvittana* feeding on *P. lanceolata* L. Different letters indicate significant differences between instars within each measure of feeding activity ($P < 0.001$). Error bars are ± 1 standard error.

Figure 3: Proportion of total time for each behaviour of female *G. jacintae* in the presence of susceptible instars of LBAM. The graph is split into pre-flight and post-flight phases. Table 1 provides definitions of behaviours.

Figure 4: Kaplan-Meier survival curves for (a) time from release to initiate first flight and (b) duration of first flight for *G. jacintae* towards susceptible instars of LBAM. Different letters in legend indicate significant differences among instars ($P < 0.05$).

Figures

Figure 1

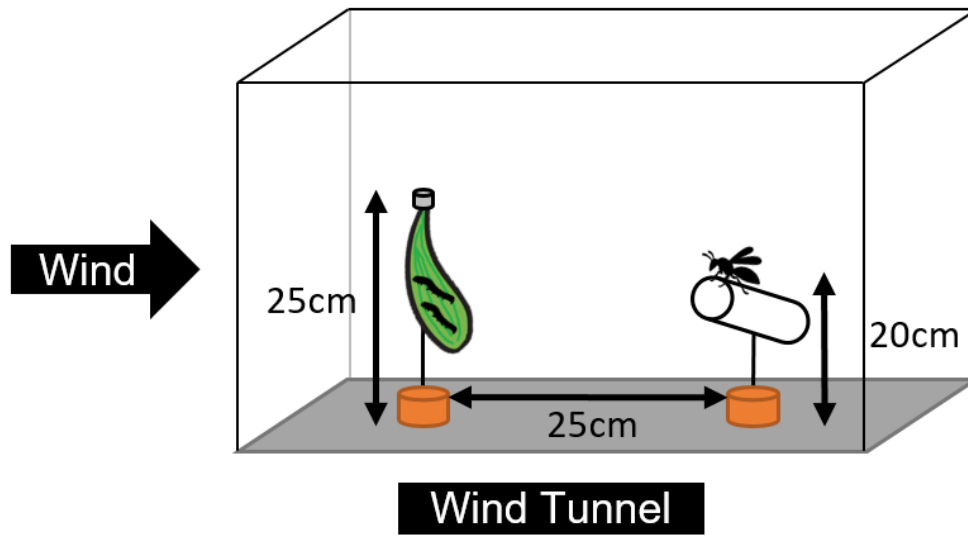


Figure 2

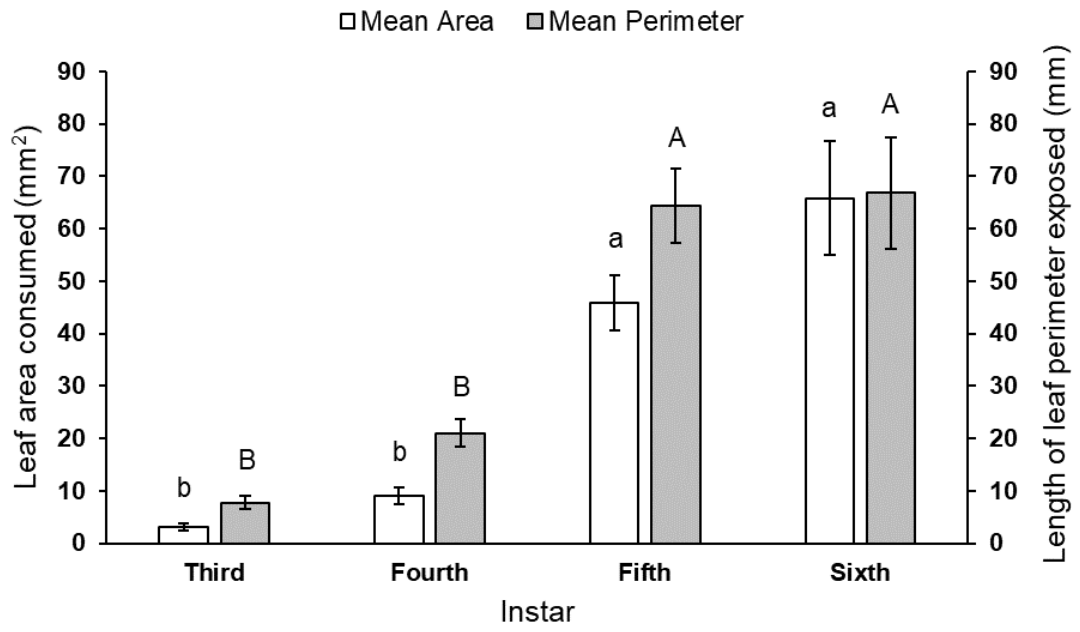


Figure 3

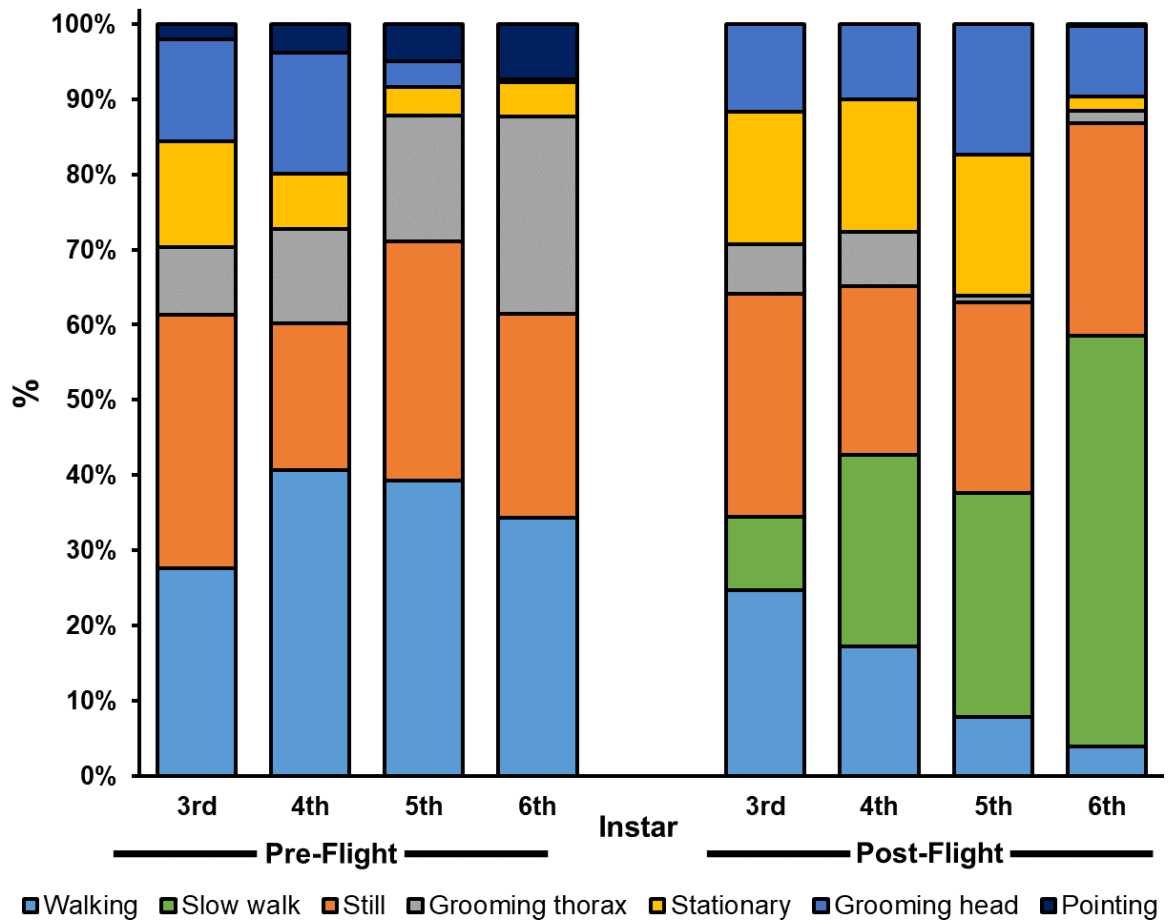


Figure 4a

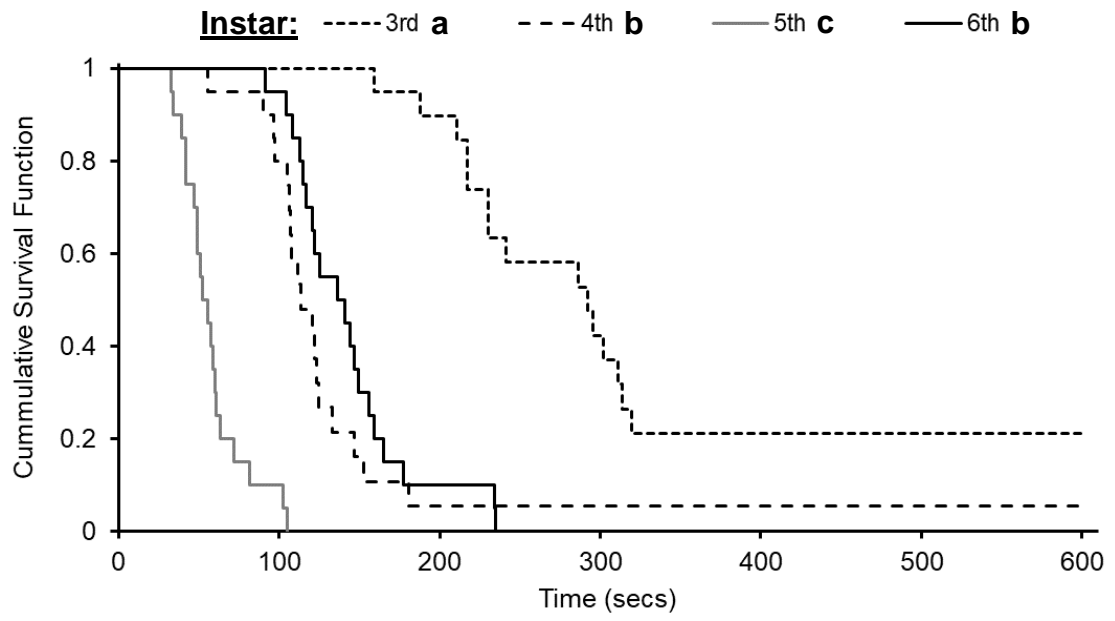
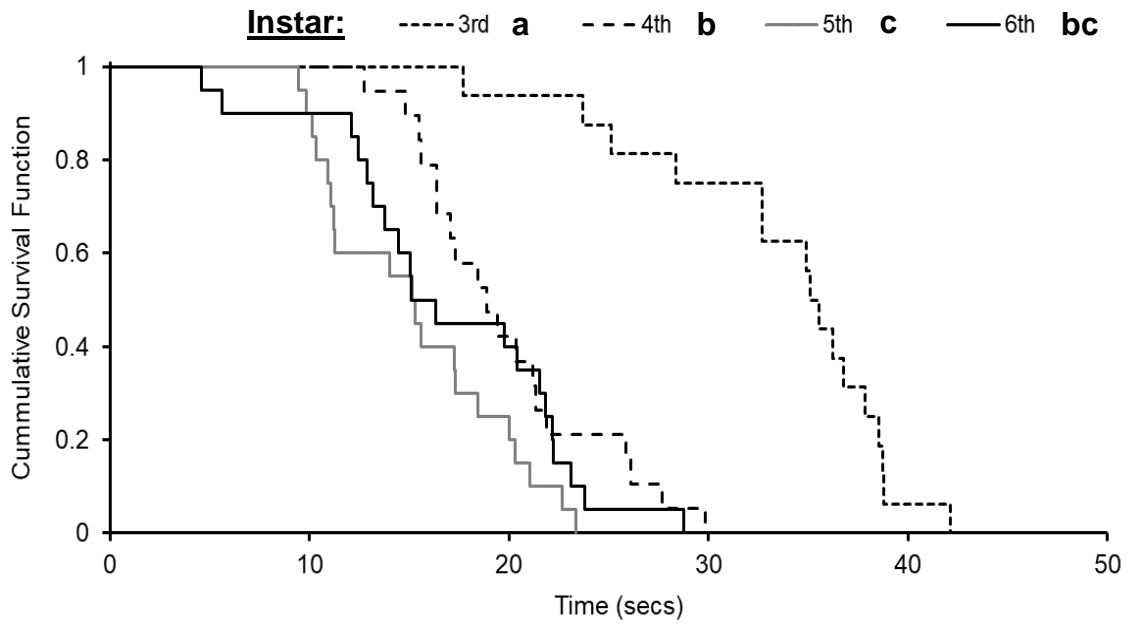


Figure 4b



Tables

Table 1. Behaviours exhibited by *G. jacintae*

Behaviour	Description
Still	Standing still on the substrate without moving antennae
Stationary	Standing still on the substrate with moving antennae, no upwind orientation
Pointing	Standing still, facing upwind with raised head and continuously moving antennae
Grooming head	Grooming antennae and other parts of head
Grooming thorax	Grooming legs, abdomen or wings
Walking	Walking while antennae are held forward of the head ¹
Slow Walking	Walking at a slower pace, with antennae retracted and occasionally in contact with the substrate ²
Flying	Flying
Attacking	Mounted on and grappling with host whilst curling abdomen and attempting to insert probing stinger into host thorax ²

¹Supplementary video 1. ²Supplementary video 2.

Table 2. Pre-flight behaviours exhibited by female *G. jacintae* according to host instar

Pre-Flight Mean Duration (secs) \pm s.e.

Behaviour	Instar				ANOVAs	
	3 rd	4 th	5 th	6 th	F	P
Grooming head	10.15 \pm 1.49 ^a	9.02 \pm 1.29 ^a	1.68 \pm 0.64 ^b	3.09 [†]	23.863	<0.001*
Grooming thorax	6.80 \pm 0.88	7.05 \pm 0.85	8.25 \pm 0.87	10.18 \pm 1.05	2.844	0.043*
Pointing	1.56 \pm 0.26 ^b	2.17 \pm 0.23 ^{ab}	2.41 \pm 0.16 ^{ab}	2.85 \pm 0.27 ^a	5.230	0.002*
Stationary	10.60 \pm 1.24 ^a	4.11 \pm 0.92 ^b	1.87 \pm 0.50 ^b	1.77 \pm 0.57 ^b	23.201	<0.001*
Still	25.34 \pm 3.74 ^a	10.95 \pm 1.99 ^b	1.38 \pm 0.79 ^c	10.54 \pm 1.54 ^b	18.729	<0.001*
Walking	20.79 \pm 1.47 ^a	22.86 \pm 2.65 ^a	19.32 \pm 1.95 ^{ab}	13.33 \pm 1.42 ^b	4.465	0.006*

Pre-Flight Mean Frequency (min⁻¹) \pm s.e.

Behaviour	Instar				ANOVAs	
	3 rd	4 th	5 th	6 th	F	P
Grooming head	0.92 \pm 0.10 ^a	0.76 \pm 0.06 ^a	0.25 \pm 0.09 ^b	0.40 [†]	31.123	<0.001*
Grooming thorax	0.42 \pm 0.05 ^b	0.86 \pm 0.12 ^a	0.96 \pm 0.11 ^a	0.98 \pm 0.05 ^a	8.031	<0.001*
Pointing	0.31 \pm 0.06 ^b	0.75 \pm 0.11 ^b	1.48 \pm 0.15 ^a	1.65 \pm 0.12 ^a	30.36	<0.001*
Stationary	0.89 \pm 0.08 ^{bc}	1.15 \pm 0.13 ^{ab}	1.52 \pm 0.12 ^a	0.67 \pm 0.0 ^c	12.56	<0.001*
Still	0.76 \pm 0.08 ^a	0.86 \pm 0.13 ^a	0.12 \pm 0.07 ^b	1.08 \pm 0.13 ^a	11.448	<0.001*
Walking	1.16 \pm 0.14 ^c	1.91 \pm 0.24 ^b	2.50 \pm 0.20 ^{ab}	2.78 \pm 0.18 ^a	13.664	<0.001*

Mean duration MANOVA: Wilks' Lambda = 0.176, $F_{(3,76)} = 9.50$, $P = 0.001$.

Mean frequency MANOVA: Wilks' Lambda = 0.082, $F_{(3,76)} = 15.95$, $P = 0.001$.

Because six ANOVA tests were carried out, the significance criterion was adjusted via the false discovery rate (FDR) procedure: P values that remained significant following this correction are indicated with an asterisk (*). † = only one occurrence of Grooming head, sixth instar. Different letters within a row indicate significant differences among host instars ($P < 0.05$).

Table 3. Post-flight behaviours exhibited by female *G. jacintae* according to host instar

Post-Flight Mean Duration (secs) \pm s.e.

Behaviour	Instar				ANOVAs	
	3 rd	4 th	5 th	6 th	F	P
Grooming head	11.35 \pm 1.21 ^{ab}	8.81 \pm 0.96 ^b	13.25 \pm 1.16 ^a	4.51 \pm 0.60 ^c	14.01	<0.001*
Grooming thorax	6.35 \pm 1.09 ^a	6.45 \pm 1.01 ^a	0.68 \pm 0.37 ^b	0.83 \pm 0.48 ^b	16.26	<0.001*
Pointing	-	-	-	2.26 [†]	N/A	N/A
Stationary	17.17 \pm 1.93 ^a	15.50 \pm 2.12 ^a	14.35 \pm 1.61 ^a	0.92 \pm 0.47 ^b	20.04	<0.001*
Still	28.78 \pm 2.07 ^a	19.70 \pm 2.08 ^b	19.36 \pm 2.32 ^b	13.59 \pm 2.12 ^b	7.395	<0.001*
Walking	23.99 \pm 2.44 ^a	15.18 \pm 2.64 ^b	5.98 \pm 1.57 ^c	1.90 \pm 0.72 ^c	22.61	<0.001*
Slow Walking	9.55 \pm 1.95 ^b	22.43 \pm 1.52 ^a	22.80 \pm 1.28 ^a	26.24 \pm 3.48 ^a	6.324	<0.001*

Post-Flight Mean Frequency (min⁻¹) \pm s.e.

Behaviour	Instar				ANOVAs	
	3 rd	4 th	5 th	6 th	F	P
Grooming head	0.75 \pm 0.08 ^a	0.82 \pm 0.06 ^a	0.48 \pm 0.03 ^b	0.64 \pm 0.07 ^a	5.115	<0.001*
Grooming thorax	0.23 \pm 0.04 ^a	0.31 \pm 0.03 ^a	0.03 \pm 0.02 ^b	0.05 \pm 0.03 ^b	19.49	<0.001*
Pointing	-	-	-	0.13 [†]	N/A	N/A
Stationary	0.82 \pm 0.08 ^a	0.76 \pm 0.10 ^a	0.67 \pm 0.08 ^a	0.11 \pm 0.05 ^b	15.51	<0.001*
Still	0.70 \pm 0.06 ^c	1.14 \pm 0.14 ^{ab}	0.87 \pm 0.08 ^{bc}	1.40 \pm 0.13 ^a	7.398	<0.001*
Walking	0.64 \pm 0.08 ^a	0.40 \pm 0.04 ^b	0.12 \pm 0.02 ^c	0.22 \pm 0.09 ^{bc}	12.19	<0.001*
Slow Walking	0.35 \pm 0.07 ^c	0.81 \pm 0.11 ^b	1.23 \pm 0.08 ^b	1.96 \pm 0.21 ^a	25.06	<0.001*

Mean duration MANOVA: Wilks' Lambda = 0.221, $F_{(3,71)} = 11.55$, $P = 0.001$.

Mean frequency MANOVA: Wilks' Lambda = 0.129, $F_{(3,71)} = 11.03$, $P = 0.001$

Because six ANOVA tests were carried out, the significance criterion was adjusted via the false discovery rate (FDR) procedure: P values that remained significant following this correction are indicated with an asterisk (*). † = only one occurrence of pointing, sixth instar. Different letters within a row indicate significant differences among host instars ($P < 0.05$).

Table 4. Occurrence of flights and attacks by *G. jacintae* according to host instar

Frequency of flight	Host instar				Total
	3 rd	4 th	5 th	6 th	
Flight	16	19	20	20	75
Total	20	20	20	20	80
Proportion flying	0.80	0.95	1.00	1.00	0.94
Frequency of attack					
Attack	7	11	16	17	51
Total	20	20	20	20	80
Proportion attacking	0.35	0.55	0.80	0.85	0.64
Frequency of attack among parasitoids that flew to the host location					
Attack	7	11	16	17	51
Total	16	19	20	20	75
Proportion attacking	0.44	0.58	0.80	0.85	0.68

Appendix 1 Supplementary video legends

Supplementary video 1. Female *G. jacintae* wasp exhibiting *walking* behaviour across a *P. lanceolata* L. leaf. *Walking* behaviour characteristics: standard pace, antennae raised and not in contact with substrate.

Supplementary video 2. Female *G. jacintae* wasp exhibiting *slow walking* behaviour before attacking and stinging a feeding light brown apple moth (LBAM) larva (*E. postvittana*) on a *P. lanceolata* L. leaf. *Slow walking* behaviour characteristics: considerably reduced pace, antennae making contact with substrate and host. Later larval LBAM instars can defensively regurgitate, which is seen in this video. They also exhibit a ‘corkscrew’ motion when attacked. In this example however, the parasitoid stung the thorax of the larva and caused paralysis.

Electronic Supplementary Information

Supplementary video 1



Accessible url: <https://www.youtube.com/watch?v=MaF89ZnAiIo>

Supplementary video 2



Accessible url: <https://www.youtube.com/watch?v=T67oFiOXttI>

CHAPTER THREE

Family planning for parasitoids: reproductive responses to leafroller host development in *Goniozus jacintae*

Title:

Family planning for parasitoids: reproductive responses to leafroller host development in *Goniozus jacintae*

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Short Title:

Bethylid reproductive behaviour

Key words:

Light brown apple moth, *Epiphyas postvittana*, parasitoid, precise sex ratio, bethylid, biological control, host size, host quality, small broods, offspring size

Statement of Authorship

Title of Paper	Family planning for parasitoids: reproductive responses to leafroller host development in <i>Goniozus jacintae</i> .
Publication Status	<input type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input checked="" type="checkbox"/> Unpublished and Unsubmitted work written in manuscript style
Publication Details	Aspin, E., Keller, M.A., and Hardy, I.C.W., 2022.

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Contribution to the Paper	Conceived the study, designed and tested the method, conducted experiments, analysed data, wrote the manuscript.			
Overall percentage (%)	75%			
Certification:	This paper reports on original research I conducted during the period of my higher degree by research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.			
Signature	<table border="1" style="width: 100%;"> <tr> <td style="width: 70%;"></td> <td style="width: 10%; text-align: center;">Date</td> <td style="width: 20%; text-align: center;">18.04.2022</td> </tr> </table>		Date	18.04.2022
	Date	18.04.2022		

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ian C. W. Hardy			
Contribution to the Paper	Assisted with data analysis and manuscript preparation.			
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	Date	24.04.2022		

Abstract

Many parasitoids alter their oviposition behaviour in response to the quality of hosts they encounter. They may make adaptive decisions concerning whether or not to parasitize the host, the number of eggs laid on a host, and the sex allocation of their offspring. Observing the response of parasitoids to hosts of different size and developmental stage can provide practical and useful information, such as how to optimise mass-rearing of parasitoids for deployment as agents of biological pest control. Here we present experimental evidence that *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae), a gregarious ectoparasitoid of larval tortricids, adjusts its reproductive response to the size and developmental stage of larvae of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae). *Goniozus jacintae* parasitises instars 3-6 of LBAM, but most readily parasitises the later, larger, larval instars. Brood sizes were bigger on larger hosts and brood sex ratios were female biased (proportion of males = 0.23) with extremely low variance, perhaps the most precise of all studied bethylids. Host size did not influence brood development time, which averaged 19.64 days, or the body size of male offspring. However, the size of females was positively correlated with host size and negatively correlated with brood size. The size of both sexes was positively related to average amount of host resource available to individuals within each brood, suggesting that adult body size is affected by scramble competition among feeding larvae. The largest brood size emerging from a host was 8 individuals on the 6th instar of LBAM, suggesting that later instars would give the highest yield of female parasitoids for mass-rearing purposes.

Introduction

Upon finding a potential host, female hymenopteran parasitoids typically assess the quality of the host for offspring development (Rehman and Powell, 2010; Hajek and Eilenberg, 2018) and make oviposition decisions in response to host condition (Visser *et al.*, 1990; Hardy *et al.*, 1992; Godfray, 1994; Bezemer and Mills, 2003; Ayala *et al.*, 2018; Li *et al.*, 2019). These oviposition decisions include the number of eggs laid (Godfray, 1987; Godfray, 1994), and the allocation of sex to offspring (West, 2009). Size-dependent selection of hosts is common in parasitoids, since the size of a host is often positively correlated with host quality via the quantity of resources available to offspring (Charnov and Skinner, 1984; Godfray, 1994; Goubault *et al.*, 2004; Rehman and Powell, 2010), which influences how many progeny can be supported per host. In general, females are selected to lay a clutch size that maximises their gain in fitness across all hosts they expect to find during their lifetime (Godfray *et al.*, 1991). In terms of sex allocation, mated haplo-diploid hymenopteran parasitoids are able to control whether their eggs remain unfertilized or become fertilized, developing into males or females, respectively (Godfray, 1994; Quicke, 1997). Understanding the reproductive strategies of parasitoids can be important for the successful implementation of biological pest control programmes, as these directly influence the number of female offspring recruited into each generation, and therefore, the degree to which target pest populations are likely to be suppressed (Hassell, 2000; Ode and Hardy, 2008).

Here we report on the responses of *Goniozus jacintae* Farrugia (Hymenoptera: Bethylinidae) to the size and developmental stages of larvae of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Danthanarayana, 1980; Aspin *et al.*, 2021). This moth feeds on a wide range of crops and other plants (Suckling & Brockerhoff, 2010), and is the most damaging insect pest of grapevines in

Australia (Scholefield and Morison, 2010). Despite the common occurrence of *G. jacintae* (Fig. 1A) as a beneficial insect, there is surprisingly little knowledge of its efficacy as a biological control agent for LBAM (Danthanarayana, 1980; Paull and Austin, 2006) and limited information on its reproductive biology (Danthanarayana, 1980; Hopper and Mills, 2015). A recent study of *G. jacintae* foraging behaviour found that females have a stronger foraging response to larger hosts, which have a higher rate of feeding and produce more feeding damage (Aspin *et al.*, 2021). This is consistent with reports of other species of *Goniozus*, that have a greater reproductive success when attacking larger hosts (Hardy *et al.*, 1992; Luft, 1993; Abdi *et al.*, 2020).

The Bethyridae are a cosmopolitan family of ectoparasitoid wasps, containing over 2000 described species within around 100 genera (Gordh and Móczár, 1990). Their hosts are predominantly coleopteran or lepidopteran larvae that often live in cryptic locations such as seed-borers and leafrollers (Evans, 1978; Mayhew and Hardy, 1998). However, some bethylid species are reported to attack hosts in the pupal stage (Pérez-Lachaud *et al.*, 2004) and even hosts from other insect orders (Zhang *et al.*, 1984). Bethyrid species have been used in research on the evolution of key life history traits, such as clutch size, sex allocation and sociality (Hardy *et al.*, 1992; Mayhew and Hardy, 1998; Goubault *et al.*, 2007; Khidr *et al.*, 2013; Abdi *et al.*, 2020). Furthermore, as many bethylid species utilise hosts that are pests of agriculturally important products, multiple species have been deployed, or considered as, biological control agents across a wide range of agroecosystems (Legner and Gordh, 1992; Batchelor *et al.*, 2006; Shameer *et al.*, 2018; Polaszek *et al.*, 2019).

Goniozus jacintae readily parasitises larval instars 3 to 6 of LBAM (Danthanarayana, 1980; Aspin *et al.*, 2021). We thus investigated whether its oviposition behaviour varies according to host size and instar, first establishing whether host head capsule size or host

weight better represents host size. Our ultimate aim was to provide a broader understanding of bethylid oviposition behaviour that could elucidate the potential of *G. jacintae* to control LBAM in the field. Furthermore, information from this study will contribute to the growing collection of other agro-ecosystems using species of *Goniozus* as agents of biological pest control (Baker, 1999; Legner and Gordh, 1992; Batchelor *et al.*, 2006; Shameer *et al.*, 2018; Polaszek *et al.*, 2019) and assist the increase in efficacy of mass-rearing bethylid parasitoids in the laboratory.

Materials and methods

Host rearing

The culture of *Epiphyas postvittana* (LBAM) used in this experiment was established at the South Australian Research and Development Institute in 1994 and has since been maintained with annual additions of wild caught moths. LBAM was reared on an artificial diet at 22 ± 2 °C under 12L:12D photoperiod, following methods reported in Yazdani *et al.* (2015).

Parasitoid rearing

A culture of *Goniozus jacintae* was established from individuals reared from parasitised LBAM that were collected in vineyards at McLaren Vale, South Australia in 2017. The wasp culture was reared at 23 ± 2 °C, 14L:10D in cages on larval LBAM that infested plantain, *Plantago lanceolata* L. (Lamiales: Plantaginaceae). Adult wasps were provided with water and honey *ad libitum*. Wasp cocoons were isolated in 50 mm × 18 mm diam. glass vials containing a drop of honey and fitted with caps that had screens for ventilation. Upon emergence, females were caged serially, 2-5 at a time, with 5 males to allow mating, and then re-isolated and held in vials for at least 1 h before being used in experiments.

Parasitoid reproduction

One hundred and sixty female *G. jacintae* were individually presented with one 4th instar LBAM larva in a glass vial for 1h so that they may obtain oviposition experience prior to the experiment. The 4th instar was chosen as it represented the mean size of LBAM larvae, and according to Danthanarayana (1980), are the most predominantly parasitised instar

by *G. jacintae*. Then, each wasp was presented with a host of known instar (3rd-6th), head capsule width and weight.

Host size may be measured in several ways, including weight and head capsule width, and both may correlate with host instar. We took both measurements for LBAM larvae of each instar used in this experiment (3rd-6th), including the head capsule measures sizes for the 6th instar which have not been reported previously (Yazdani *et al.*, 2014). Head capsule width was measured under a dissecting microscope at a magnification of 40× with a calibrated ocular micrometre (precision ± 0.0125 mm). Host weight was measured using an A&D HR-250AZ analytical balance with a 0.1mg resolution (A&D Company, Limited, Tokyo, Japan).

After oviposition, the female parasitoid was removed and the host and parasitoid clutch were maintained at 22 ± 2 °C under 12L:12D photoperiod until brood emergence. Upon emergence of the adult parasitoids, the following measurements were recorded: brood size (= number of adult offspring), sex ratio (= proportion of offspring that were males), time from oviposition to adult eclosion (= developmental time) and length of thorax (an indicator of parasitoid body size).

Statistical Analysis

We used generalised linear modelling (GLM) and mixed modelling (GLMM) techniques (Dobson, 1983; McCullagh and Nelder, 1983; Aitkin *et al.*, 1989) which allow for the analysis of data with non-normal error distributions, such as binomial or Poisson, without prior transformation. Log-linear analyses, utilising the log-link function, were used to determine the relationship between host weight and head capsule size as well as host weight and brood size. Parasitoid sex ratios were analysed using logistic regression and adopted a logit-link function. Broods consisting only of males on emergence were

excluded from analyses (one brood of 4 individuals and one brood of 6 individuals) as they were most likely produced by virgin mothers (following Hardy & Cook, 1995). Variance in brood sex ratio was summarized using Heterogeneity Factors and the variance ratio, R, and departures from binomial distributions were assessed using the Meelis test (test statistic U) (Krackow *et al.*, 2002). Development time of parasitoid offspring was analysed using the Cox's proportional hazards model. A generalised linear model and a generalised linear mixed model analysis were conducted to determine the influence of host weight on the size of male and female parasitoids, respectively. GLM and GLMM analyses were conducted using the statistical software GenStat (version 20, VSN International, Hemel Hempstead, UK).

Results

Head capsule width, host weight and host instar

Larval instars are identified by head capsule width (Yazdani *et al.*, 2014). Head capsule width was associated with host weight (log-linear ANCOVA: $F_{(2,157)} = 418.64$, $P < 0.001$; Fig. 2) in a curvilinear relationship (quadratic term: $F_{(1,157)} = 132.77$, $P < 0.001$; Fig 2) that explained 84% of the variance in head capsule width. Head capsule width reached a maximum of approx. 1.4 mm. As larvae with head capsules of this width ranged widely in weight, between approximately 0.01 and 0.035mg (Fig. 2), host weight was used as the measure of host size in subsequent analyses.

Brood size and host weight/instar

Goniozus jacintae brood size increased with host weight (log-linear ANCOVA: $F_{(5,154)} = 73.18$, $P < 0.001$; Fig. 3) in a curvilinear relationship (quadratic term: $F_{(1,154)} = 28.07$, $P < 0.001$; Fig. 3). Broods laid on larger instars (5 and 6) did not differ significantly in size (aggregation of factor levels: $F_{(4,155)} = 0.02$, $P = 0.890$).

Sex ratio

Goniozus jacintae brood sex ratios were strongly female biased: the mean proportion of offspring that were male was 0.23 (S.E. ± 0.01). The maximum number of males recorded in any brood was 1 and sex ratio variances were significantly under-dispersed (HF = 0.09; Meelis test: $R = 0.022$, $U = -8.77$, $P < 0.001$; Table 2). Among instars 4, 5 and 6, sex ratios decreased significantly as brood size increased ($F_{(3,156)} = 162.06$, $P < 0.001$; Fig. 5). But the sex ratios did not differ between host instars 4, 5 and 6 (aggregation of factor

levels: $F_{(3,156)} = 0.85$, $P = 0.495$). Broods produced on 3rd instar hosts consisted of either one or two offspring and their sex ratios were either zero (a single female) or 0.5 (one male and one female), resulting in a positive relationship between sex ratio and brood size over this narrow brood size range (Fig. 5). Sex ratios of broods produced on 3rd instar hosts were significantly different to broods produced on all other instars ($F_{(1,156)} = 142.24$, $P < 0.001$).

Developmental time of brood

There was no difference in parasitoid development time on different host instars (Cox PH model, $\chi^2 = 2.78$, d.f. = 3, $P = 0.427$). The mean development time from oviposition to adult eclosion was 19.64 days, S.D. = 0.88.

Size of emerging parasitoids

Female *G. jacintae* were larger than males; mean thorax lengths for male and female *G. jacintae* were (1.13, S.D. = 0.083) and (1.31, S.D. = 0.097), respectively. For adult males, there was no significant relationship between thorax length and host weight ($F_{(4,71)} = 0.33$, $P = 0.858$; Fig. 4A), nor was male size related to brood size ($F_{(4,71)} = 0.32$, $P = 0.865$). Conversely, the body size of females was influenced by both host weight ($F_{(1,88)} = 4.44$, $P = 0.038$, Fig. 4B) and brood size ($F_{(1,84)} = 7.03$, $P = 0.01$). There was also a significant interaction between these main effects ($F_{(1,81)} = 7.46$, $P = 0.008$), with larger females developing from larger hosts, and smaller females developing from larger broods.

The statistical interaction between host weight and brood size indicates that these are not separate influences on adult female size. We calculated a resource index (host

weight/brood size) as a proxy for how much food resource, on average, was available to each individual in each brood. The effects of resource index, host larval instar and offspring sex on parasitoid size were then explored using a generalised linear mixed model, with brood identity included as a random factor (Bolker *et al.*, 2009). Thorax length was significantly influenced by both resource index ($F_{(1,104)} = 5.83, P = 0.017$) and sex of the wasp ($F_{(1,244)} = 445.07, P < 0.001$), such that the mean size of a parasitoid increased with increasing resource index, and males were smaller than females (Fig. 4C), but thorax length was not influenced by host instar ($F_{(3,84)} = 1.20, P = 0.316$).

Discussion

Goniozus jacintae adjusts its brood size in response to variation in host size; females produce larger broods on larger hosts. This is consistent with prior reports on this species (Danthanarayana, 1980; Hopper and Mills, 2015) and on other *Goniozus* species (Gordh *et al.*, 1983; Hardy *et al.*, 1992; Abdi *et al.*, 2020) having greater reproductive success when attacking larger hosts. It is also consistent with the finding that *G. jacintae* have a stronger foraging response as hosts develop through instars 3 to 6, reflecting their growth in size (Aspin *et al.*, 2021).

The host represents the sole nutritional resource for immature parasitoids. Larger hosts are preferential for the development of parasitoid larvae, since they contain more resources than their smaller counterparts (Godfray, 1994; Mackauer *et al.*, 1997; Farahani *et al.*, 2016). Smaller hosts may result in greater mortality and/or the production of smaller parasitoid offspring, with fitness measures such as fecundity and longevity also being lower among smaller adults (Godfray, 1994; Quicke, 1997; Mayhew, 2016; Zhang *et al.*, 2022). Hence, identifying the most suitable host size for a potential biological control agent could enhance the success of mass-rearing parasitoids in the laboratory.

Host size influenced the size of emerging *G. jacintae* offspring, with larger females emerging from larger hosts. Fitness of female parasitoids is typically positively influenced by their body size (Hardy *et al.*, 1992; Kazmer and Luck, 1995; Ellers *et al.*, 1998; Sagarra *et al.*, 2001; Samková *et al.*, 2019). Larger females exhibit higher fertility and longevity compared to smaller ones (Visser, 1994; Harvey *et al.*, 2001; Samková *et al.*, 2019; Wang *et al.*, 2021), as well as greater foraging efficiency when searching for hosts or food resources (Visser, 1994; Kazmer and Luck, 1995; Sarfraz *et al.*, 2009). In addition, larger females have higher success in the outcome of conflicts for host resources

against smaller, competing females (Petersen and Hardy, 1996; Hardy *et al.*, 2013). Thus, it can be inferred that when a female *G. jacintae* is accepting a host for oviposition, host size will play a key role in determining not only the size of her brood but the size of the female offspring within that brood.

The size of female offspring was also significantly influenced by brood size; smaller females emerged from larger broods, a trend also reported in the congener *Goniozus nephantidis* (Muesebeck) when clutches were artificially created on hosts of a fixed size (Hardy *et al.*, 1992). However, the opposite pattern was seen in broods that were laid naturally (Hardy *et al.*, 1992). In contrast to solitary parasitoids, where only one offspring per host can survive and develop, gregarious offspring may share a host – the sole nutritional resource – with their siblings and even the offspring of conspecific females (Godfray, 1994). Parasitoid growth and development varies depending on both the quality and quantity of the host resource available (Mackauer *et al.*, 1997; Cusumano *et al.*, 2016; Pekas *et al.*, 2016). Hence, scramble competition may arise between parasitoid larva on the same host, with potential impacts on offspring mortality (Salt, 1961; Brodeur and Boivin, 2004; Fox and Messina, 2018), fitness (Hardy *et al.*, 1992; Bernstein *et al.*, 2002; Pereira *et al.*, 2017), and size (Visser, 1996; Bezemer and Mills, 2003; Malabusini *et al.*, 2022).

Conversely, the size of male *G. jacintae* offspring was not related to either host size or brood size when these were treated as separate explanatory variables. However, it was influenced by these properties when combined into an index of *per capita* resource availability, as was female size. This suggests that, as above, there may be scramble competition between offspring within a brood for food as a resource, with direct consequences on offspring size. This competition may influence male offspring size to a lesser extent than females, since males require fewer resources than females due to their

smaller size. As is common in bethylids, adult *G. jacintae* males emerge from their cocoons before females in preparation for mating (Hardy *et al.*, 1999; Hardy *et al.*, 2000; Amante *et al.*, 2017; E. Aspin, pers. obs.). There may be little advantage for males in acquiring more resources to become larger, as development to a larger size may extend development time and result in the male missing the opportunity to emerge before females and secure mating opportunities (reviewed in Boulton *et al.*, 2015; Wang *et al.*, 2019; Teder *et al.*, 2021). Furthermore, as there is typically no more than 1 male in a *G. jacintae* brood, larger body size will not normally enhance competitive ability with male siblings.

The sex ratio of *G. jacintae* is female biased (mean proportion of males = 0.23), similar to that of most bethylids, most likely due to high levels of sibling mating and the resulting selection from strong local mate competition (Green *et al.*, 1982; Mayhew and Hardy, 1998; Tang *et al.*, 2014; Abdi *et al.*, 2020). In addition, the sex ratio of *G. jacintae* has extremely low variance (significantly less than binomial), perhaps the most precise sex ratio of all bethylid species; all broods with a size greater than one contained only one male. Notably, the variance ratio for *Goniozus jacintae* ($R = 0.022$) is lower than estimates obtained for several congeners: *G. nephantidis*, $R = 0.743$ (Hardy and Cook, 1995); *G. legneri*, $R = 2.39$, (Khidr *et al.*, 2013); *G. nigrifemur*, $R = 0.37$; *G. emigratus*, $R = 0.42$ (Hardy *et al.*, 1998).

Sex allocation is a behaviour of interest for the application of biological control and the mass rearing of bethylids, as the number of female offspring recruited into each generation positively influences the degree to which target pest populations are likely to be suppressed (Ode and Hardy, 2008). It is commonly known that parasitoids make adaptive decisions about sex allocation (reviewed in Charnov, 1982; Waage, 1986; West 2009; Whitehorn *et al.*, 2015), and that selection favours mothers that are able to produce

precise sex ratios, as this does not produce any superfluous males and instead promotes the number of dispersing females (Green *et al.*, 1982; Hardy, 1992; West and Herre, 1998; Khidr *et al.*, 2013). However, there are multiple factors that influence selection for, and the attainment of, precise sex ratios, such as the order in which sexes are produced when clutches are laid and developmental mortality (Green *et al.*, 1982; Nagelkerke and Hardy, 1994). Mortality of parasitoid larva during the developmental stage increases the variance of observed sex ratios at eclosion, introducing the risk that no males survive to maturity, resulting in a brood of virgin females with very limited fitness under single foundress local mate competition (reviewed in Nagelkerke and Hardy, 1994; Hardy *et al.*, 1998). The advantage of precise sex ratios can vary considerably depending on the different distributions of mortality within a brood (Nagelkerke and Hardy, 1994). In addition, parasitoids exhibit different sequence patterns when laying a clutch; some species lay female eggs first whereas others lay male egg(s) first (reviewed in Hardy, 1992). In the current study, all single egg broods produced females, and all 2-egg broods produced one male and one female, suggesting that this bethylid may fit in the group of parasitoids that lay male eggs last, although empirical assessment will be required to confirm this. Therefore, in order to obtain a fuller understanding of how the observed *G. jacintae* brood sex ratios arise, the sequence of sex allocation during the oviposition of a clutch and, especially, developmental mortality, should be assessed.

This study provides new information on the reproductive behaviour of a relatively unstudied potential biocontrol agent as well as complementing findings from existing work on bethylids (Griffiths and Godfray, 1988; Hardy *et al.*, 1992; Hardy and Mayhew, 1998; Luft, 1993; Hardy *et al.*, 2000; Polaszek *et al.*, 2019). Although some aspects require further investigation, we have demonstrated that (1) like other bethylids, *G. jacintae* has greater reproductive success on larger hosts and exhibits female biased sex

ratios (2) these sex ratios have extremely low variance, seemingly lower than all previously studied bethylids, and finally, (3) female parasitoid offspring size is influenced by brood size and host weight whilst male size is not, but the body size of both sexes is positively determined by the *per capita* availability of resources during development. Such information is key for designing and implementing effective biological control programmes for LBAM, for instance, when considering which larval instar would produce the most parasitoid offspring per host during mass-rearing procedures.

Acknowledgements

We thank Dr Hieu Trung Bui for assistance with insect culture maintenance, Georgia Black for the editing of Figure 1 and Dr Maryam Yazdani for co-supervision of E.A. The culture of LBAM was obtained from the South Australian Research and Development Institute (SARDI). E.A. was funded by the University of Nottingham - University of Adelaide joint PhD programme.

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Figure legends

Figure 1: Development of *G. jacintae* on light brown apple moth. Successive stages of development of a brood of *G. jacintae* on *E. postvittana*: **(A)** Host encounter: female *G. jacintae* on a paralysed 6th instar LBAM larva on a plantain leaf, **(B)** Day 1: eggs of *G. jacintae* laid on host's integument, **(C)** Day 6: larvae of *G. jacintae*, **(D)** Day 8: late instar larvae of *G. jacintae* and the head capsule of the consumed host, **(E)** Day 10: pupating larvae of *G. jacintae* inside their silken cocoons. Photo **(A)** has had the background changed to greyscale for clarity; the original leaf colour is green.

Figure 2: The relationship between head capsule size and weight of *E. postvittana*.

Figure 3: The relationship between brood size and host weight, classified by host instar. Data points for each instar are shown as symbols and the log-linear models fitted for each instar are defined as the following: 3rd instar: long dash and dot line, 4th instar: round dotted line, 5th instar: solid black line, 6th instar: dashed line.

Figure 4: Parasitoid size and resource availability. Relationship between emerging parasitoid size and host weight, classified by host instar, for male **(A)** and female **(B)** offspring. Effect of increasing resource index on parasitoid size for male and female offspring **(C)**.

Figure 5: The relationship between brood size and offspring sex ratio, classified by host instar. For instar 3, the fitted line is extrapolated to illustrate the bounded nature of

the relationship: note that broods on 3rd instar hosts never exceeded 2 offspring. Brood sex ratios of broods developing on host instars 4, 5 and 6 did not differ significantly and were combined across instar classes. Lines were fitted by logistic ANCOVA.

Figures

Figure 1

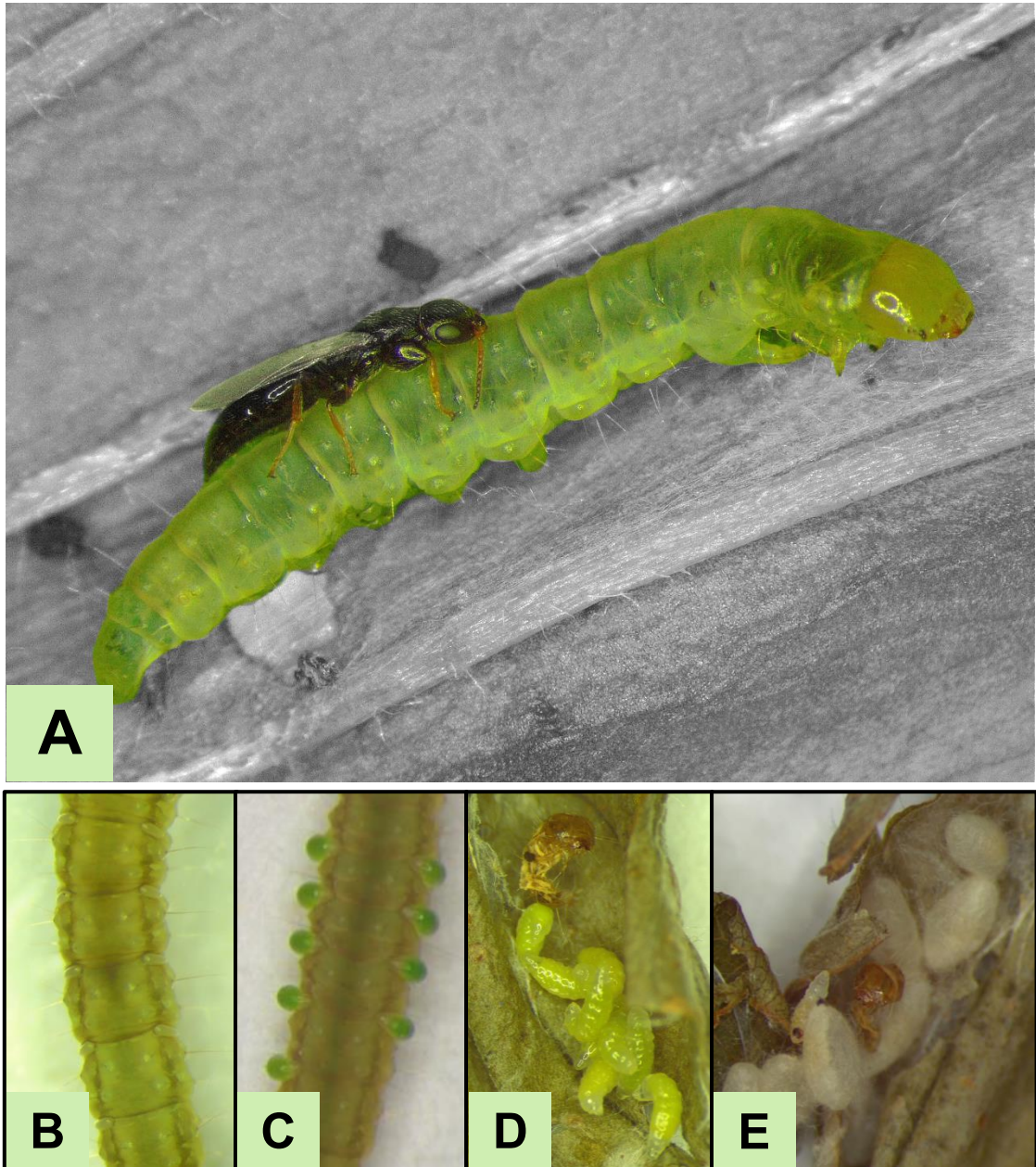


Figure 2

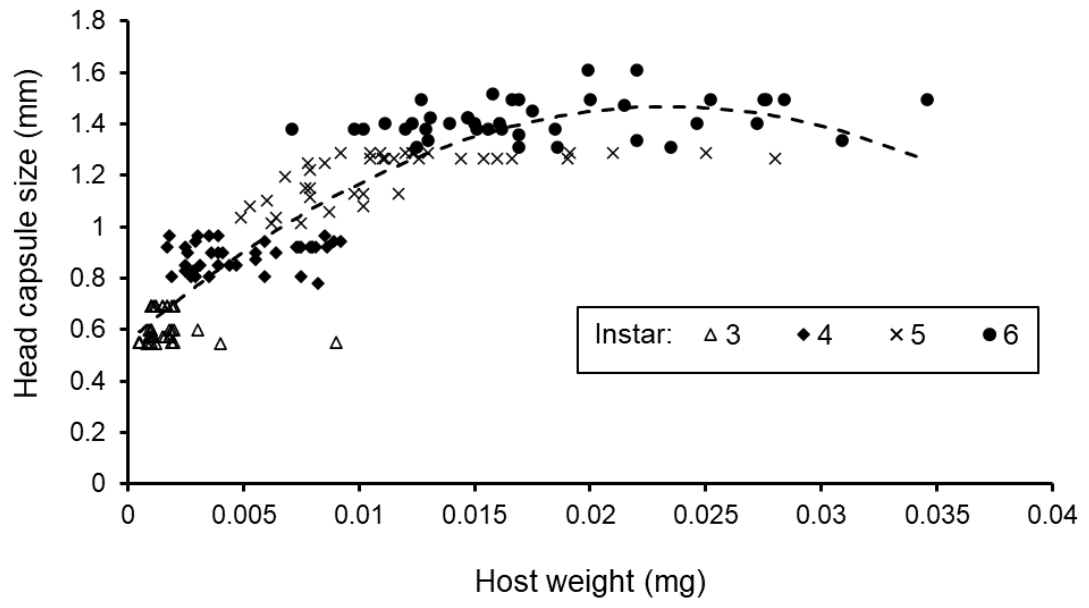


Figure 3

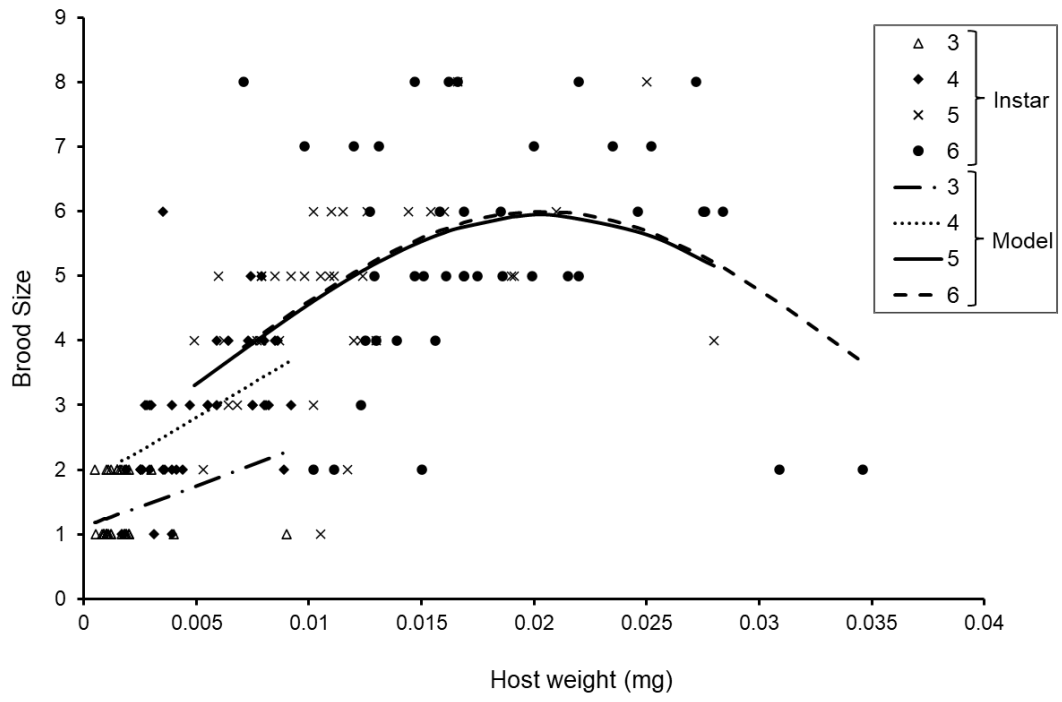


Figure 4

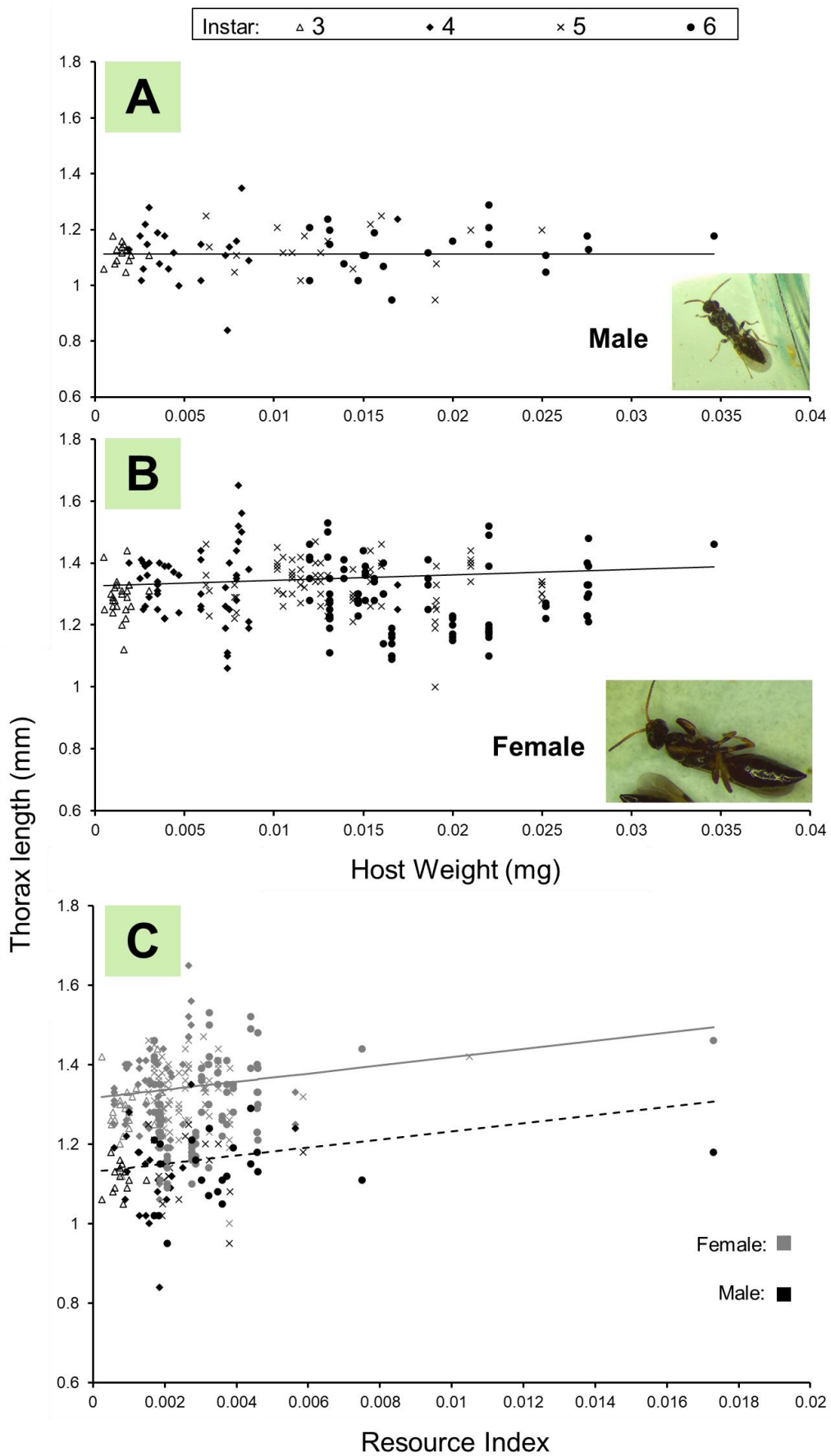
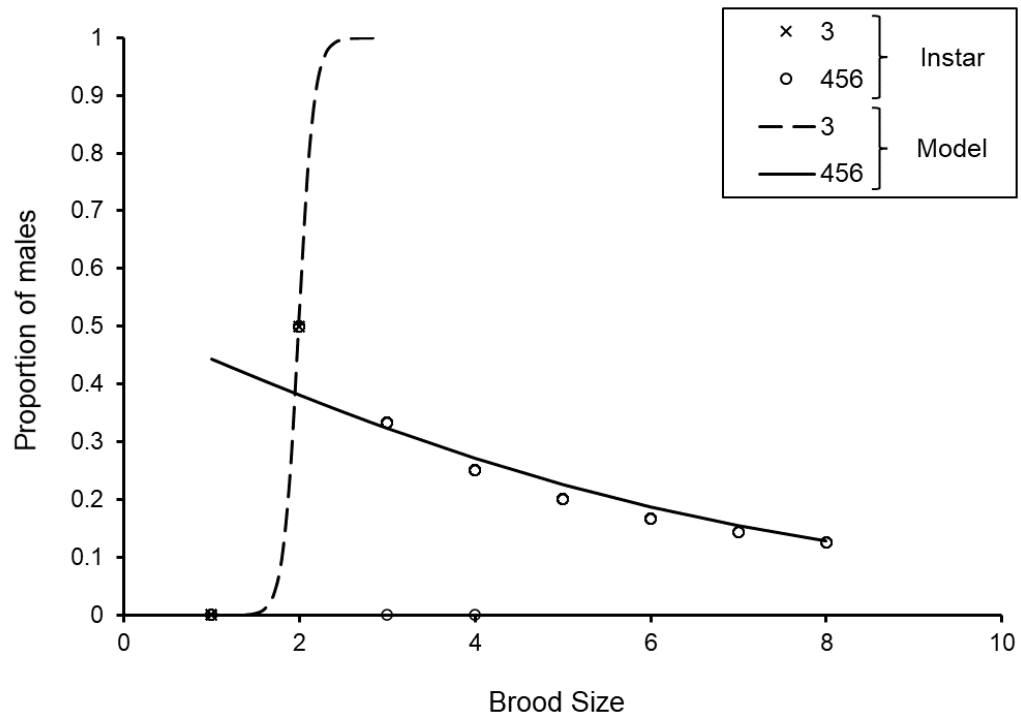


Figure 5



Tables

Table 1. Head capsule widths (mm) of *E. postvittana* reared at 22 °C.

Instar	Mean	Std. Dev.	Range	n
3	0.610	0.059	0.552-0.690	40
4	0.890	0.055	0.782-0.966	40
5	1.203	0.095	1.012-1.288	39
6	1.420	0.075	1.311-1.610	41

Table 2. Sexual composition of realised broods of *Goniozus jacintae*, and a test of sex ratio variance. Values lower than 1 for the variance ratio ‘*R*’ indicate sex ratio precision (less than binomial sex ratio variance). ‘*U*’ is the test statistic from the Meelis test.

Brood Size	Frequency	Frequency of number of males per brood		<i>R</i>	<i>U</i>
		0	1		
1	32	32	-	-	-
2	32	-	32	0.000	-5.523
3	19	1	18	0.081	-3.370
4	21	1	20	0.066	-3.426
5	25	-	25	0.000	-3.893
6	17	-	17	0.000	-3.134
7	6	-	6	0.000	-1.784
8	8	-	8	0.000	-2.073
Overall Totals	160	34	126		
Approx. proportion (%)		21	79		
Overall: <i>R</i> = 0.022, <i>U</i> = -8.77, <i>P</i> < 0.001					

CHAPTER FOUR

Don't judge a 'bug' by its cover: responses of the ectoparasitoid *Goniozus jacintae* to hosts containing the endoparasitoid *Dolichogenidea tasmanica*

Title:

Don't judge a 'bug' by its cover: responses of the ectoparasitoid *Goniozus jacintae* to hosts containing the endoparasitoid *Dolichogenidea tasmanica*

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Short Title:

Bethylid host discrimination behaviour

Key words: Interspecific competition, light brown apple moth, *Epiphyas postvittana*, biological control, parasitoid, bethylid, multiparasitism, host quality, small broods

Statement of Authorship

Title of Paper	Don't judge a 'bug' by its cover: responses of the ectoparasitoid <i>Goniozus jacintae</i> to hosts containing the endoparasitoid <i>Dolichogenidea tasmanica</i>
Publication Status	<input type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input checked="" type="checkbox"/> Unpublished and Unsubmitted work written in manuscript style
Publication Details	Aspin, E., Keller, M.A., and Hardy, I.C.W., 2022.

Principal Author

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Contribution to the Paper	Conceived the study, designed and tested the method, conducted experiments, analysed data, wrote the manuscript.			
Overall percentage (%)	75%			
Certification:	This paper reports on original research I conducted during the period of my higher degree by research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.			
Signature	<table border="1" style="width: 100%;"> <tr> <td style="width: 80%;"></td> <td style="width: 20%;">Date</td> <td>18.04.2022</td> </tr> </table>		Date	18.04.2022
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Assisted with data analysis and manuscript preparation.			
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	Date	19.04.2022		

Name of Co-Author	Michael A. Keller			
Contribution to the Paper	Assisted with design of method, insect culture maintenance, and gave comments on manuscript.			
Signature	<table border="1" style="width: 100%;"> <tr> <td style="width: 80%;"></td> <td style="width: 20%;">Date</td> <td>24 April 2022</td> </tr> </table>		Date	24 April 2022
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Abstract

In nature, multiple parasitoid species often exploit the same stages of a host species, leading to competitive interactions amongst them. These interspecific interactions can influence the size and composition of ecological communities. Some parasitoids can identify whether an encountered host is already parasitised by another species, and may choose to reject or accept it, termed host discrimination. The host discrimination ability of a parasitoid that could potentially serve as a biological control may contribute to its efficacy in suppressing target pest populations. Female *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae) were tested for their ability to discriminate between unparasitised light brown apple moth (LBAM) larvae, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) and LBAM larvae parasitised by *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae). The two parasitoids co-exist in natural habitats, but *D. tasmanica* is numerically dominant in vineyards. *Dolichogenidea tasmanica* parasitises the early larval instars of LBAM, whilst *G. jacintae* parasitises the later instars. Both parasitise the 3rd instar of LBAM, suggesting that interspecific competition may occur. We tested the response of *G. jacintae* to hosts that had been parasitised either 1h or 168h previously by *D. tasmanica*. We also tested different sequences of host treatment exposure to *G. jacintae*. We found that *G. jacintae* attacked larger hosts more and laid more eggs on larger hosts, but that the probability of oviposition on the second host encountered was influenced by parasitism status of both the current and the prior host. Clutch sizes laid on the first host were larger than clutches laid on the second host, but the number of eggs laid on the second host was influenced by host parasitism status. Finally, the proportion of attacks leading to oviposition was highest in unparasitised hosts and lowest in late-stage (168h) *D. tasmanica* parasitised hosts. Collectively, these results

show that *G. jacintae* has some host discrimination abilities and adjust its responses according to prior experience, but does not avoid multiparasitism entirely.

Introduction

After being attacked by a female parasitoid for the first time, a parasitised host may remain *in situ* and be at risk of attack by other foraging parasitoids (Visser *et al.*, 1990; Godfray, 1994; Wajnberg *et al.*, 2008). Foraging parasitoids may encounter hosts of varying quality throughout their lifetime, and are expected to make optimal foraging decisions related to their maximum fitness gain (Godfray, 1994; Nonacs, 2001; Wajnberg *et al.*, 2000). Although it is usually beneficial for a parasitoid to avoid depositing eggs onto hosts that are already parasitised, there are some instances in which it can be an adaptive strategy; this depends on the relative costs and benefits of oviposition, such as whether the availability of unparasitised hosts is high or low (van Alphen and Visser, 1990; Godfray, 1994; Quicke, 1997). Ultimately, a female parasitoid must judge the quality of a host and decide whether or not to utilize it for reproduction, termed host discrimination.

In insect parasitoids, host discrimination is defined as the ability of a female to distinguish between parasitised and unparasitised hosts (van Lenteren, 1981). This behaviour can be categorised into three groups: (1) self-discrimination, where a female recognises hosts parasitised by herself, (2) conspecific discrimination, where a female recognises hosts parasitised by another female of the same species, and (3) allospecific or interspecific discrimination, where a female recognises hosts parasitised by another species (Mackauer, 1990).

Parasitoids are key biological control agents that contribute to the suppression of pest populations in both natural and agricultural ecosystems. Efficient foraging behaviour is

an important determinant of their reproductive potential, and thus their fitness (van Alphen, 1986; Takabayashi *et al.*, 2006). *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), or the light brown apple moth (LBAM), feeds on a variety of crops and other plants (Suckling and Brockerhoff, 2010), and is the most damaging insect pest of grapevines in Australia (Scholefield and Morison, 2010). The parasitoids *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) and *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae) are reported to be the first and second most common parasitoids of LBAM (Danthanarayana, 1983). *Dolichogenidea tasmanica* parasitises the first to third larval instars of LBAM (Paull and Austin, 2006; Yazdani *et al.*, 2015a), whilst *G. jacintae* parasitises the third to sixth instars (Danthanarayana, 1980; Aspin *et al.*, 2021; Aspin *et al.*, unpublished data). Both parasitoids have been successfully reared on another species of tortricid, *Merophyas divulsana* (Walker) (Lepidoptera: Oecophoridae) (Feng *et al.* 2017, H.T. Bui, unpublished data), suggesting that these parasitoids may not be naturally monophagous.

The overlap in host range of the parasitoids indicates that they are competitors, which could influence their abundance in vineyards and other natural habitats. Indeed, *D. tasmanica* is the most commonly collected parasitoid of LBAM in New Zealand and Australia (Charles *et al.*, 1996; Suckling *et al.*, 1998; Paull and Austin, 2006), and it has been noted that the prevalence of *G. jacintae* in South Australian vineyards is much lower in comparison to *D. tasmanica* (M.A Keller, E Aspin, pers. obs.). This population size difference may be due to factors involving the ability of each parasitoid species to persist in a commercial vineyard setting, e.g. whether ample nectar sources or alternative hosts are available. However, the difference in abundance between these species may also relate to differences in the stages (larval instars) of hosts attacked, and how they respond to hosts that have already been encountered and parasitized.

This study investigates the response of the gregarious ectoparasitoid *G. jacintae* to larvae of the light brown apple moth, *E. postvittana*, that are either unparasitised or previously parasitised by the solitary endoparasitoid, *D. tasmanica*. *Goniozus jacintae* and *D. tasmanica* both parasitise the 3rd instar of LBAM (Danthanarayana, 1980; Paull and Austin, 2006; Yazdani *et al.*, 2015a; Aspin *et al.*, 2021). We thus investigated the response of *G. jacintae* to unparasitised hosts versus hosts parasitised by *D. tasmanica* at the 3rd instar stage. As parasitoids may come across hosts containing allospecific parasitoids at different stages of development in the field, we tested hosts that had been parasitised at two different time points: 1h post-parasitisation and 168h post-parasitisation by *D. tasmanica*. Experience is known to influence parasitoid foraging behaviour (Vet and Groenewold, 1990; Visser *et al.*, 1992; Turlings *et al.*, 1993; Le Lann *et al.*, 2008), thus we also tested different sequences of host treatment exposure to *G. jacintae*.

Materials and methods

Insect rearing

The culture of *E. postvittana* (LBAM) used in this experiment was established at the South Australian Research and Development Institute in 1994 and has since been maintained with annual additions of wild moths. LBAM was reared on an artificial diet at 22 ± 2 °C under 12L:12D photoperiod, following methods reported in Yazdani *et al.* (2015a).

The cultures of *G. jacintae* and *D. tasmanica* were established from individuals reared from parasitised LBAM that were collected in vineyards at McLaren Vale, South Australia in 2017. Parasitised *E. postvittana* larvae were collected at least once every 2 months from the field, and adult parasitoids emerging from these larvae were added to the cultures to maintain genetic diversity. The wasp cultures were reared at 23 ± 2 °C, 14L:10D in cages on larval LBAM that infested plantain, *Plantago lanceolata* L. (Lamiales: Plantaginaceae). Adult wasps were provided with water and honey *ad libitum*. Wasp cocoons were isolated in 50 mm × 18 mm diam. glass vials containing a drop of honey and fitted with caps that had screens for ventilation. Upon emergence, females were caged serially, 2-5 at a time, with 5 males to allow mating, and then re-isolated and held in vials for at least 1 h before being used in experiments.

Host discrimination

An experiment was conducted to determine whether hosts parasitised by *D. tasmanica* were equally attractive to ovipositing *G. jacintae* compared to unparasitised larvae, and whether time interval or order of exposure influenced host discrimination ability. The experimental treatments were larval status (parasitised by *D. tasmanica* or unparasitised), sequential order, and time between parasitisation by *D. tasmanica* and exposure to *G.*

jacintae (1h or 1 week/168hrs). These time intervals were chosen to represent the condition of the host instantly after parasitisation by *D. tasmanica* versus when a *D. tasmanica* egg has hatched and a single parasitoid larva is established within the host.

One larva feeding on a plantain leaf was exposed to a 1-2 day old *D. tasmanica* female in a 50 mm × 18 mm diam. glass vial. When the parasitoid had stung the larva and been removed, the larva was left in the vial with a fresh plantain leaf for 1h or 168h depending on the time treatment and kept in an insectary at 23 ± 2 °C, 14L:10D before use in experiments.

1 day old naïve *G. jacintae* females were presented with individual larval LBAM that had been parasitised by *D. tasmanica* after 1h or 168h depending on the treatment, in a 50 mm × 18 mm diam. glass vial containing a drop of honey. The rate of attack (attacked larva or rejected larva) and number of eggs laid on the host were recorded. After the encounter, the larva was removed, and the female wasp was kept and presented with an unparasitised host after 1h. This process was repeated in different sequential orders (unparasitised larva first or *D. tasmanica* parasitised larva first) and with different time treatments (*D. tasmanica* parasitised 1h or 168h prior to exposure).

A control, where an unparasitised larva was exposed to a *G. jacintae* female, followed by another unparasitised larva, was also conducted. A pair of healthy 3rd instar LBAM larvae of similar size were chosen for each replicate, and their weights measured. Larval LBAM that were parasitised by *D. tasmanica* were dissected after the experiment to ensure that an egg or larva could be found, and that female wasps were fertile. A *D. tasmanica* wasp egg was found in 98 out of 100 dissected larvae (98%), indicating that eggs could be found with a high level of confidence. Twenty five replicates were conducted for each interval (a total of 150 replicates, including controls).

Statistical Analysis

Data were analysed using generalised linear modelling techniques (Dobson, 1983; McCullagh and Nelder, 1983; Aitkin *et al.*, 1989), specifically logistic and log-linear analyses of covariance (ANCOVA), which allow for the parametric analysis of data with non-normal distributions of errors. When response variables were binary (e.g. the behaviour was either present (= 1) or absent (= 0)), a binomial distribution of errors was assumed. For analyses of small integer response variables, quasi-Poisson distributions or errors were assumed. After the maximal model, including all measured explanatory variables, was fitted, a stepwise model simplification process was used to assess significance as variables were excluded, until the minimum adequate model was obtained. For logistic models, the significance of each variable was assessed by change in deviance, G , which approximates to the chi-squared distribution. For log-linear models, significance was assessed by the F-ratio. Descriptions of abbreviations for each variable can be found in Table 1. Different models were conducted to consider parasitoid responses to the first host only, then the second host in combination with the known conditions of the first host. Differences were considered significant at $P < 0.05$.

A life table approach (Varley and Gradwell, 1960; Kidd and Jervis, 2005; Malabusini *et al.*, 2022) was used to identify how failures to progress between successive stages of brood production contributed to the overall probability of reproductive failure.

Results

Reproductive failure

Out of 300 LBAM presented to *G. jacintae* in this experiment, 268 were attacked, and 227 were then oviposited on (Table 2). The stage of failure that contributed most to the overall probability of failure differed across treatments: for unparasitised and 1h *D. tasmanica* parasitised hosts it was the paralysis stage, and for 168h *D. tasmanica* parasitised hosts it was the oviposition stage (Table 2).

The treatment which received the highest percentage of hosts attacked and paralysed was 168h *D. tasmanica* parasitised hosts, but the treatment which received the highest frequency of oviposition on hosts after attacks was unparasitised hosts (Table 2). It is important to note that LBAM parasitised by *D. tasmanica* at the 168h post-oviposition mark would have continued to grow and develop at a reduced rate for one week, making them the largest hosts available to parasitoids in the experiment.

First host encounter

The probability of *G. jacintae* attacking and paralysing *Host1* ($n = 150$) (see Table 1 for descriptions of abbreviations) was significantly influenced by host size (logistic ANCOVA, *Host1Wt*: $F_{(1,148)} = 15.95$, $P < 0.01$), but not by host parasitism status (*Status1*: $F_{(1, 148)} = 0.29$, $P = 0.588$) or the interaction between these two main effects ($F_{(2, 147)} = 1.77$, $P = 0.184$).

The probability of *G. jacintae* ovipositing onto *Host1* that had been paralysed ($n = 128$) was not influenced by host size (logistic ANCOVA, *Host1Wt*: $F_{(1,55)} = 1.16$, $P = 0.282$), status (*Status1*: $F_{(0,56)} = 1.64$, $P = 0.200$), or any interaction between the two ($F_{(2,$

$_{54}) = 0.16, P = 0.689)$ for the 1h time interval ($n = 57$). However, the likelihood of oviposition onto *Host1* was influenced by host size (*HostWt1*: $F_{(2, 68)} = 5.27, P = 0.022$) and host status (*Status1*: $F_{(1, 69)} = 6.75, P = 0.009$), but not an interaction between the two ($F_{(2, 68)} = 0.00, P = 0.990$) for the 168h time interval ($n = 71$).

When eggs were laid onto *Host1* ($n = 118$) the number was influenced by host size (log-linear ANCOVA, *Host1Wt*: $F_{(1, 50)} = 28.34, P < 0.001$), but not host status (*Status1*: $F_{(1, 50)} = 0.01, P = 0.943$) nor the interaction between them ($F_{(2, 49)} = 1.6, P = 0.212$) for the 1h time interval ($n = 52$). Further, the number of eggs laid onto *Host1* was influenced by host size (*Host1Wt*: $F_{(2, 63)} = 28.53, P < 0.001$), and the interaction between them ($F_{(2, 63)} = 13.27, P < 0.001$), but not host status (*Status1*: $F_{(2, 63)} = 0.33, P = 0.568$), for the 168h time interval ($n = 66$).

Second host encounter

Step-wise logistic regression allowed for the analysis of *G. jacintae* behaviours towards *Host2* whilst considering the characteristics of the present host, the previous host, and the responses of the parasitoid to the previous host (*Host1*). Out of 150 *G. jacintae* females observed from all replicates, 129 attacked *Host2*, and 109 oviposited onto *Host2*.

Goniozus jacintae were more likely to attack larger hosts; the model identified the weight of the host (*Host2Wt*) as a significant predictor of attack by *G. jacintae* during the second host encounter, and *Status1*, or status of the first host presented, was marginally non-significant (Table 3; Fig. 1).

The probability of *G. jacintae* laying eggs was influenced by host status; the likelihood of oviposition onto *Host2* was significantly influenced by whether or not the parasitoid had attacked the first host or not (*Attk1*) and the parasitism status of the second

host (*Status2*). *Host2Wt* was marginally non-significant (Table 4). The proportion of hosts oviposited on was highest for unparasitised hosts (*UnP*), followed by hosts parasitised by *D. tasmanica* 1h prior to exposure to *G. jacintae* (*EarlyP*), and then hosts parasitised 168h prior to exposure (*LateP*) (Fig. 2).

The number of eggs *G. jacintae* laid on *Host2* was found to be influenced by numerous factors: the parasitism status of both the first and second host encountered (*Status1*, *Status2*; Table 5, Fig. 3), whether or not the parasitoid had oviposited upon the first host and if so, the number of eggs laid (*Ovip1*, *NumberEggs1*; Table 5), and the weight of the second host (*Host2Wt*; Table 5; Fig. 4). Overall, the number of eggs laid on the first host encountered was higher than the number of eggs laid on the second host (Fig. 5).

Discussion

In recent years there have been numerous publications on *D. tasmanica* relating to its efficacy as a parasitoid of LBAM, including its host discrimination ability (Suckling *et al.*, 2012; Yazdani *et al.*, 2014; Feng *et al.*, 2015; Yazdani and Keller, 2015; Yazdani *et al.*, 2015b; Yazdani *et al.*, 2015a; Yazdani and Keller, 2016). However, despite its (albeit less common) occurrence as a beneficial insect, there is little equivalent knowledge of the efficacy of *G. jacintae* in controlling LBAM (Danthanarayana, 1980; Paull and Austin, 2006; Aspin *et al.*, 2021; Aspin *et al.*, unpublished data), and no prior information on its interactions with *D. tasmanica*, such as its ability to discriminate between unparasitised and previously parasitised hosts.

Goniozus jacintae are more likely to attack larger hosts and also lay more eggs on larger attacked hosts. This is consistent with previous reports that *G. jacintae* show a preference for larger hosts whilst foraging (Aspin *et al.*, 2021; Aspin *et al.*, unpublished data), most likely due to larger hosts providing a greater quantity of nutritional resource for developing parasitoid larvae (Godfray, 1994; Mackauer *et al.*, 1997; Aspin *et al.*, unpublished data).

The likelihood of oviposition on the second host encountered in the experiment was influenced by its parasitism status. Hosts that had been parasitised by *D. tasmanica* 168h before being exposed to *G. jacintae* were oviposited on the least frequently, followed by hosts parasitised by *D. tasmanica* 1h before exposure, with unparasitised hosts being the most frequently oviposited on. This trend is reflected in the proportion of attacks leading to oviposition; 168h before exposure hosts had the lowest prevalence of attacks leading to oviposition, followed by 1h before exposure hosts and then unparasitised hosts. A likely explanation is that the parasitoid attacks an encountered

hosts based primarily on their size, with a preference for larger hosts. The parasitoid is then able to examine the paralysed host, subsequently either accepting or rejecting the host for oviposition depending on further aspects of its quality (e.g. parasitism status). This would explain why the majority of hosts that were parasitized 168h beforehand were attacked but then were not oviposited upon: these were the largest hosts but also the ones containing the most developed *D. tasmanica* larva.

Goniozus jacintae oviposition behaviour was influenced by host parasitism status. Not only did the status of the current host influence the number of eggs laid on it, but the status of prior hosts encountered also influenced current clutch size decisions. This indicates that *G. jacintae* exhibits some degree of learning, adjusting decisions according to prior experience. Host discrimination is generally achieved via the detection of internal or external cues left by the first parasitoid and/or their offspring, such as marking pheromones or internal host quality changes associated with parasitism (Vinson, 1976; Mackauer, 1990; Godfray, 1994; Ruschioni *et al.*, 2015). In the present case, *G. jacintae* females are likely to be able to detect chemical differences between more and less recently parasitized hosts, for instance via chemical cues in the host's haemocoel (*Goniozus* species and other bethylids are commonly observed to feed on host fluids accessed by puncturing the integument with their mandibles, e.g. Pérez-Lachaud *et al.*, (2004); Lupi *et al.*, (2017), or changes to the composition of the hosts integument.

Goniozus jacintae laid more eggs on hosts that had been parasitised by *D. tasmanica* 168h prior to encounter compared to hosts parasitised by *D. tasmanica* 1h prior to encounter, but laid most eggs on unparasitised hosts. This could be an attempt by the wasp to generate a greater number of parasitoid larvae, overwhelming the established endoparasitoid within the host (Cusumano *et al.*, 2016), as well as the fact that 168h parasitised hosts were the largest the wasp came across in this study. However,

multiparasitism may also be due to the lack of alternative hosts for the confined female. In the case of an endoparasitoid and an ectoparasitoid developing on the same host, the ectoparasitoid will typically have a higher probability of outcompeting the endoparasitoid (Flanders, 1971; Godfray, 1994; Harvey *et al.*, 2013). However, some multiparasitised hosts were kept for a short time after the experiment and all died, with no parasitoids of either species emerging (E. Aspin, unpublished data). Thus, despite a 168h parasitised host appearing to be the highest quality host available, due to their size, they are in fact an unsuitable host for the development of *G. jacintae* offspring. This may be an example of an ‘evolutionary trap’ (Mesterton-Gibbons *et al.*, 2021), where host attributes that would otherwise indicate high suitability for *G. jacintae* development instead result in developmental mortality.

Feng *et al.* (2015) reported that larvae of *D. tasmanica* outcompeted those of the endoparasitoid *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae), irrespective of order or time interval between attacks, and host larvae attacked by both parasitoids died more frequently before a juvenile parasitoid completed its development. Similar to *G. jacintae*, the prevalence of *T. unimaculatus* in vineyards is lower than that of *D. tasmanica*, as *T. unimaculatus* occurs mainly in adjacent native vegetation (Feng *et al.*, 2017). As *D. tasmanica* is more prevalent in vineyards and parasitises the early larval instars of LBAM, a *G. jacintae* female, which parasitises the later instars of LBAM, may come across hosts already parasitised by *D. tasmanica* at various stages of development. If the encounter results in multiparasitism of the host, it is likely that smaller hosts could die from the injection of venom or mechanical injury caused by two parasitoid attacks (Cebolla *et al.*, 2018), and if not, they may succumb to overexploitation induced by scramble competition of multiple parasitoid larvae feeding on them, such that neither parasitoid species is able to complete its development. This could explain why *G. jacintae*

are the less common species in vineyards, as there may not be enough suitable (unparasitised mid- to late-instar) LBAM larvae to sustain large populations. Moreover, as both parasitoids in this study can be laboratory-reared on alternative hosts, such as the tortricid *M. divulsana* (M.A. Keller, H.T. Bui, unpublished data) it can be hypothesised that *D. tasmanica* may also dominate the parasitism of other suitable hosts for *G. jacintae* such that there is no refuge from potential multiparasitism interactions.

As discussed by Bakker *et al.*, (1985), from a functional point of view, multiparasitism behaviour is surprising; if ovipositing into a previously parasitised host is not beneficial to a parasitoid's reproduction, natural selection might be expected to promote the mutual recognition of cues, such as host marking pheromones, and the subsequent avoidance of multiparasitism in the field. In this experiment, *G. jacintae* was able to adjust its oviposition behaviour in response to prior experience with *D. tasmanica* parasitised hosts. Despite this, many eggs were oviposited onto unsuitable hosts. Ultimately, if *G. jacintae* encounter hosts parasitised by *D. tasmanica* from the earlier instar range, the parasitoid can only reject the host, retain its eggs and continue to forage, or multiparasitise the host, depleting its supply of mature eggs and resulting in the likely death of all parasitoid offspring and the host itself. The means by which *G. jacintae* populations may persist is for females to find hosts that have not been found and parasitised by *D. tasmanica* and that have developed into larger instars. The fact that *G. jacintae* does not avoid multiparasitism altogether may indicate limited host discrimination ability but it is important to consider that the behaviour of *G. jacintae* females under laboratory conditions may differ from their behaviour in the field; host discrimination and host rejection could be more common when hosts are abundant and when females are free to leave a host and forage elsewhere.

Understanding how interspecific competition affects pest suppression is a key aspect for biological control (Boivin and Brodeur, 2006), and it is important to consider the fitness-related effects of interspecific competition between two potential biocontrol agents. Further study is required to elucidate the population-level consequences of multiparasitism between *D. tasmanica* and *G. jacintae*, such as choice tests to determine how *G. jacintae* responds to a selection of unparasitised or parasitised hosts. However, it is likely that the common parasitism of early instar hosts by *D. tasmanica* severely restricts the abundance of *G. jacintae* in the field and also that multiparasitism by *G. jacintae* reduces population recruitment of *D. tasmanica*.

Acknowledgements

We thank Tom Reader for discussion of the statistical analysis. The culture of LBAM was obtained from the South Australian Research and Development Institute (SARDI). E.A. was funded by the University of Nottingham - University of Adelaide joint PhD program.

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Figure legends

Figure 1: The relationship between the proportion of *Host2* attacked and paralysed and the weight of *Host2*.

Figure 2: The proportion of *Host2* oviposited on given the *Status* of the second host being: unparasitised (*UnP*), parasitised by a *Dolichogenidea tasmanica* 1h prior to exposure to *Goniozus jacintae* (*EarlyP*), or parasitised by *D. tasmanica* 168h prior to exposure to *G. jacintae* (*LateP*).

Figure 3: Mean number of eggs laid on *Host2* given the *Status* of the first (*Status1*) or second (*Status2*) host being: unparasitised (*UnP*), parasitised by a *Dolichogenidea tasmanica* 1h prior to exposure to *Goniozus jacintae* (*EarlyP*), or parasitised by *D. tasmanica* 168h prior to exposure to *G. jacintae* (*LateP*).

Figure 4: The relationship between the mean number of eggs laid on *Host2* and the weight of *Host2*.

Figure 5: The relationship between the mean number of eggs laid on *Host1* (*NumberEggs1*) and the mean number of eggs laid on *Host2* (*NumberEggs2*). Dashed line signifies a line of equivalence.

Figure 1

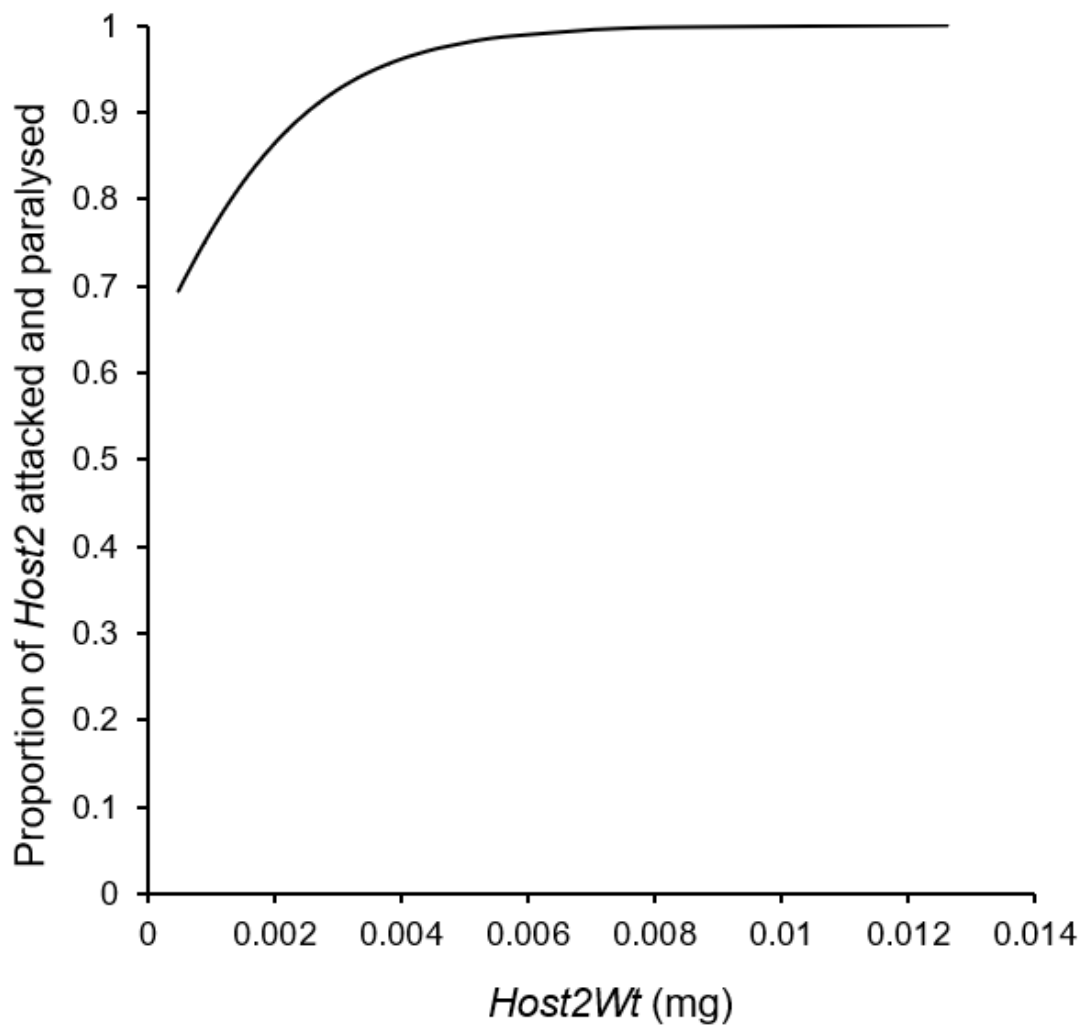


Figure 2

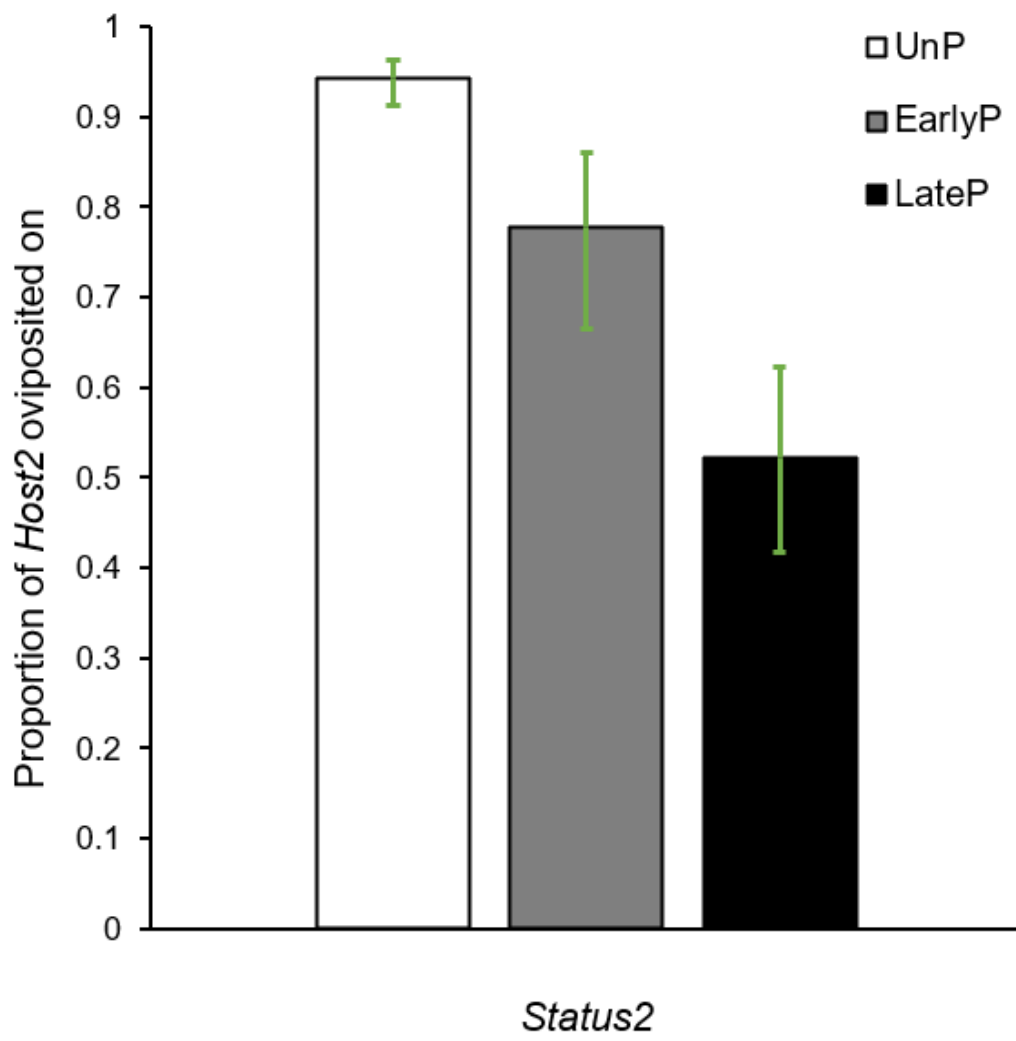


Figure 3

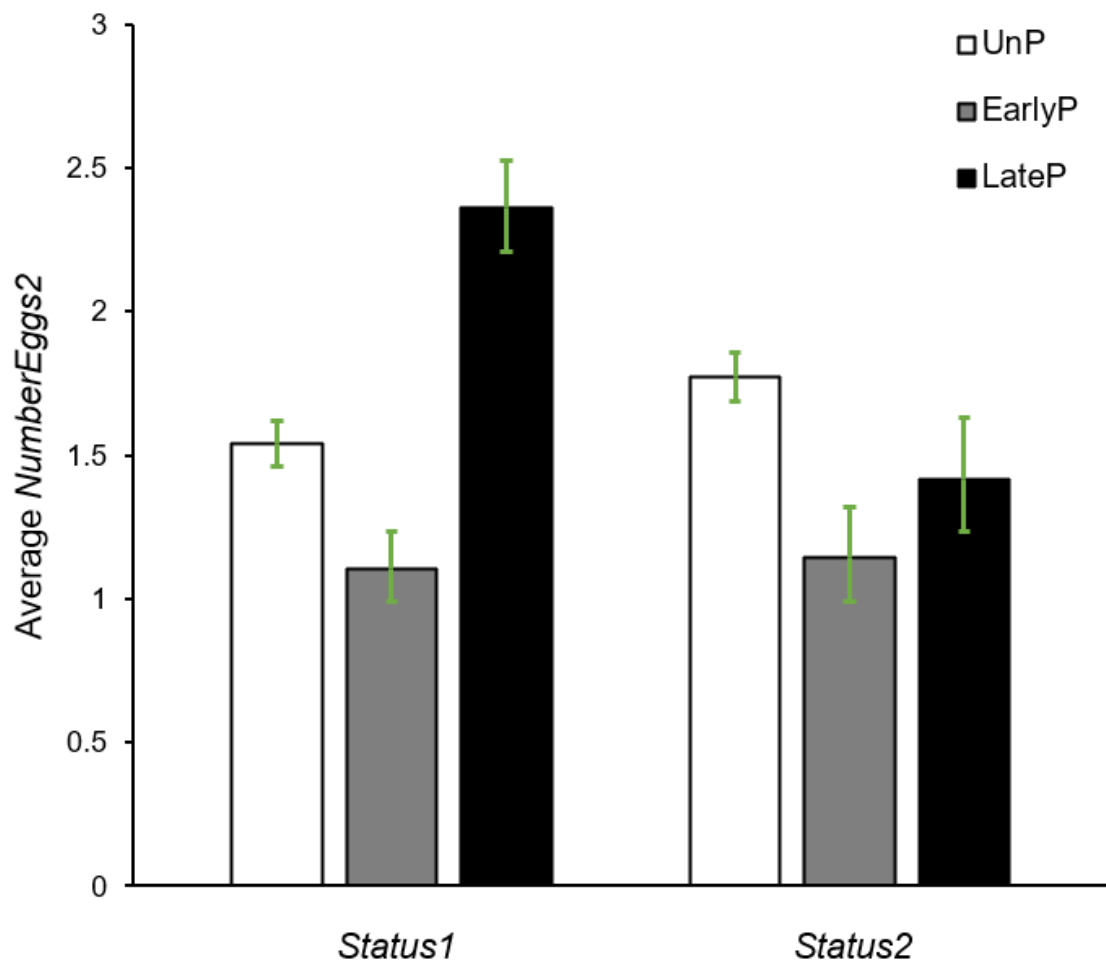


Figure 4

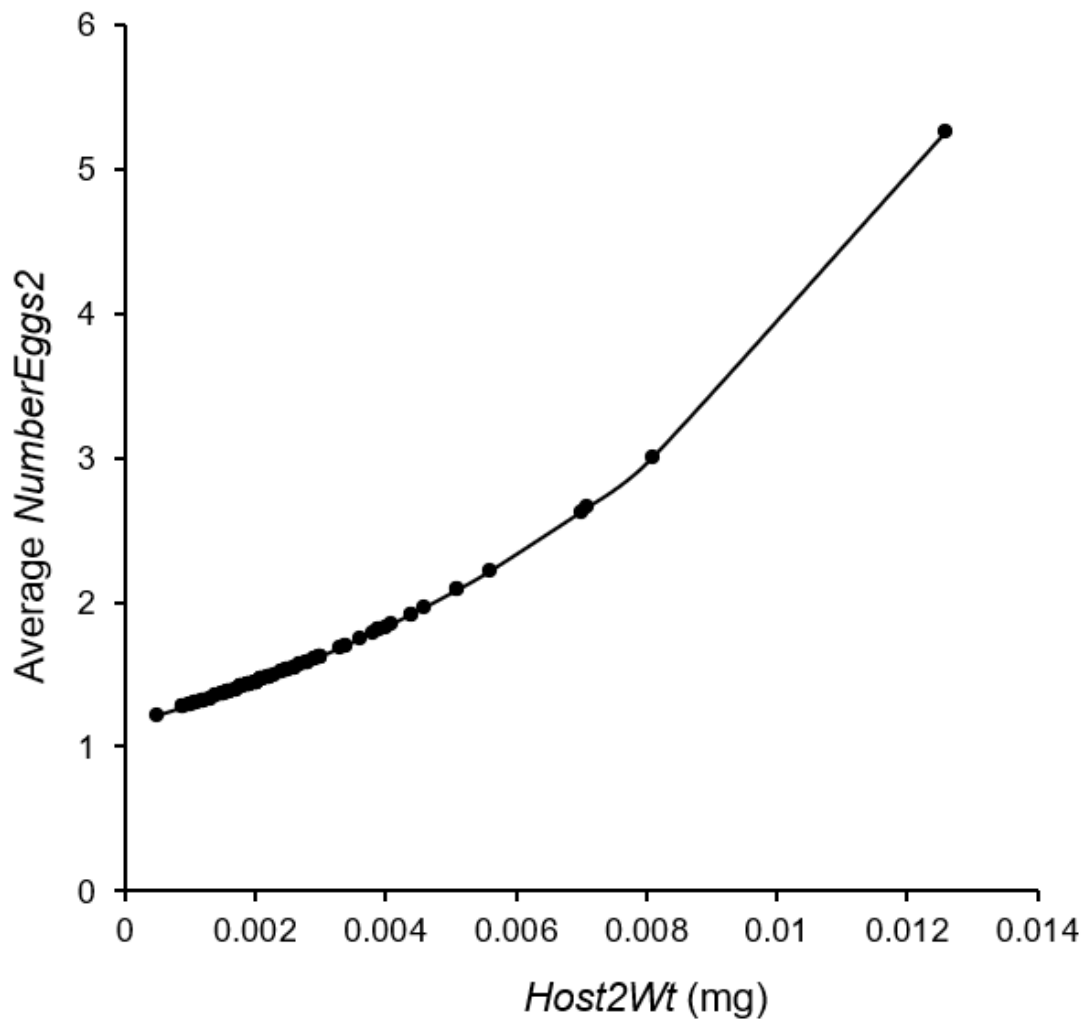
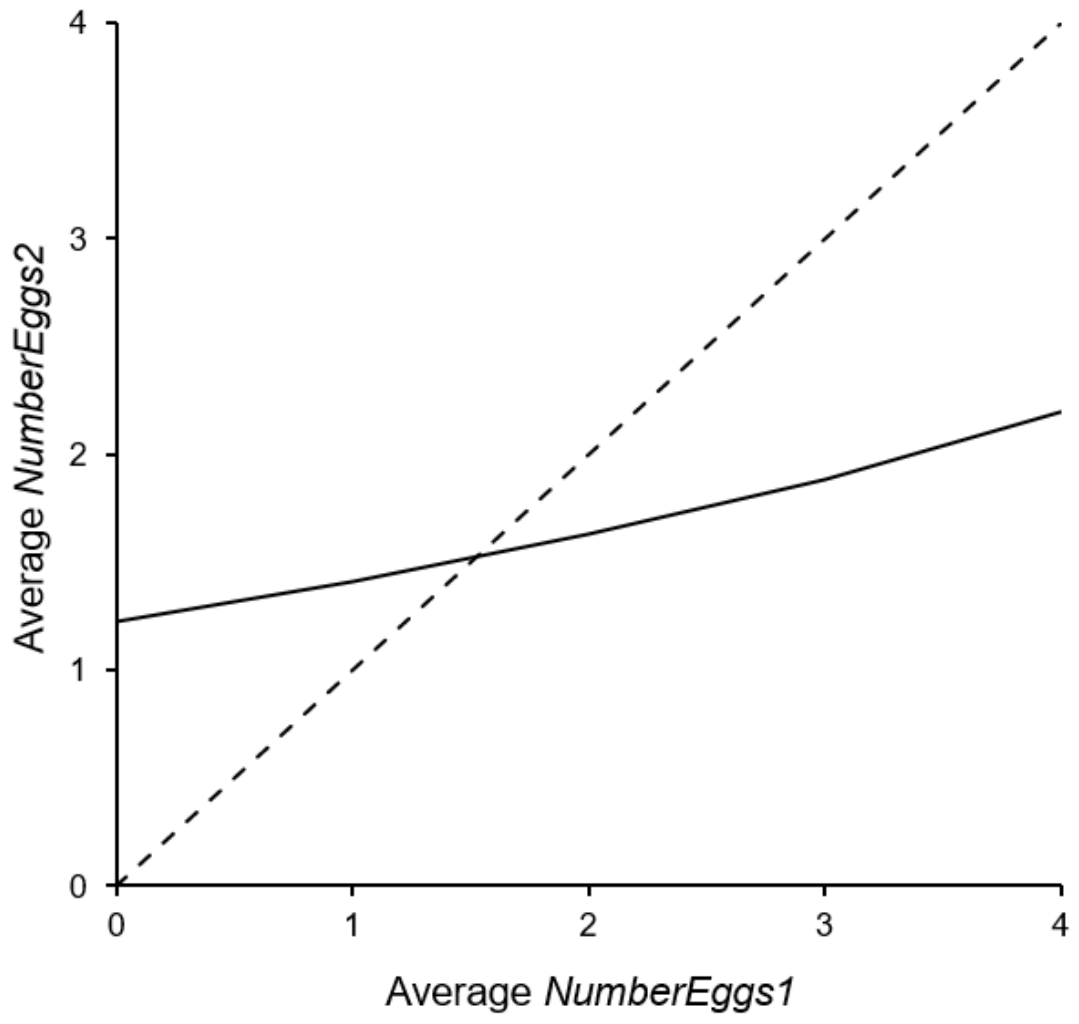


Figure 5



Tables

Table 1. Definitions of abbreviations

Abbreviation	Description
<i>Host1</i>	First LBAM larva presented to <i>G. jacintae</i> in host discrimination experiment
<i>Host1Wt</i>	Weight of first host presented to <i>G. jacintae</i>
<i>Status1</i>	Status of first LBAM larva presented, one of the following: unparasitised (<i>UnP</i>), 1h <i>D. tasmanica</i> parasitised (<i>EarlyP</i>), or 168h <i>D. tasmanica</i> parasitised (<i>LateP</i>)
<i>Attk1</i>	Whether or not <i>Host1</i> was attacked by <i>G. jacintae</i>
<i>Ovip1</i>	Whether or not <i>Host1</i> was oviposited upon by <i>G. jacintae</i>
<i>NumberEggs1</i>	The number of eggs oviposited onto <i>Host1</i> by <i>G. jacintae</i>
<i>Host2</i>	Second LBAM larva presented to <i>G. jacintae</i> in host discrimination experiment
<i>Host2Wt</i>	Weight of second host presented to <i>G. jacintae</i>
<i>Status2</i>	Status of second LBAM larva presented, one of the following: unparasitised (<i>UnP</i>), 1h <i>D. tasmanica</i> parasitised (<i>EarlyP</i>), or 168h <i>D. tasmanica</i> parasitised (<i>LateP</i>)
<i>Attk2</i>	Whether or not <i>Host2</i> was attacked by <i>G. jacintae</i>
<i>Ovip2</i>	Whether or not <i>Host2</i> was oviposited upon by <i>G. jacintae</i>
<i>NumberEggs2</i>	The number of eggs oviposited onto <i>Host2</i> by <i>G. jacintae</i>

Table 2. Life table of *G. jacintae* host acceptance. The number of larvae at each stage of acceptance that failed to be parasitised were used to obtain *k*-values (*k*₁ and *k*₂), calculated as $k = (\log_{10} \text{ number before failure} - \log_{10} \text{ number after failure})$, which indicate the probability of failure of parasitism during successive stages of host acceptance (for each treatment and also overall). *K* is the sum of contributions of failures at each stage to the overall probability of failure ($K = k_1 + k_2$). ‘*’ indicates the stage of failure that contributed most to the overall failure for each treatment and also overall.

Treatment	Hosts presented	Hosts paralysed	% hosts not paralysed	<i>k</i> ₁ (paralysis failure)	Hosts oviposited on	% hosts not oviposited on	<i>k</i> ₂ (oviposition failure)	<i>K</i> (overall reproductive failure)
Unparasitised LBAM	200	173	13.5	0.063*	166	4.05	0.018	0.081
LBAM parasitised by <i>D. tasmanica</i> – 1h	50	38	24	0.119*	30	21.05	0.103	0.222
LBAM parasitised by <i>D. tasmanica</i> – 168h	50	47	6	0.027	31	34.04	0.181*	0.208
Overall	300	268	10.67	0.049	227	15.30	0.072*	0.121

Table 3. Factors effecting the probability of *attack* on the *second* host presented. G = change in deviance. This analysis used all replicates ($n = 150$). Variables that were significant when the model was fitted are marked with a ‘*’. See Table 1 for definitions of the abbreviation for each variable.

Variable	GLM Logistic Model		
	df	G	P (approx.) [†]
<i>Characteristics of 1st host</i>			
<i>Host1Wt</i>	1	0.00	0.997
<i>Status1</i>	2	2.59	0.075 [†]
<i>Response to 1st host</i>			
<i>Attk1</i>	1	1.07	0.301
<i>Ovip1</i>	1	0.02	0.892
<i>NumberEggs1</i>	1	0.00	0.971
<i>Characteristics of 2nd host</i>			
<i>Host2Wt</i>	1	7.28	0.007*
<i>Status2</i>	2	0.79	0.455

[†] = *Status1* is marginally non-significant. Values given by logistic analyses are not exact (Crawley, 1993; Warton & Hui, 2011), suggesting possible biological significance.

Table 4. Factors effecting the probability of *oviposition* on the *second* host presented. G = change in deviance. This analysis used replicates where all second hosts had been attacked ($n = 129$). Variables that were significant when the model was fitted are marked with a '*'. See Table 1 for definitions of the abbreviation for each variable.

Variable	GLM Logistic Model		
	df	G	P (approx.) [†]
<i>Characteristics of 1st host</i>			
<i>Host1Wt</i>	1	0.08	0.777
<i>Status1</i>	2	1.29	0.274
<i>Response to 1st host</i>			
<i>Attk1</i>	1	10.02	0.002*
<i>Ovip1</i>	1	0.37	0.541
<i>NumberEggs1</i>	1	0.02	0.890
<i>Characteristics of 2nd host</i>			
<i>Host2Wt</i>	1	3.15	0.076 [†]
<i>Status2</i>	2	14.38	<0.001*

[†] = *Host2Wt* is marginally non-significant. Values given by logistic analyses are not exact (Crawley, 1993; Warton & Hui, 2011), suggesting possible biological significance.

Table 5. Factors effecting the *number of eggs laid* on the *second* host presented. G = change in deviance. This analysis used replicates where all second hosts had at least one egg laid onto them ($n = 109$). Variables that were significant when the model was fitted are marked with a '*'. See Table 1 for definitions of the abbreviation for each variable.

Variable	GLM Log-Linear Model		
	df	F	P (approx.) [†]
<i>Characteristics of 1st host</i>			
<i>Host1Wt</i>	1, 108	2.77	0.099
<i>Status1</i>	2, 108	15.91	<0.001*
<i>Response to 1st host</i>			
<i>Attk1</i>	1, 108	0.07	0.796
<i>Ovip1</i>	1, 108	4.57	0.035*
<i>NumberEggs1</i>	1, 108	11.69	<0.001*
<i>Characteristics of 2nd host</i>			
<i>Host2Wt</i>	1, 108	6.73	0.011*
<i>Status2</i>	2, 108	3.86	0.024*

CHAPTER FIVE

General Discussion and Conclusions

Summary of Results

The Research Objectives of this thesis were to:

Part One: Provide a foundation of knowledge for the behavioural ecology of *Goniozus jacintae*, a relatively unstudied parasitoid of the Light Brown Apple Moth (LBAM) (Chapters 2 & 3).

Part Two: Investigate the interspecific interactions between *Dolichogenidea tasmanica* and *G. jacintae*, two common parasitoids of LBAM, when competing for the same host (Chapter 4).

Findings: Part One

***G. jacintae* exhibits host-stage dependent foraging behaviour towards LBAM:** pre- and post-flight behaviours were different and varied according to host instar, flight duration was shortest when exposed to 5th instar larvae, the newly described *slow walking* behaviour was only exhibited in close proximity to a potential host and was seen more around larger hosts (Chapter 2).

***G. jacintae* has a preference for larger instars of LBAM:** female parasitoids produced bigger broods on larger hosts (with the highest number of individuals being reared on 6th instar), brood sex ratios were female biased with extremely low variance, body size of offspring was positively related to the amount of host resource available, suggesting scramble competition occurs among feeding larvae (Chapter 3).

Findings: Part Two

***G. jacintae* exhibits some learning and host discrimination abilities towards hosts parasitised by *D. tasmanica*:** the probability of oviposition on the second host encountered was influenced by parasitism status of both the current and previous host. Clutch size laid on the second host was influenced by parasitism status, and the proportion of attacks leading to oviposition was highest in unparasitised hosts and lowest in late-stage *D. tasmanica* parasitised hosts. *Goniozus jacintae* laid more eggs on larger hosts and laid more eggs on the first host encountered, suggesting they do not avoid multiparasitism entirely (Chapter 4).

Each of the preceding empirical chapters have their own discussions, so the aim of this chapter is to integrate the components of this thesis and discuss potential avenues for further study, specifically subjects that I had aimed to address when I initially designed this project.

Goniozus jacintae as a parasitoid of LBAM

Findings from this study indicate that *G. jacintae* is able to parasitise the 3rd-6th larval instars of LBAM and exhibits varied foraging behaviours depending on the size of the host it is presented with. The parasitoid prefers larger hosts and is capable of producing up to 8 offspring from a brood. *Goniozus jacintae* sex ratios are female biased with extremely low variance, which increases the number of female offspring recruited to the next generation. Finally, *G. jacintae* shows learning and host discrimination behaviour towards hosts parasitised by *D. tasmanica* but does not avoid multiparasitism altogether.

There are some contrasting indications from these results to consider when evaluating the efficacy of *G. jacintae* as a potential biological control agent of LBAM. Whilst *G. jacintae* is effective at parasitising LBAM in the laboratory, there are interspecific interactions between *G. jacintae* and *D. tasmanica* that could be influencing the prevalence of *G. jacintae* in the field. As discussed in Chapter 4, the fact that *D. tasmanica* parasitises early instars of LBAM may leave few suitable hosts for *G. jacintae* to attack in a vineyard ecosystem. Therefore, whilst this study has concluded, the overarching investigation of *G. jacintae* as an effective biocontrol agent has not.

I will now discuss ideas for further work involving *G. jacintae* as a study organism, as whilst some aspects of *G. jacintae* behavioural ecology were covered in this project, many others remain and are available for scrutiny.

Contest behaviour

A potential avenue for the further study of intraspecific competition in *G. jacintae* is contest behaviour. Pairs of individuals often compete for indivisible

resources, such as a host, via agnostic contest behaviour (Huntingford and Turner, 1987; Mesterton-Gibbons and Adams, 1998; Riechert, 1998; Hardy *et al.*, 2013; Cusumano *et al.* 2016). The likelihood of a contest is promoted when foraging females remain with the host or within patches of hosts for considerable amounts of time, making the likelihood of two females simultaneously encountering a host higher. It is often that, in dyadic contests with two females, one female will be considered the 'owner' of the resource if it came across the host first or has already oviposited onto it, with the other female acting as an 'intruder' which attempts to displace the owner and gain monopoly of the resource.

Intraspecific competition between individuals of the same species, in particular animal contests over indivisible resources, has played a major part in the development of game theory and the foundation of evolutionary behavioural ecology (Parker 1974; Maynard-Smith and Parker, 1976; Pérez-Lachaud *et al.*, 2004; Batchelor *et al.*, 2005; Kokko, 2013; Palaoro and Briffa, 2017). Models predict that two main categories influence behaviour in dyadic contests; factors associated with the difference in abilities between contestants concerning the acquisition and retention of resources, known as resource holding potential (RHP), and factors associated with resource value to the contestant (resource value, RV) (Maynard-Smith and Parker, 1976; Kokko, 2013; Palaoro and Briffa, 2017). Contestants are expected to compete more intensively for resources with higher value. A basic example is that a larger host will possess more RV to a contestant than a small host (Hardy and Briffa, 2013). However, the value that each contestant places on a given resource can vary (Stockermans and Hardy, 2013). For instance, a female that is older than its contestant and/or has a higher egg load will place a higher value on the host resource (RV). Similarly, a female with a larger body size which encountered the host first ('owner') has a higher resource holding potential (RHP) compared to a female with contrasting traits. In the case of multi-species interactions, it could be that a host larva as a resource has varying RV depending on whether it has already been parasitised by another individual of the same species, or even another species altogether.

A series of studies have already been conducted on other *Goniozus* species, chiefly *G. nephantidis* (Muesebeck) and *G. legneri* (Gordh), concerning

the outcomes of female-female contests due to asymmetries in RV and RHP (Hardy and Blackburn, 1991; Stokkebo and Hardy, 2000; Humphries *et al.*, 2006; Hardy and Goubault, 2007; Stockermans and Hardy, 2013; Mathiron *et al.*, 2018). These studies report some of the conditions that a female may fight for a host under, as well as the conditions when it may forfeit a host to an intruder. However, no prior research has been conducted concerning how host parasitisation status influences the RV of the host to foraging females. As *G. jacintae* females have already been witnessed fighting over a host (E. Aspin, pers. obs.), it is likely that the species exhibits contest behaviour interactions similar to those known in other members of the genus, and results from this thesis show that *G. jacintae* exhibits host discrimination abilities (Chapter 4).

In the presence of conspecifics, the optimal decision of an individual regarding resources are likely to depend on the decisions taken by others (Maynard-Smith, 1974). As discussed previously, it is known that hymenopterous parasitoids have the ability to discriminate between hosts using surface markers and other diagnostic cues, as well as the ability to decide whether to selectively superparasitise hosts under certain circumstances (van Lenteren, 1981). Considering these two strategies, it would be valuable to deduce the outcome of dyadic competition between two females when the host has already been parasitised. The host could be manipulated in a variety of ways to consider the influence of parasitism on RV.

For instance, chemical markers deposited on the surface of the host have the potential to deteriorate over time, making them less detectable by foraging females. Hence, their discriminatory ability may also diminish if some time has passed since host parasitisation, which in turn could influence perceived RV of the host. In addition, if the host two females are contesting for has been parasitised by another species of parasitoid, such as *D. tasmanica*, this could have important influences on the perceived host RV. Experiments which evaluate how host parasitisation status influences dyadic contest outcomes are necessary to facilitate the understanding of how parasitoids would overcome both intra and interspecies interactions in the field.

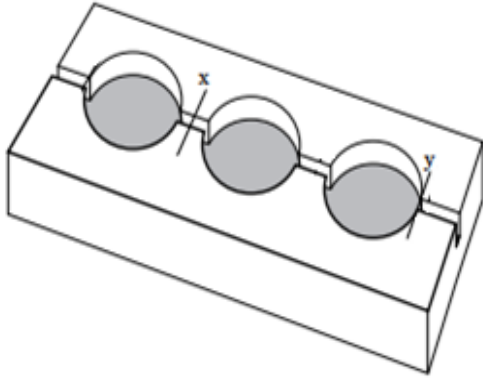


Fig. 1. Experimental arena. Three circular chambers (diameter = 1.8cm, depth = 0.6cm) set 1cm apart in an opaque plastic block with a transparent lid. A 0.1cm wide slot, through which *G. jacintae* can pass through, connects the chambers. Prior to experiments two opaque barriers are pushed into each side of the slot before they meet at point 'x', which isolates and bisects the chamber. When an experiment begins, one barrier will be withdrawn to position 'y'.

experimental arenas excavated from polyethylene blocks and covered by transparent Plexiglass lids (Fig. 1). 'Owner' and larva would be placed into one half of the bisected central chamber and left to acclimate for 30 mins, allowing for the 'owner' to inspect the condition of the larva she has been presented with. Meanwhile, another female wasp of similar size with no prior experience of the larva, termed 'intruder' would be added to the unoccupied half of the bisected central chamber and left to acclimate. Upon beginning the experiment, the barriers used to separate 'owner' and larva from the 'intruder' female would be withdrawn to point 'y', and observations would begin for 1 hour. During this time the contest behaviour between the 'owner' and 'intruder' females could be recorded.

As the removal of the barrier to point 'y' allows for movement of wasps into an empty side chamber, this could be used as a diagnostic for whoever 'wins' a

An example of a contest behaviour experiment for the study organisms scrutinised in this thesis could involve the adaptation of experimental designs used for previous work in other *Goniozus* species (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000; Hardy *et al.*, 2013). Female *G. jacintae* would be exposed to 3rd instar LBAM larvae (the larval instar that both parasitoids will attack) of varying condition. These females would be defined as 'owners' as they would have prior experience of the host used in the experiment. 'Owner' females would be exposed to larvae of different conditions: unparasitised, parasitised by a conspecific (another *G. jacintae*) or parasitised by an allospecific (a *D. tasmanica* female). All contest experiments would take place in

contest. Any interactions between females would be recorded, such as ‘fights’ (determined by biting and grappling behaviour), ‘chases’, or ‘non-aggressive’ (being in close proximity to one another without fights or chases). After fights or chases, one female may attempt to escape the chamber containing the host and exit to the empty chamber. This would determine the ‘winner’ of a contest. In the case of non-aggressive host take-overs, if they occur, the female in closest proximity to the host would be deemed the ‘winner’.

If host parasitisation status does indeed influence the perceived RV of a host, it would be expected that ‘owners’ would be less likely to defend an allospecific/conspecific parasitised host versus an unparasitised host. This experiment could be taken further by adding asymmetry between ‘owner’ and ‘intruder’ RHP, e.g. by having one female be larger than the other, and observing how this determines the outcome of the contest.

Intraguild Predation

In entomological literature, it is generally accepted that larvae of an ectoparasitoid will have a higher probability of outcompeting endoparasitoid larvae, usually via the ectoparasitoid consuming the endoparasitoid (Flanders, 1971; Godfray, 1994; Harvey *et al.*, 2013). The act of killing and eating a species that uses similar and often limited resources, and are thus potential competitors, is termed intraguild predation (IGP), and is common in ecological systems (Polis *et al.*, 1989; Raso *et al.*, 2014; Frances and McCauley, 2018; Pahl *et al.*, 2020) (Fig. 2). IGP differs from classical predation as the act reduces potential exploitation competition. Guild is defined as “a group of species that exploit the same class of

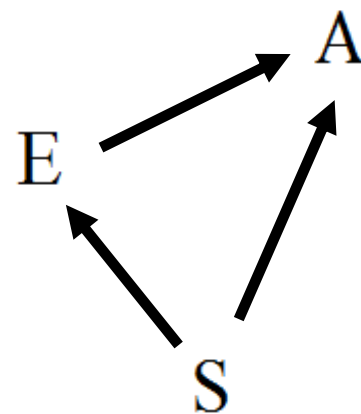


Fig. 2. Intraguild predation is a combination of exploitative competition by guild members for a shared resource (S) and predation of one guild member (E) by the other (A). For coexistence to occur in an intermediately productive system, E must be a superior exploiter of S (relative to A), and to compensate for its poorer exploitative ability, A must be capable of using E as a secondary resource. (Borer, 2002).

environmental resources in a similar way” (Root, 1967), which in this example is parasitoids sharing and competing for resources from the same host.

Endoparasitoids often hold more extrinsic exploitative ability, i.e. the ability to find hosts and produce progeny, compared to ectoparasitoids (Flanders, 1971; Force, 1970; Harvey *et al.*, 2013; Cusumano *et al.*, 2016). In theory, for the ‘worse’ exploiter to be sustained and not driven to extinction, a trade-off is required. Coexistence can occur in IGP systems as the ‘worse’ exploiter (A in Fig. 1) uses the better exploiter, or ‘intraguild prey’ (E in Fig. 1) as a second resource (Borer, 2002). However, coexistence will only occur at intermediate productivity (Holt and Polis, 1997; Holt and Bonsall, 2017). At low productivity, only the intraguild prey species will persist as it can successfully produce progeny on fewer resources, whereas at high productivity the intraguild predator will drive the intraguild prey to extinction via a combination of competition and predation (Holt and Polis, 1997; Borer, 2002; Fonseca *et al.*, 2017).

For the example of this host-parasitoid system, theory would predict that the ‘intraguild predator’, *G. jacintae*, would outcompete and prey on the ‘intraguild prey’, *D. tasmanica*, which has a better ability of exploiting the shared host resource as an endoparasitoid. However, some aspects of host-parasitoid systems are not currently incorporated into IGP theory, such as the fact that endoparasitoids often attack younger hosts than ectoparasitoids (Askew, 1975; Harvey *et al.*, 2013).

In an IGP formulation, the endoparasitoid is able to gain an advantage by attacking younger host stages (Briggs, 1993; Borer, 2002). The benefit of this is that a more developed endoparasitoid larvae has a higher chance of survival against competition from ectoparasitoids, which attack later host stages. Hence, it is predicted that the ectoparasitoids probability of survival decreases as the endoparasitoids age increases. Borer (2002) provided evidence for this theory by reporting that an ectoparasitoids (*Aphytis melinus*) juvenile survival rate decreased as a function of the age of the juvenile endoparasitoid (*Encarsia perniciosi*) in a shared host (California red scale). When *E. perniciosi* parasitised very young hosts, larvae were increasingly

invulnerable to attack by *A. melinus* and more likely to survive to emergence (Borer, 2002). These findings are contrary to common assumptions that ectoparasitoids are always able to consume endoparasitoids in a shared host. Hence, the relative timing of attacks on the shared host by ectoparasitoids and endoparasitoids could have important consequences for coexistence in parasitoid systems.

From research conducted in this thesis, it is known that both *D. tasmanica* and *G. jacintae* parasitise the 3rd larval instar of LBAM (Chapters 2, 3 & 4), that *G. jacintae* exhibits interspecific host discrimination behaviour (Chapter 4), and that multiparasitism of a LBAM host by both the parasitoids results in the death of a host (E. Aspin., unpublished data). The presence of *D. tasmanica* in vineyards is also much higher than that of *G. jacintae*. It could be postulated that the theory of an IGP interaction could be challenged by this study system, such as that seen in Borer (2002). Thus, future experiments which assess the outcomes of IGP and interspecific competition within a single LBAM host could generate further understanding of competitive interactions between *D. tasmanica* and *G. jacintae* in the field.

A method to analyse IGP between the parasitoids used in this study could be for a 3rd instar LBAM larva to be parasitised by a *D. tasmanica* wasp, then left to develop until exposure to a *G. jacintae* female at a chosen time point. These times could be 1h post-parasitisation (external cues from the parasitisation would still be present), 2 days (the point around which a *D. tasmanica* egg would hatch; Feng *et al.*, 2015) and then longer intervals such as 5, 10, 15 and 20 days (to encapsulate the full development of a *D. tasmanica* larva). After exposure to a *G. jacintae* female at one time point and if oviposition has been witnessed, the larvae would be left to develop until parasitoid emergence or host death. Proportion of survival of each species of parasitoid would be recorded, as well as observations of *G. jacintae* laying decisions such as clutch size, sex ratio and egg development time (if applicable). As *G. jacintae* is an ectoparasitoid, eggs that are laid would be visible and would indicate successful oviposition. However, *D. tasmanica* lays a single egg inside the host that is not visible to the naked eye. To ensure the host is parasitised by both species, controls would be conducted; the same *D.*

tasmanica wasp used per replicate would be exposed to another unparasitised larva of the same age/size as the one used in the experiment. After exposure, the larvae would be dissected in water under a microscope for observation of an egg. This should provide a confidence level of successful parasitisation witnessed per observed sting.

Avoidance of pupating LBAM

During this project, it was observed that *G. jacintae* occasionally reject 6th instar LBAM larvae, even when no other potential hosts were available (E. Aspin, pers. obs.). This is despite findings that *G. jacintae* prefers to parasitise the larger larval instars of LBAM, and that 6th instar LBAM gave the highest yield of female parasitoids out of all instars tested (Chapter 3). When the rejected hosts were kept, they would proceed to pupate within one or two days.

Avoidance of laying eggs on a host that is soon to pupate would be beneficial for a parasitoid, since a moulting host would shed its cuticle and any parasitoid eggs that are attached, but it is currently unknown how this is achieved. As *G. jacintae* are capable of discriminating between unparasitised and parasitised hosts (Chapter 4), it can be hypothesised that the parasitoid could be using a similar mechanism of host discrimination in this case.

An insect's integument consists of hydrocarbon compounds, primarily n-alkanes, olefins and methylalkanes (Lockey, 1991; Khidr *et al.*, 2013), which are utilised as olfactory cues to recognise and discriminate between hosts or kin, especially in social insects (reviewed in Howard and Blomquist, 2005; Lim and Ben-Yakir, 2020). Recognition cues are associated with their chemical stability, low volatility and structural diversity, making them a common chemical presence found on the cuticle (Blomquist *et al.*, 1987; Dani *et al.*, 2001; Ginzel and Blomquist, 2016). Perhaps during pupation, the composition of these compounds changes in LBAM larvae, making the presence of some recognition cues absent and/or other compounds present. However, further empirical assessment of both *G. jacintae* host discrimination behaviour and the role of cues in host pupation are required to address this observation.

A behavioural experiment could be conducted to observe the response of *G. jacintae* to hosts that are soon to pupate. Preliminary experiments would need to be conducted to determine the mean time a LBAM larva takes to pupate under controlled conditions. After this is established, *G. jacintae* females could be exposed to 6th instar LBAM larvae that have 5, 4, 3, 2 or 1 day(s) remaining until pupation. Laying decisions of the parasitoid would be observed, such as host acceptance or rejection, the number of eggs laid (if accepted) and the length of host inspection time by the parasitoid. After exposure to a *G. jacintae* female at one time point, regardless of host acceptance/rejection, the larvae would be left to develop until either parasitoid emergence (if applicable), host death or pupation of the host occurs. Results from this experiment would determine if *G. jacintae* avoids ovipositing on larvae that are close to pupation, and if so, it could identify the development stage at which *G. jacintae* begins to reject 6th instar larval LBAM. This behaviour may be observed as increased prevalence of host rejection or less eggs laid on accepted hosts as they approach pupation.

A further application of this experiment may be possible if the exact compounds involved with LBAM pupation cues are identified. The chemical profile of the cuticular hydrocarbon compounds (CHCs) present on insects is commonly identified using coupled gas chromatography-mass spectrometry (GC/MS) (e.g. Kather *et al.*, 2015; Buellbach *et al.*, 2018; Kathe *et al.*, 2021). Samples of 6th instar LBAM larvae that have 5, 4, 3, 2 or 1 day(s) remaining until pupation could be tested using GC/MS. This would allow for the identification of any changes (if they do occur) in the CHC profile of individual LBAM larvae during the time up until pupation. If any prominent changes are present, manipulation experiments could be performed to determine if the CHC profile of larval LBAM is indeed used by *G. jacintae* females to detect imminent pupation in 6th instar larva. For example, an early 6th instar LBAM larva could be manipulated via gene therapy (e.g. Chung and Carroll, 2015) to express the hydrocarbon compounds present on a (soon to pupate) late 6th instar larva before exposure to a female *G. jacintae*, then behavioural response of the parasitoid could be observed. However, the reality of this experiment can only

be determined by the presence of currently unknown cues within the CHC profile of a late 6th instar LBAM larva.

Overall Conclusions

The life histories of bethylids provide valuable study systems for research into biological control agents, particularly host-parasitoid interactions. However, it is important to not consider single host – single parasitoid interactions in isolation. The dynamics of interspecific competition with one potential biological control agent and another must be analysed to mitigate the risk of disruption of natural enemy actions in the field.

When addressing the wider implications of this study, we must first consider the effectiveness of *G. jacintae* when using LBAM as a host. *Goniozus jacintae* only temporarily paralyse LBAM and partially guard broods on LBAM (Hopper and Mills, 2015). This is despite permanent paralysis of hosts and brood guarding until offspring pupation being a common occurrence in other *Goniozus* (Hardy and Blackburn, 1991; Sreenivas and Hardy, 2016). This, as well as the influence of *D. tasmanica* on the availability of unparasitised LBAM in the field, postulates that alternative host species are present for *G. jacintae* besides LBAM. Other *Goniozus* species have been beneficial in controlling pests of sugarcane (Graham and Conlong, 1988), almond (Legner and Gordh, 1992), coconut (Shameer *et al.*, 2018) and date palm (Polaszek *et al.*, 2019) but this control is not 100% effective and is not always long-lived. Due to it being able to produce larger broods on larger hosts, the mass-rearing of *G. jacintae* in a laboratory is feasible. Therefore, considering the information above, *G. jacintae* could be used for augmentative biological control, or biocontrol that requires an introduction of a biocontrol agent to temporarily suppress pest populations in a system, such as LBAM in grapevine crops. The efficacy of this biocontrol would be improved if it was a component of an IPM program which employed other methods to control the pest (e.g. LBAM mating disruption via pheromone traps) and promote the presence of parasitoids and other natural enemies (e.g. planting of nectar sources in crop inter-row). It is important to note that many more aspects of *G. jacintae* behavioural ecology

and interspecific interactions remain to be investigated before drawing firm conclusions on its use as a biocontrol agent.

Ultimately, interspecific competitive interactions between parasitoids shape the structure of ecological communities (Cusumano *et al.*, 2016), and without consideration of these interactions, one cannot hope to formulate a robust biological control programme. Investigations conducted in this study have produced results that contribute to the improvement of biological control practices for LBAM, and can be applied to a wider scope of integrated pest management practices, such as information relevant to the mass-rearing of parasitoids and new information on bethylid foraging behaviour.

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APPENDICES

I – III

Appendix I – Insect Rearing & Management

Host Plant

Plantain, *Plantago lanceolata* L. (Lamiales: Plantaginaceae), was the chosen host plant for rearing *E. postvittana* (LBAM) used for this study due to numerous benefits associated with the plant. The plant itself is low in cost, widely available and easy to grow in controlled greenhouse conditions. In addition, the body weight of female LBAM is consistently higher on plantain in comparison to other host plants (Danthanarayana, 1983; Danthanarayana *et al.*, 1995), and fecundity of females is greater by a factor of 1.5-2.5 on plantain, with the maximum fecundity (1492 eggs per female) being recorded on plantain (Suckling and Brockerhoff, 2010).

Rearing LBAM

The culture of LBAM used in this experiment was established at the South Australian Research and Development Institute in 1994 and has since been maintained with annual additions of wild moths. LBAM was reared on an artificial diet at 22 ± 2 °C under 12L:12D photoperiod, following methods reported in Yazdani *et al.* (2014).



Figure 1. LBAM rearing containers



Figure 2. LBAM egg mass on plantain leaf

The diet instructions are as follows:

- 500g soaked lima beans (250g dried beans left in 500ml water overnight)
- 80g brewer's yeast
- 2.5g sorbic acid
- 5g methyl-p-hydroxy benzoate
- 600ml water

The above mixture was added to a beaker and sterilised in an autoclave for 20 minutes at 120 °C. When the mixture cooled to 70 °C, the following was added:

- 8g ascorbic acid
- 4ml formaldehyde
- 32g agar dissolved in 1000ml of water

The mixture was then stirred well and blended. The hot diet was poured to a depth of 1-2cm into 100 ml plastic cups that served as rearing containers (Fig. 1). After cooling, rearing containers were sealed and kept at 4 °C until use. Prior to use, rearing containers were sterilised using UV light to prevent contamination.



Figure 3. LBAM female (left) and male (right) pupae



Figure 4. Female LBAM pupae phases of development

Three to five individual egg masses of similar size (Fig. 2) were cut from adult holding cups and inserted into the diet layer of the rearing containers. Pupae were harvested from the containers after LBAM eggs had completed development, which was approximately between 30-35 days.

Pupae were sexed by examining the anatomy of the abdominal segments; females had 3 segments whilst males had 4 (Fig. 3, 4), before being placed into separate emergence cages and supplied with a 10% honey solution via a wick.

To allow mating when required, five newly emerged male and female adult moths were placed into a 285ml plastic cup with vertical ridges containing a dental wick soaked in honey solution, covered with a sheet of voile. Cups were left in natural light and room temperature for seven days to allow for mating to occur and for eggs to be laid on the ridges of the cups.

Parasitoid rearing

Cultures of *G. jacintae* and *D. tasmanica* were established from individuals collected from 2018-2020 in South Australian vineyards. Larval LBAM were collected in the field by searching for their trademark damage and leaf rolls on plantain and grape leaves (Fig. 5, 6). All collected larvae were held in individual rearing cups containing plantain foliage at 24 °C and checked daily for the instance of parasitoid emergence.



Figure 5. Larval-damaged grapevine leaf (*Vitis vinifera*) containing a LBAM larva



Figure 6. Larval-damaged grapevine leaf (*Vitis vinifera*) containing a *D. tasmanica* cocoon

Approximately 20-30 individual LBAM were placed into insectary cages 2-3 days prior to the addition of parasitoids to infest a pot containing plantain and to allow the larvae to settle.

Any newly emerged parasitoids from larvae collected in the field were released into cages that contained plantain infested with first to second instar LBAM larvae for *D. tasmanica* cultures or fourth to fifth instar LBAM larvae for *G. jacintae* cultures and maintained in an insectary at 23 ± 2 °C, 14L:10D, with daily provision of fresh honey as a nectar source (Fig. 7).

Newly formed wasp cocoons would be extracted from the insect cages and held in 50 mm × 18 mm diam. glass vials containing a drop of honey and fitted with caps that had screens for ventilation. Upon emergence, females were collected and caged overnight with 5 males for mating. Females were subsequently re-isolated in glass vials and provided with honey and water *ad libitum* 1h before use in experiments.


For general rearing, each pot of infested plantain would be replaced every 2 weeks and re-infested with new LBAM larvae. A series of multiple insect cages was kept for each parasitoid species in the event of colony collapse. Field collected adult parasitoids were added to each culture periodically to maintain genetic diversity.



Figure 7. A sure sign of success: newly emerged *G. jacintae* feeding on honey in a rearing cage

Appendix II – Career and Research Skills Training

CaRST Completion Certificate



THE UNIVERSITY
of ADELAIDE

This is to certify that

Miss Emma Aspin

has completed

120 hours

of Career and Research Skills Training

27 Feb 2020

*Dr Monica Kerr,
Director, Career and Research Skills Training*

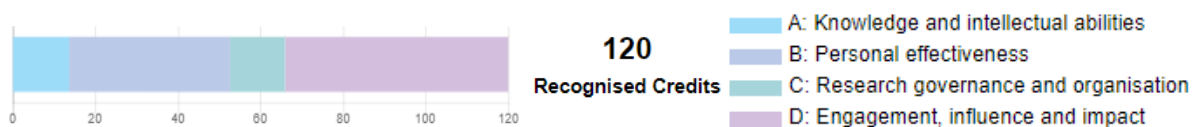
Date

adelaide.edu.au CRICOS Provider Number 00123M

seek LIGHT

CaRST Record

Below is a record of activities that were completed during my PhD candidature as per the requirements of the Career and Research Training (CaRST) programme.



Date	Activity	Domain	Hours
23/1/2018	Postgraduate Research Induction	B	2
7/2/2018	School of AFW Safety Session	C	4
6/3/2018	Global IQ Workshop	B	1.5
13/3/2018	IBP-R Diagnostic Workshop	A	2
14/3/2018	'Why Waite' Volunteering	D	4.5
22/3/2018	Literature Review Workshop	D	4.5
3/4/2018	Figshare for Researchers	A	1.5
23/4/2018 & 26/7/2018	Women in Stem Careers (WiSC) Inklings Sessions	B	7
4/5/2018	EndNote training	A	1
7/6/2018	WiSC Networking Event	B	3
19/6/2018	Research Data Management	C	4.5
21/08/2019 & 23/06/2020	Three Minute Thesis Competitions	D	11
29/8/2018	WiSC Industry Panel Q+A	B	3
5/9/2018	Royal Adelaide Show Outreach	D	6
19/9/2018	Postgraduate Symposium	A	8
24/9/2018	Professional Development Day	B	10.5
12/10/2018	UoN-UoA Joint PhD Symposium - Malaysia	D	6
25/10/18 & 24/10/2019	National School Wine Show	D	9.5
1/11/2018	Waite in the Spotlight Q+A Panel	A	2.5
24/4/2019	Career Control for Researchers	B	14
22/5/2019	Alternate Science Careers Q+A Panel	B	2
12/09/2019	Media training and Professional Development	D	5
17/10/2019	Travel Scholarship Application	C	3
3/12/2019	Australian Entomol. Society Conference - Brisbane	D	6
24/2/2020	Demonstrating Staff Health & Safety Workshop	C	3

Appendix III – University Achievements

A PhD is so much more than the hours spent in the lab, squirrelling away. I learnt that it is vital to balance ‘work’ and ‘play’, with an emphasis on *balance*; one side should not take priority over the other.

During my placement in Australia, I found myself involved in multiple endeavours that took me away from the office and developed a range of skills, values and lessons as a scientist.

Some of these activities have led to the accumulation of awards for my research, besides the publication of scientific papers. I am a firm believer that research should be communicated to a broader audience in an engaging and understandable format, rather than locked away behind pay-wall protected journals and written in garbled, complicated language.

“Saving our planet is now a communications challenge.

We know what to do, we just need the will.”

– Sir David Attenborough

Science communication is vital for spreading scientific fact to the masses. We can no longer live in a world where general society aren’t involved in scientific decisions, policies and their impacts. For this to be achieved the concepts and how they are put across need to be understandable by all, not just the intellectual ‘elite’.

In addition, the spread of misinformation could severely harm the true message of science. I’m sure I’m not alone in my hopes to ensure that there’s another generation who are inspired to take up scientific roles in the future.

So, I leave here a sort of legacy: a reminder to myself to be proud of what I achieved during my time in Oz, to not be ashamed for standing out from the crowd, and an example of how I hope to continue communicating science.

Women in Stem Careers (WiSC) Certificate – 2018



THE UNIVERSITY
of ADELAIDE

This is to certify that

Emma Aspin

has graduated from the

**Women in STEM
Careers Program 2018**

with high achievement

Professor Anton PJ Middelberg

*Executive Dean
Faculty of Engineering, Computer and
Mathematical Sciences*

8 October 2018

Date

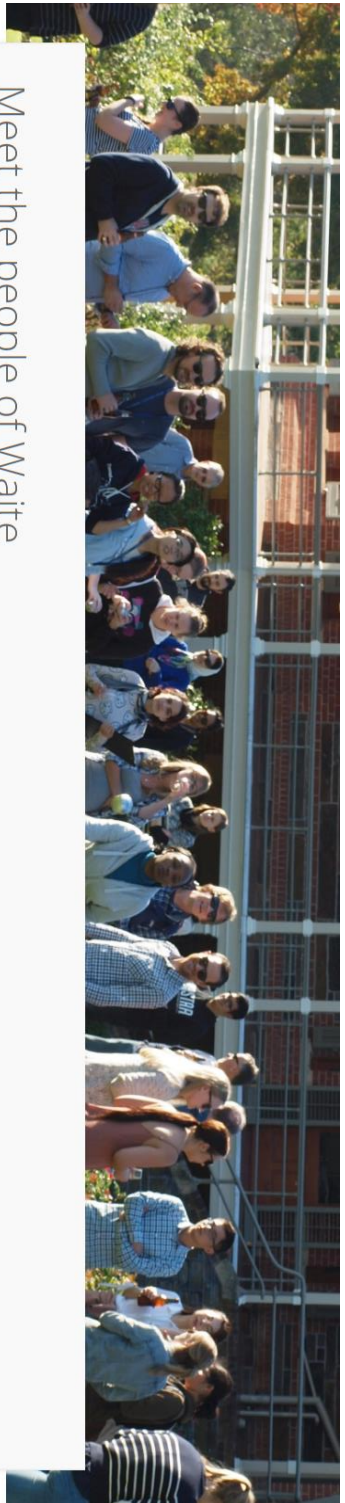
adelaide.edu.au

CRICOS Provider Number 00123M

Meet the People of Waite, Creator & Writer – 2019 to 2020

Interviewed and wrote articles for 8 participants. More content in link below.

Accessible link: <https://www.thewaite.org/about/meet-the-people-of-waite/>



Meet the people of Waite

Location: Waite Research Precinct, Urrbrae SA 5064

There are roughly 1,500 people on the Waite campus – from students to professors, technicians to human resource managers, and even volunteers. They work and study across a range of campus partner organisations.

This series of articles will introduce you to some members of the Waite community – who they are, what they do, and why it matters!

About the Author:



Emma Aspin is a second-year PhD student from the UK with a passion for science communication. Upon arrival to the Waite, Emma was astounded by the diversity of workers, students and scientists across the campus and knew that there were some great stories to be told! As well as having some fun, the column is a great opportunity to discuss your work and have a chance to reach the public or even future collaborators.

If you would like to be featured in the column and have time to chat to Emma sometime, drop her an email:

emma.aspin@adelaide.edu.au

Three Minute Thesis Media Release – 2019

Accessible link: <https://blogs.nottingham.ac.uk/teachingpartnerships/2020/03/06/insect-wars-emma-aspins-3-minute-thesis/>

WAITE RESEARCH SHOWCASED IN JUST 3 MINUTES

Jul 17, 2019 | News, Students, University of Adelaide

The Three Minute Thesis (3MT) celebrates the exciting research conducted by PhD students. It is a skills development competition that challenges Higher Degree by Research students to explain their research project to a non-specialist audience in just three minutes and with only a single static slide permitted.

The School of Ag, Food and Wine held its heat on Tuesday July 16 and showcased some of the excellent students here at Waite. The standard was high across all of the six participating students – well done to all involved!

The quality presentations definitely made it a tough job for Judges Kathy Ophel-Keller, Steve Tyerman and Stuart Matthews!

Winners for the School heat were:

- Emma Aspin – Peoples Choice and 1st place: *Insect wars: parasitoid vs pest*
- Jin-Chen Li – 2nd place: *Getting rid of 'Greenness' in wine*
- Amanda Philpot – 3rd place: *Building a better psyllium seed case*

Emma and Jin-Chen will now compete in the Faculty of Science 3MT final on Wednesday 21st August. The winner and runner up of the Faculty final will then compete in the University Final to be held as part of the Research Tuesdays Lecture series on Tuesday 10 September 2019.

For more information, rules, prizes, and tickets to the finals, please visit: <http://www.adelaide.edu.au/3mt/>



Three Minute Thesis Certificate – Winner 2019



Three Minute Thesis Certificate – People’s Choice 2019



School of Agriculture, Food & Wine

2019 Three Minute Thesis Competition (3MT)

People’s Choice

awarded to

Emma Aspia

Professor Eileen Scott
Deputy Head of School of Agriculture, Food
and Wine

adelaide.edu.au

seek LIGHT

Waite Postgraduate Symposium Media Release – 2019

Sally & Andrew Smith Prize & PAWS People’s Choice Prize

ANNUAL POSTGRADUATE SYMPOSIUM 2019

Oct 3, 2019 | News, Students, University of Adelaide

Last week the School of Agriculture, Food and Wine held their Annual Postgraduate Symposium. This event provides a forum for mid-candidature students to give an update on their research in preparation for the Annual Review of Progress. It also provides an opportunity for the Waite community to become familiar with the broad range of interesting problems our postgraduates are tackling.

This year, 34 postgraduate students gave 10-minute presentations on their projects, grouped into themes that broadly align with the School's research strengths. Sessions were chaired by members of the Postgraduate Association of Waite Students (PAWS) Committee.

The program also featured guest speaker Lieke van der Hulst a freelance science communicator who studied for her PhD at Waite, analysing grapevine response to smoke exposure. Nowadays, Lieke is working in the WIC winemaking team at Waite as well as writing about the South Australian wine industry for multiple online outlets.

Prizes were also awarded to students in a number of categories. This event was organised by the PAWS Committee and the School Postgraduate Committee.

Well done to all the students and congratulations to all the following prize winners:

Emma Aspin - PAWS audience choice day 1

Sally and Andrew Smith Award prize for best presentation at the symposium – selected by School Academics on behalf of Waite Research Institute



UoN & UoA Joint PhD Press Release – 2020

Moving half way around the world by yourself can seem like a daunting task. On top of that, you have the occupation of being a PhD student – this can mean challenging hours in the lab, a lot of working alone and sometimes an unbalanced work/leisure ratio... how would anyone deal with that?



I consider myself the 'social butterfly' type. You can find me on the ENFP side of Myers-Briggs, or a 'Peacock-Dove' of the 'DOPE' test (yes, really). Literally everything in my genetic makeup deters me from being unsociable, so I reached out, determined to find friends in my new home.

Luckily, that was achieved with ridiculous ease. Thanks to being part of a cohort with the UoN-UoA Joint PhD scheme, I had a few points of contact to get in touch with straight away. We all have the scheme in common and any questions you have, they've had before! It's incredible how quickly you can go from meeting strangers you're studying with to becoming fast friends.

I also wanted to get involved with a society. I'm based on the Waite campus in Adelaide, so it made sense for me to join the Postgraduate Association for Waite Students (PAWS). From there, I was able to meet people from across the campus who came from a whole range of different countries and backgrounds. The charm in that is I've been able to sample delicious food across a range of cuisines and be invited to parties for all sorts of diverse holidays – like Iranian New Year!

I started off as Secretary for PAWS, but after 1.5 years on the committee I became President (ooh, la dee dah). I'm now in charge of running events across the campus for students as well as community events. Our most recent one was a bake sale to raise funds for Bushfire Relief; everyone knows how devastating the impact bushfires have been in recent years in Australia. Thanks to the efforts of the Waite community (and a crudely drawn cupcake sign), we managed to raise over \$500!

Another benefit of having international friends is that, again, you're all in the same boat. You share a bond of knowing you're in a new place and you're all eager to go and explore or be involved in as many new things as possible. That was definitely my case; when I landed in Australia, I vowed to myself that I would learn how to surf. After ticking that box, I've gone on to learn bouldering and scuba diving. Australia is definitely an adventurous person's paradise!

Despite the fact that you're thousands of miles away from home, I think it's incredibly easy to settle in Adelaide. Of course, it wouldn't have been the same without the mix of people I've become friends with, and everyone's experiences will be different. However, Adelaide is definitely a nucleus of activity and opportunity.

I've had a whirlwind of a time, all whilst working on a project I'm passionate about and being able to communicate it via news articles, community outreach and conferences. The Joint DTP has allowed me to collaborate with supervisors across two continents and for their expertise to combine and help refine my project. I'm super proud of how far I've come and what I've achieved – my confidence and presentation skills have really gone through the roof since I started, and I have a couple of awards to show for it!

If I had to sum up my three top experiences, I would do it very concisely in a list quite like this:

- The camping trips (Uluru, Flinders National Park, the Grampians, Kangaroo Island, Yorke Peninsula)

- The wildlife (Koalas on the Waite campus, the sound of cockatoos and kookaburras filling the air, possums sneaking up to steal food from the BBQ)
- And last but not least... the people: those who I met when I first arrived, those who moved on along the way and those I have yet to meet

More of what I've been up to:

- Spokesperson at EcoVineyard Workshop in the Barossa Valley
- <https://www.abc.net.au/news/rural/2019-10-31/bringing-australian-bush-to-vineyards-could-reform-wine-industry/11655940>
- <https://www.thewaite.org/waite-research-showcased-in-just-3-minutes/>
- <https://www.thewaite.org/annual-postgraduate-symposium-2019/>



VOTE FOR EMMA IN THE AUSTRALIAN FAMELAB SEMI-FINAL

Apr 23, 2020 | [News, Students, University of Adelaide, Women of Waite](#)

Waite PhD student Emma Aspin this week participated in the Australian FameLab semi-finals.

You can view the video here: <https://www.fwam.com.au/victoria-semi-final/>

You can vote for Emma in the 'Audience Choice' category of these awards. As far as she knows, Emma is the only student from SA that took part (and is in the Victorian semi-final as SA doesn't have its own round this year).

FameLab is a live science communication competition that aims to discover charismatic early career scientists who can inspire people to see the world from new perspectives. It is run annually in Australia and in over 25 countries across the world. In 2020, FameLab is produced in Australia by the [Foundation for the Western Australian Museum](#).

Hurry – voting closes this Saturday!



FameLab Certificate – Wildcard Winner 2020

Accessible link: <https://www.youtube.com/watch?v=1NMywevkwGc>



South Australia Young Achiever Awards Nomination – 2020



Nominee

Nominated for

The University of Adelaide STEM Award

Awarded to

Emma Aspin

A handwritten signature in blue ink, appearing to read 'S Marshall'.

The Honourable Steven Marshall MP
Premier of South Australia



Three Minute Thesis Media Release – 2020

Accessible link: <https://vimeo.com/439706661>

EMMA ASPIN INTO UNIVERSITY 3MT FINAL

Aug 28, 2020 | News, University of Adelaide, Women of Waite

Congratulations to Waite PhD student Emma Aspin who is one of two Faculty of Science finalists through to the University of Adelaide's 3 Minute Thesis (3MT) final.

The 3MT is a national competition celebrating the exciting research conducted by PhD candidates.

PhD candidates have just three minutes and one slide to effectively explain their research to a non-specialist audience. In the best interests of public health, the 2020 3MT Competition has moved to a virtual format, and competitors have submitted a pre-recorded video of their 3MT presentation.

Emma's presentation, *The Goldilocks Grub*, outlines her research on parasitoid wasps and their caterpillar hosts. She joins nine other finalists competing for the main prize and one will go on to compete in the virtual Asia Pacific finals.

The University of Adelaide 3MT Finals will be held at 5.30pm on Tuesday, 8 September at a special Research Tuesdays event.

The presentations will be judged by a panel of experts and we also invite you to have your say in the People's Choice Award, which will be tallied live on the night. If you would like to view the videos prior the event, head to the [Three Minute Thesis](#) web page.

GOOD LUCK EMMA!



www.adelaide.edu.au/3mt/finals

Adelaide Graduate Award Certificate – 2021



DIRECTOR'S OVERVIEW

While 2020 was obviously an unusual and challenging year, WRI researchers continued to do outstanding work by bringing to bear skillsets from a range of disciplines.

I was heartened by the depth of community spirit shown by our members in this past year, perhaps best embodied by our graduate students who self-organised and rallied the university community, to ensure our international students and Early Career Researchers were supported during the COVID-19 lockdowns and restrictions. Particular mention for Melanie Ford, Emma Aspin and the whole Agriculture Postgraduate Society (AgPOGS) Team whose energy at a time of great uncertainty reflected the best of human qualities.

Exciting new ventures in 2020 included the award of seven ARC Discovery grants (our highest number ever awarded in a single year), strategic, multidisciplinary bids submitted in gene technologies and barley, the renewal of large breeding programs and centre agreements, and the development of new areas to us such as space horticulture, synthetic biology and the use of machine learning in crop, animal and soil management.

The WRI continues to perform a key enabling role, supporting its people through investment in professional development, coaching and mentoring programs, unique research infrastructure and services that attract leading researchers and enable research excellence, and strategic support for individuals, partnerships and Centres in key strength areas to maximise the opportunities for innovative and significant research.

The WRI took on several new roles in 2020, including driving the support of closer links with the SA State Government's Primary Industry and Regions Department (PIRSA), where through partnership we possess that whole of research value

chain from blue sky research through development, to extension and adoption on the farm. Activities included the development of new proposals in drought resilience and oat research. In 2021, amongst other activities, we will work with PIRSA on eliminating the threat of fruit fly to our Horticulture sector.

In early 2021 we bid farewell to our engine room – and some might say the heart and soul of WRI for many years – as Carolyn Mitchell, our executive officer, and Keryn Lapidge, our communications officer, both take up new opportunities elsewhere. I would personally like to thank Carolyn and Keryn for their many years of service, since 2011 and 2016 respectively, and the dedication they have shown in making WRI the success that it is. I am sure all WRI members will join with me in wishing them well with the next phase of their careers.

Lastly, I would like to pay tribute to all of our members' efforts in 2020. Research is not the easiest of career choices and involves often more rejection than success (especially when it comes to research funding applications), so much hard work goes unnoticed and unacknowledged. Therefore, when success does come, it should be celebrated. I hope you enjoy reading about some of their many highlights for 2020.

Our doors are always open so please get in touch if you want to explore new opportunities with us.

Professor Matthew Gilliham
Director, WRI

Note: Postgraduate Association for Waite Students (PAWS) was mistakenly named as its old title, Agriculture Postgraduate Society (AgPOGS).

We raised over \$5,000 AUD to support Waite HDR students struggling in the face of the pandemic and distributed care packages during lockdowns throughout 2020.