Why are some parasitoids of light brown apple moth so uncommon in vineyards?

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Dedicated to my dear wife Yan Ma and our daughter Luyu Feng

ABSTRACT

The light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is a key insect pest that belongs to one of the largest families of Lepidoptera, the Tortricidae, which has over 10,000 described species. This family includes numerous major pests of crops, forests, and ornamental plants. Hence an understanding of factors that affect parasitism of *E. postvittana* is potentially relevant to many other pest species and agroecosystems. Although a number of species are known to parasitise *E. postvittana*, only few of them were recorded attack *E. postvittana* in vineyards. Moreover, little is known about the interactions between *E. postvittana* and the parasitoids that are associated with it in crop and non-crop habitats. Therefore，this study addressed the question, “why are some parasitoids that attack light brown apple moth so uncommon in vineyards?” My thesis presents an investigation of the activities of parasitoids in vineyards and adjacent native vegetation in the Adelaide Hills wine region, and provides insights into the contribution they make towards natural biological control of the light brown apple moth.

This project aimed to investigate: (1) parasitism rates of *E. postvittana* in vineyards and adjacent native vegetation; (2) competitive interactions between parasitoids that attack *E. postvittana*; (3) the influence of host plants on foraging behavior and parasitism by parasitoids that attack *E. postvittana*; and (4) temperature dependent development of *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae), a common parasitoid species that attacks *E. postvittana*.

Field experiments showed that *T. unimaculatus* was most active in non-crop native vegetation, whereas *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) was the most common parasitoid of larval *E. postvittana* in vineyards. Molecular identification of larval tortricids that were parasitised by either of the two parasitoids species indicated these two parasitoids share a range of tortricid hosts in both vineyards and natural habitats. These results indicated that the two key parasitoids have different patterns of habitat use between vineyard and adjacent fields.

In order to investigate why parasitoids are not equally distributed between vineyards and native vegetation, two further series of studies were conducted. The first investigated the extent of interspecific differences in host discrimination and the outcome of interspecific competition between *D. tasmanica* and *T. unimaculatus*. Both wasp species did not show differential behavioural responses to un-parasitised hosts or those that were parasitised by the other species. But immature *D. tasmanica* out-competed immature *T. unimaculatus*, irrespective of the order or interval between attacks by the two species.

The second series of experiments examined the effects of host plants on the behaviour of *D. tasmanica* and *T. unimaculatus*. The effects of selected native and non-native host plants on the foraging preferences and efficiency of the two parasitoids were investigated through behavioural observations in a wind tunnel, and an experiment in the field. The results indicated that plants play a role that affects the habitat preferences of the two parasitoid species by influencing their foraging behaviour, and contribute to their distributions among habitats.

By studying the temperature dependent development of *T. unimaculatus* under constant temperatures, its mean developmental time from egg to adult emergence was found to be shortest at 24.4 days at 28.9 ℃. The data were fitted to a non-linear model, which showed that the number of generations of *T. unimaculatus* is equal or greater than *E. postvittana* in three out of four locations in Australia, and the development of *T. unimaculatus* is faster when the temperature is above 16.0 ℃. Thus temperature affects the extent of synchronization between populations of *T. unimaculatus* and *E. postvittana*.

Overall, this research contributes to understand the contributions that parasitoids make to natural biological control of *E. postvittana*. I concluded that native vegetation adjacent to vineyards is not always a reliable source of natural enemies for control of *E. postvittana* in vineyards and, more generally, that native vegetation is not always a reliable source of natural enemies in crops. Based on the results, the different habitat preference of the two parasitoid species is likely to be influenced by different degrees of host-species and habitat preferences, including responses to plants, and possibly specific life history differences between the two parasitoid species. The results of this research are also expected to be useful for understanding natural biological control of many other pest species.

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PREFACE

The research discussed in this thesis has led to the generation of four journal manuscripts that will be published in journal papers and four conference papers

Journal papers

Yi Feng, Olena Kravchuk, Harpinder Sandhu, Stephen D. Wratten, Michael A. Keller. The activities of generalist parasitoids are segregated between crop and adjacent non-crop habitats. For submit to: Biocontrol.

Yi Feng, Stephen Wratten, Harpinder Sandhu, Michael Keller. Interspecific competition between two generalist parasitoids that attack *Epiphyas postvittana* (Lepidoptera: Tortricidae). Accepted for publication: Bulletin of Entomological Research.

Yi Feng, Stephen Wratten, Harpinder Sandhu, Michael A. Keller. Plants affect the foraging success of two parasitoids that attack *Epiphyas Postvittana*. Submitted for publication: PLoS ONE.

Yi Feng, Michael A. Keller. Temperature dependent development of *Therophilus unimaculatus* on *Epiphyas postvittana.* For submit to: Austral Entomology.

Conferences papers

15-16 Nov. 2012, Australian Grape and Wine Research Symposium 'Crush 2012', *oral presentation* 'Biological control of light brown apple moth in vineyards: What affects the diversity of parasitic wasps?'

13-18 Jul 2013,15th Australian Wine Industry Technical Conference in Sydney, *poster presentation* 'Parasitic wasps that attack light brown apple moth: why do some species occur in vineyards and not others?'

29 Sept.-2 Oct. 2013, Australian Entomological Society Conference 2013 in Adelaide, *oral presentation* 'Interspecific competition between two generalist parasitoids that attack *Epiphyas postvittana* (Lepidoptera: Tortricidae)'.

28 Sept.-1 Oct. 2014, Australian Entomological Society Conference 2013 in Canberra, *oral presentation* 'Towards biological control of a generalist insect herbivore: the activities of generalist parasitoids are segregated between crop and adjacent non-crop habitats'.

References for chapter 1 and 6 are included in the References section; references for chapter 2-5 are included within each of those chapters

CHAPTER ONE

General Introduction and literature Review

Biological control of invertebrate pests by resident natural enemies is an ecosystem service that contributes to sustainable agriculture (Wratten et al., 2013, Wilby and Thomas, 2002). Enhancing the diversity of natural enemies can facilitate more effective biological control of insect pests (Wilby and Thomas, 2002). However, the simplification, fragmentation and disturbance of an agricultural landscape can reduce the diversity and abundance of specialist natural enemies such as parasitoids. Interactions between populations of natural enemies and their host species often occur at landscape scales beyond cultivated fields (Kareiva and Wennergren, 1995). Therefore, conservation of natural enemies in agro-ecosystems requires a landscape management perspective (Tscharntke et al., 2007). In agro-ecosystems, parasitoids need resources that are mostly found in undisturbed vegetation (DeBach and Rosen, 1991, Landis et al., 2000). There is a growing body of research that has demonstrated that various characteristics of uncultivated habitats in agro-ecosystems can influence the abundance and diversity of natural enemies. The quantity, quality and connectivity of uncultivated habitats can all influence the persistence and diversity of parasitoid populations (Kruess and Tscharntke, 1994, Zabel and Tscharntke, 1998, Holzschuh et al., 2010), especially near crop edges (Williams and Martinson, 2000, Bianchi and Van der Werf, 2003, Pfannenstiel and Unruh, 2003, Miliczky and Horton, 2005, Miliczky and Horton, 2007). In addition, the age and size of natural habitats near an agricultural field have been shown to influence the activity of natural enemies (Thies and Tscharntke, 1999, Denys and Tscharntke, 2002). But in some cases, parasitoid diversity and parasitism may not be enhanced by increasing habitat complexity at certain landscape scales (Menalled et al., 1999). Moreover, factors that influence the activities of natural enemies, espically parasitic wasps in cultivated habitats are not thoroughly understood. These

factors might include the competitive interactions between parasitoids (e.g. Harvey et al., 2013); varying characteristics of host plants (e.g. Price et al., 1980); and alternative host species (e.g. Unruh et al., 2012); the effect of hyperparasitoids (e.g. Sullivan and Volkl, 1999); regular patterns of seasonal change (e.g. Teraoka et al. 2004), abiotic factors(e.g. Fink and Volkl, 1995), and the differential dispersal abilities of parasitoids (Schellhorn et al. 2014). In addition, spatial organisation at the landscape scale, and the biotic and abiotic characteristics of cultivated and uncultivated habitats in highly disturbed landscapes can substantially influence the degree of pest suppression (Tscharntke and Kruess, 1999, Landis et al., 2000). The extent of these differences and their impact on interactions among parasitoids could affect the spatial distribution of activities of these natural enemies in agro-ecosystems (Figure 1).

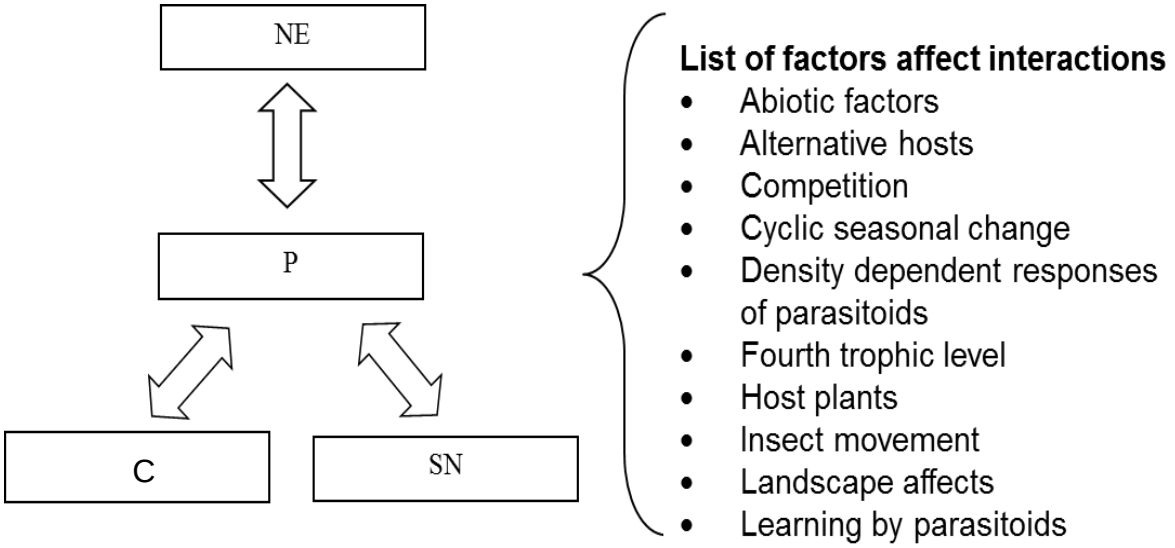

Figure1. Conceptual framework showing a number of biotic and abiotic factors that could affect the activities of pests and associated natural enemies in crops and the adjacent natural habitats. NE: natural enemies; P: pest; C: crops; SN: semi-natural habitats with native vegetation.

The light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) is an Australian indigenous polyphagous insect that has been introduced and become a pest in New Zealand, the United Kingdom, Hawaii and California (Suckling and Brockerhoff, 2010). *E. postvittana* is in the Tortricidae (Lepidoptera), which is one of the largest families of Lepidoptera with over 10,000 described species. There are numerous major pests of crops, forests, and ornamental plants within the Tortricidae (Brown, 2005). Hence, studies that address the ecology and management of *E. postvittana* are likely to have relevance to a range of related pest species.

Epiphyas postvittana is one of the most destructive insect pests of wine grapes in Australia (Scholefield and Morison, 2010). *E. postvittana* not only causes direct feeding damage to leaves, flowers and fruits, but more importantly, its feeding damage can lead to secondary damage caused by *Botrytis cinerea* and other fungi (Nair, 1985). The host plant range of *E. postvittana* includes species in at least 123 genera in 55 families (Geier and Briese, 1980, Danthanarayana, 1975).

In parts of Australia, *E. postvittana* can be found throughout the year. The lower threshold for development of larval LBAM is reported to be 7.5°C (Danthanarayana, 1983). At 25°C its populations have the maximum growth rate. Population growth continues between 7.5 $^{\circ}$C and 31°C (Danthanarayana et al., 1995). There are usually three to four generations of *E. postvittana* annually: winter generation, spring generation and one or two summer generations (Danthanarayana, 1975). Overlap is observed between generations. The major flight periods in Southern Australia are September to October, December to January, February to March, and April to May. For the winter generation, the first instars hatch from eggs laid in autumn. They feed on broad-leaf weeds and cover crops during winter. This generation can move onto grapevines at budburst. The spring generation is initiated from eggs laid by the

overwintering generation. The larvae of this spring generation can reach large numbers during flowering of the grapevine and cause extensive loss of newly set flowers. Moths that emerge in December lay eggs on the mid-sections of shoots. These eggs become the summer generation that cause up to 10% damage to bunch of grapes from mid-January. This generation pupates on the grapevine in early autumn (Nicholas et al., 1994).

There are at least 25 hymenopteran parasitoid species reported to be associated with *E. postvittana* in Australia (Paull and Austin, 2006). Among these, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) is the most commonly reared species in cultivated habitats, including vineyards (Paull 2007). It is also the most common parasitoid of *E. postvittana* in New Zealand (Suckling et al., 1998, Paull, 2007, Charles et al., 1996, Suckling and Brockerhoff, 2010). Another species, *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae) is found more in natural habitats in which it coexists with *D. tasmanica*; (*T. unimaculatus* is described as *Bassus* sp. in Paull and Austin (2006) and Paull (2007). Only a few of the larval parasitoids that attack *E. postvittana* are common in vineyards (Paull, 2007). Therefore, to understand if some key parasitoid species have specific habitat preferences and the the factors that may influence this, it is necessary to conduct a systematic investigation to reveal how these parasitoids interact with their shared host in both vineyards and the adjacent habitats.

In this thesis, the interactions of *E. postvittana* and its associated larval parasitoids in vineyards and the adjacent habitats were investigated by addressing the following questions:

- Are the parasitoid species that attack larval *E. postvittana* and other leafrollers segregated between vineyards and adjacent habitats as suggested by previous research?
- Could competition between these parasitoids influence their activities in vineyards?
- Could the host plants of *E. postvittana* influence the foraging behavior and success of these parasitoids, and therefore influence their abundance in vineyards?
- How does temperature affect the development of these parasitoids in vineyards?

1.1 Activities of parasitoids in vineyards and adjacent habitats

In agro-ecosystems, farmland occurs as a mosaic of cultivated and un-cultivated habitats that differ significantly in various ways. These include landscape effects, plant diversity and local abiotic factors. For biological control, semi-natural habitats provide natural enemies with resources such as overwintering shelter (Cortesero et al., 2000b, Landis et al., 2000), alternative hosts (Pfannenstiel and Unruh, 2003, Williams and Martinson, 2000) and alternative foods (Wratten et al., 2004, Berndt and Wratten, 2005, Begum et al., 2006, Berndt et al., 2006, Lee and Heimpel, 2008, Gámez-Virués et al., 2009). These resources are often limited in highly disturbed and simplified crop habitats (DeBach and Rosen, 1991, Landis et al., 2000, Van Emden, 1965). Therefore, semi-natural habitats are considered to be refuges for regional species pools of natural enemies (Rand et al., 2006, Wilby and Thomas, 2002, Landis et al., 2000). However, this has not been well elucidated for natural enemies that are associated with *E. postvittana*.

Although parasitoids have been widely used as bio-control agents, the interactions of parasitoids that share the same host species in cultivated and un-cultivated habitats remain unclear (Miliczky and Horton, 2005, Dennis and Fry, 1992, Alhmedi et al., 2011). A number of tortricid species are reported in Australian vineyards and natural habitats (Gillighan 2014). The trophic links between parasitoids and their host leafrollers in Australian vineyards and natural habitats are poorly documented. To investigate the activities of parasitoids and their hosts in different habitats, it is important to effectively sample and identify the species. Insect sticky traps (for example, Miliczky and Horton, 2005, Thomson and Hoffmann, 2010) and direct sampling methods (Dennis and Fry, 1992, Alhmedi et al., 2011) have been widely used in community level studies of predators and parasitoids. In a study investigating the effect of remaining native vegetation on the abundance of natural enemies in vineyards, woody vegetation was observed to be positively correlated with the abundance of various groups of natural enemies that are active in vineyards. The abundance of relatively larger mobile parasitoids has been suggested to be unaffected by local vegetation (Thomson and Hoffmann, 2010). However, the absence or presence of highly mobile adult parasitoids may not be a good indicator of actual parasitism. Therefore, sentinel systems with live host insects that feed on plants can be used in studies to efficiently sample the activities of natural enemies in agro-ecosystems (Suckling et al., 2001, Pfannenstiel et al., 2012, Unruh et al., 2012, Paull et al., 2013).

The diversity of Australian tortricids and their parasitoids has not been thoroughly elucidated, and there are no keys based on morphological characters that can be used to identify larval tortricids to species. Therefore DNA-based methods must be used to investigate accurately the trophic links between leafrollers and parasitoids. In

studies reported this thesis, rigorous controlled experiments and molecular identification tools were combined to investigate the direct and indirect interactions between natural enemies that attack *E. postvittana* and related tortricids in South Australian vineyards. One of the major obstacles when investigating ecological interactions between parasitoids and their host insects is accurate identification of species. At maturity, endoparasitoids that feed internally on their hosts as larvae emerge and leave cadavers of their hosts, which are impossible to identify accurately using morphological characters. Molecular methods, such as DNA barcoding with short DNA sequences in a standardised region of the genome, are effective tools for identifying species (Hajibabaei et al., 2006, Smith et al., 2008) and detecting trophic links in complex food webs (Rubinoff et al., 2011, Santos et al., 2011). Molecular operational taxonomic units (MOTUs), which are groups of organisms that can be distinguished in taxonomic studies without necessarily being assigned a taxonomic rank, are widely used to investigate biological diversity and trophic links amongst groups whose taxonomy is poorly understood (Smith et al., 2005, Smith et al., 2009, Blaxter et al., 2005, Floyd et al., 2002, Santos et al., 2011).

1.2 Competitive interactions between larval parasitoids

Competition for host species among parasitoids can affect the community structure of natural enemies. Understanding of the dynamics of competition among potential biological control agents, such as parasitoids, that share the same host species and habitat is important for evaluating their efficiency (Bogran et al., 2002, De Moraes and Mescher, 2005, Mackauer, 1990, Harvey et al., 2013, Gurr et al., 2004, Orre-Gordon et al., 2013). There are two types of interspecific competition between parasitoids: 1) extrinsic competition, which includes all direct and indirect interactions between parasitoids while foraging for hosts, and 2) intrinsic competition, which

involves the indirect or direct competition between immature parasitoids that takes place within an individual host (Harvey et al., 2013). Little is known about the extent and nature of such competition and its influence at the community level for parasitoids (Force, 1985, Godfray, 1994, Harvey et al., 2013). Competitive interactions between parasitoids may not prevent their co-existence (Aluja et al., 2013, Hawkins, 2000, Van Nouhuys and Hanski, 2005). Various circumstances, such as the lack of alternative host species, can cause a single parasitoid species to dominate the parasitism of a host species (Pijls and Van Alphen, 1996). Competition between multiple parasitoid species may, at least theoretically, result in a decline in the efficiency of pest control (Collier et al., 2002, Collier and Hunter, 2001). At the start of this thesis, little was known about how the two parasitoids *D. tasmanica* and *T. unimaculatus* interact with each other. Experiments on competition between *D. tasmanica* and *T. unimaculatus* are reported in this thesis.

1.3 Effects of plant attributes on the foraging success of parasitoids

Plants in agro-ecosystems and the surrounding areas are important for the survival and abundance of natural enemies. Plants affect the foraging success of parasitoids. Parasitoids locate and attack hosts through a series of behavioural steps that lead females to the vicinity of their potential hosts. These steps involve habitat location, host location, and host acceptance (Vinson, 1976). During these steps, plants affect the foraging behaviour of parasitoids in several ways. 1) Herbivore-induced plant volatiles (HIPVs) emitted from host infested plants attract a parasitoid to the location of its host (Vet and Dicke, 1992, Geervliet et al., 1994). 2) The architecture of plants influences the host finding behaviour of parasitoids (Geitzenauer and Bernays, 1996, Bergman and Tingey, 1979, Cortesero et al., 2000a, Carter et al., 1984, Letourneau, 1990, Grevstad and Klepetka, 1992). 3) Parasitoids have innate behavioural

mechanisms to respond to different host plant species and they can also learn cues associated with host availability when they are engaged in host locating behaviour (Vet et al., 1995, Wackers and Lewis, 1994). Learning can affect the foraging efficiency of parasitoids under both laboratory (Geervliet et al., 1998, Lewis and Takasu, 1990) and field conditions (Papaj and Vet, 1990). To understand how plants affect the foraging efficiency of parasitoids, both the plant attributes and the adaptive learning behavior of parasitoids should be considered. A study of how host plants and experience could influence parasitism of larval *E. postvittana* is reported in this thesis.

1.4 Abiotic factors influence the activity of natural parasitoids

There are number of abiotic factors that can influence the activities of parasitoids in agro-ecosystems. These includ temperature (Amat et al., 2006), light levels and moisture (Smith and Rutz, 1991), and other environmental conditions (Fink and Volkl, 1995). In vineyard agro-ecosystems, vineyards and adjacent vegetation are distinguishable habitats that differ in both biotic and abiotic conditions. Different parasitoid species may respond to varying abiotic conditions in different habitats, such as temperature and moisture, differently. An investigation of how abiotic factors affect the activities of parasitoids could provide insights into their distribution in vineyards and other habitats. An investigation of the effect of temperature on the development of *T. unimaculatus* is reported in this thesis.

1.5 Summary of project

Little was known about how larval parasitoids that attack the immature stages of *E. postvittana* interact in vineyard ecosystems, and it was not clear whether adjacent vegetation of the vineyard is functioning as a source of parasitoids in the vineyards.

Therefore an investigation of the parasitism of *E. postvittana* by larval parasitoids in both vineyards and adjacent habitats was conducted in two consecutive years (2011-2012 and 2012-2013). Regular insect sampling and a molecular identification protocol for larval tortricids were used to determine the host ranges of naturally occurring larval parasitoids in both vineyards and natural habitats. The results are reported in Chapter 2. This study laid the foundation for the rest of the studies that are reported in this thesis.

The aim of the studies reported in Chapter 3 was to elucidate the competitive interactions between the two major larval parasitoids *of E. postvittana*, *D. tasmanica* and *T. unimaculatus,* and examine how competitive interactions could affect their co-existence in vineyards. A series of laboratory experiments was conducted. The first examined whether hosts already parasitised by one species are equally attractive to the other species compared to un-parasitised larvae. The second examined how the intrinsic competition that occurs within a parasitised host affects the survival of parasitoid larva.

Chapter 4 presents experiments to understand how different plant species affect the foraging efficiency of *D. tasmanica* and *T. unimaculatus*, and the levels of parasitoid activity in vineyards. Both laboratory and field experiments were conducted. The first experiment examined whether naïve female *D. tasmanica* and *T. unimaculatus* show innate preference for different host plant species infested with larval *E. postvittana.* The second examined whether a previous oviposition experience on a host fed on a selected plant species altered the host plant preferences of the parasitoids. The third examined the foraging efficiency for *E. postvittana* of the parasitoids when *E. postvittana* fed on different plant species. The fourth investigated whether parasitism of *E. postvittana* varies among host plant when they occur together in vineyards.

In chapter 5 I present the effects of temperature on *T. unimaculatus* under laboratory conditions at seven constant temperatures. The potential for synchronization between populations of *T. unimaculatus* and *E. postvittana* under different climatic conditions were explored with a non-linear model.

The main findings of this project are synthesised and integrated in Chapter 6. A general discussion is presented to bring together conceptually factors that could affect the activity of natural enemies in both vineyards and natural habitats. The results of this study and its potential contribution to biological control of *E. postvittana* are synthesised. Possible future studies are also discussed.

CHAPTER TWO

The activities of generalist parasitoids can be segregated between crop and adjacent non-crop habitats

Statement of Authorship

The activities of generalist parasitoids can be segregated between crop and adjacent non-crop habitats

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For submission to Biocontrol

YF conceived and designed the experiments, carried out the experiments, generated sequencing data and conducted molecular analyses, analysed the data, and wrote the manuscript. MK conceived and designed the experiments and analysed the data. OK analysed the data; Other authors provided conceptual and editorial advice throughout the project.

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Abstract

Non-crop habitat adjacent to crops may be important for enhancing the activity of natural enbemies in crops. However, it is not clear whether parasitoids that actively attack pests in crops are directly associated with those active in adjacent non-crop habitats. We hypothesised that parasitic wasps that utilise the same hosts can co-exist through partitioning activities between crop and non-crop habitats in an agro-ecosystem. We tested this hypothesis using the light brown apple moth, *Epiphyas postvittana*, in vineyards and adjacent native vegetation as the model system. We experimentally measured the parasitism rate of larval *E. postvittana* at six and eight sites in both vineyard and the adjacent native vegetation in two consecutive years. Wild larval Tortricidae were also collected at each experimental site to assess their diversity and related parasitoids. Parasitised hosts were then identified using a PCR-based protocol to examine the parasitoids' host ranges. The parasitoid *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae) was most active in non-crop native vegetation, whereas *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) parasitised the most larvae in vineyards. Parasitism of *E. postvittana* by *D. tasmanica* was higher on grape than on plantain, which indicates that host plants influence activities in different habitat. Both species shared the same range of tortricid host insect species. Overall, our results indicate the two key parasitoids that attack *E. postvittana* differ in their partten of habitat use. The native vegetation adjacent to crops may not enhance the activity of some natural enemies for pest control in an agricultural ecosystem.

Key-words: agroecosystem; host specificity; native vegetation; parasitoids; Tortricidae; natural enemy

Introduction

Mordern agriculture production has intensively reduced non-crop habitat areas and simplified agriculture lanscapes. Therefore, most agricultural ecosystems occur as a fragmented mosaic of crop and non-crop habitats in which arthropod biodiversity and the efficiency of natural biological control are declining (Bianchi et al. 2006; Meehan et al. 2011). The remaining non-crop habitats, espically woody vegetation in agriculture landscapes could be important to enhance the activities of natural enemies and their effectiveness in sustainable pest suppression (Landis et al. 2000). Non-crop habitats may provide natural enemies with resources such as overwintering shelter (Cortesero et al. 2000; Landis et al. 2000a), alternative hosts (Pfannenstiel and Unruh 2003; Williams and Martinson 2000) and food (Begum et al. 2006; Berndt and Wratten 2005; Berndt et al. 2006; Gámez-Virués et al. 2009; Lee and Heimpel 2008; Wratten et al. 2004). These resources are usually limited in highly disturbed and simplified crop habitats (DeBach and Rosen 1991; Landis et al. 2000a; Van Emden 1965). Therefore, non-crop habitats may enhance the abundance and activity of predatory species for crop habitats (Landis et al. 2000a; Rand et al. 2006; Wilby and Thomas 2002). Moreover, the abundance, activity or impact of natural enemies at the boundary of crop and non-crop habitats, so-called ‘edge effects’, are often higher than that in field interiors (Duelli et al. 1990; Dyer and Landis 1997; Rand et al. 2006; Thies and Tscharntke 1999; Tylianakis et al. 2004). This could be due to the proximity of alternative resources in the adjacent habitats (Rand et al. 2006). Predatory groups often move between adjacent habitats in agro-ecosystems. These movements could be driven by spatiotemporal variability in resource availability and resource utilization patterns (Holt and Hochberg 2001; Oksanen 1990; Ries et al. 2004). However, the increase of natural enemy activity is

not always linked to effective pest control (Gurr et al., 2000; Olson and Wackers 2007; Holland and Fahrig, 2000). In addition, it is not always clear whether natural enemies that are active in non-crop habitats actually contribute to pest suppression in adjacent crop habitats.

Studies investigating and comparing communities of predator or parasitoid species in crop and non-crop habitats are typically performed by collecting data from insect traps (for example, Miliczky and Horton 2005) or through direct sampling methods (Alhmedi et al. 2011; Dennis and Fry 1992). The abundance of relatively larger mobile parasitoids has been suggested to be unaffected by local vegetation when assessed using yellow sticky traps in crops (e.g., Thomson and Hoffmann 2010). However, in practice it is hard to accurately identify flying insects collected on yellow sticky traps. Moreover, the absence or presence of highly mobile adults may not be a good indicator of actual parasitism. Sampling of parasitoids using sentinel plants and host insects has been demonstrated to be an effective way to measure their activity in many studies (Suckling et al. 2001; Furlong et al. 2007; P Mackey & Unruh 2012; Unruh et al. 2012). Therefore, controlled experiments are necessary to investigate thoroughly the direct interactions between natural enemies and their shared host species.

Host–parasitoid systems have long been used as model systems for studying ecological and evolutionary questions. The light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), and its larval parasitoids in vineyard ecosystems were the focal species. *E. postvittana* belong to the family Tortricidae, which is one of the largest families of Lepidoptera, with over 10,000 described species. This family includes numerous major pests of crops, forests, and ornamental plants (Brown 2005). Hence an understanding of factors that affect

parasitism of *E. postvittana* is likely to be relevant to many other pest species and agroecosystems. Larval *E. postvittana* is a polyphagous leafroller that is known to feed on plants from 123 genera in 55 families (Suckling and Brockerhoff 2010). It is one of the most damaging insect pest species on grapes in Australian vineyards (Scholefield and Morison 2010). At least 26 species of parasitoids and hyperparasitoids are reported to be associated with *E. postvittana* in Australia (Paull 2007). *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) is recorded as being the most abundant parasitoid of larval *E. postvittana* (Stephens et al. 1998; Suckling et al. 1998), accounting for 70 % or more of the observed parasitism in vineyards (Paull and Austin 2006). Another larval parasitoid, *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae), has been documented as common in the least-disturbed native vegetation (Paull 2007). Both *D. tasmanica* and *T. unimaculatus* could attack *E. postvittana* that has already been parasitized by the other species, while larval *D. tasmanica* outcompete larval *T. unimaculatus* when they occur in the same host (chaper 3).

a number of leaf roller species other than *E. postvittana* are present in vineyards and natural habitats. Little is known about the trophic links between parasitoids and their host leaf rollers in Australian vineyards and natural habitats, in part because there are no accurate morphological characters that can distinguish larvae of tortricid species, and parasitoids kill larvae before adults emerge. Molecular methods, especially DNA barcoding using short DNA sequences of a standardised region of the genome, have become useful tools for identifying species (Hajibabaei et al. 2006; Smith et al. 2008) and detecting trophic links in complex food webs (Rubinoff et al. 2011; Santos et al. 2011). In addition, molecular operational taxonomic units (MOTUs) can be employed when undescribed species are encountered. These are

groups of organisms that can be designated in taxonomic studies without necessarily being assigned a formal taxonomic rank. MOTUs based on DNA barcoding are widely used to investigate biological diversity and trophic links among poorly understood organisms (Blaxter et al. 2005; Floyd et al. 2002; Santos et al. 2011; Smith et al. 2009; Smith et al. 2005).

We aimed to investigate whether parasitoids attack the early larval stages of *E. postvittana* equally between vineyards and adjacent habitats. Field experiments were conducted in both vineyards and adjacent vegetation. Sentinel plants infested with young larval *E. postvittana* were used to determine the levels of parasitism. In addition, we tested whether host plants affect parasitism by comparing parasitism levels between two sentinel host plant species. To complement our field experiments, leaf rollers and related parasitoids were sampled twice per month in both the vineyards and adjacent native vegetation during the experimental periods. A PCR-based protocol was used to determine the trophic links between indigenous leaf rollers and related larval parasitoids. A phylogenetic tree-based method was used to identify leaf roller MOTUs that are attacked by the two major parasitoids, *D. tasmanica* and *T. unimaculatus*.

Materials and methods

Study site

To examine tparasitism of *E. postvittana* in both vineyards and the adjacent woody habitats, two series of field experiments was conducted at six and eight vineyards in Adelaide Hills, South Australia over two years (Table 1). Naturally occurring leafrollers and their associated parasitoids were also surveyed additional natural conservation site. The eight vineyards were all located in the Adelaide Hills region in

South Australia. The closest and farthest distances between two sites were 5.6 and 51 km, respectively. Pesticides of low toxicity to natural enemies including sulphur, tebufenozide and chlorothalonil were used at some sites.

Plants and insects

To investigate parasitism of *E. postvittana* by indigenous parasitoids, two sentinel plant species were used, the grapevine, *Vitis vinifera* L., and plantain, *Plantago lanceolata* L. *Plantago lanceolata* is a common host plant for *E. postvittana* in south Australia (Paull 2007). Potted plantain was grown from seed, several plants per pot, in a glasshousethree months before the experiment. Both grape and *P. lanceolata* are easy to grow so that sentinel potted plants could be standardised for age and quality.

Shiraz grape was used for the 2011 experiment and Chardonnay for the 2012 experiment. Chardonnay was chosen because population density of *E. postvittana* on this variety were observed to be relatively higher that others (Paull 2007). Shiraz was chosen due to their availability and the shortage of Chardonnay. Both the plantain and grape plants were planted in nursery pots (300 mm × 120 mm ×150 mm) in a glasshouse and were moved to a field cage to acclimatise to natural conditions four weeks before the field experiments.

The culture of *E. postvittana* was maintained at 22 ± 2 ºC under a 12 L: 12 D cycle (for details, see Yazdani et al. *in press*). To obtain eggs of *E. postvittana*, six female and six male moths were held in a plastic cup with vertical ridges for mating and oviposition. A dental wick soaked in a 10% honey solution was placed in each cup to provide water and food for the moths. The cups were maintained under

natural light at room temperature. The moths laid their eggs in masses of 30-40 on the cup ridges, which were cut into small pieces (3×1 cm) to transfer them to plants.

General methods for field experiments

Each plant was infested with approximately 30 LBAM eggs. We covered these inoculated plants with perforated plastic bread bags to prevent the neonate *E. postvittana* from escaping. The larvae were allowed to settle on the plants for three days before moving them to the field. A 4 l plastic bottle provided water for the plants through two 10 mm siphoning ropes that were buried in the soil. Previous experiments showed that this construction maintains a water supply for the plants for up to two weeks. Due to the hot and dry weather, the bottle was refilled with water once per week during the experiments. To prevent the plants from falling over, the potted plants were tied together with the water bottle.

The plants were collected after two weeks in the field. Next, larvae from the same plant were transferred to labelled plastic rearing cups (440 ml, 64 mm x 118 mm diam) containing fresh grape or plantain leaves and a piece of dry tissue paper. The larvae were reared at 22 ± 2 °C under a 14 L:10 D light/dark cycle to determine the parasitism rate. The state of each larva was monitored until it produced a parasitoid, pupated, or died.

The 2011 parasitism experiment

A field experiment was conducted at six vineyards in the Adelaide Hills, in South Australia. The experiment were conducted twice. Plants infested with first-instar *E. postvittana* were added to the field over three consecutive days at a rate of two vineyards per day on 7-9 November and 2-4 December 2011 for the first and second

sample periods, respectively. In each vineyard, potted plants were placed at three locations: in the vineyard interior (40 m from the border), the vineyard edge (along the border row of vines), and in the native vegetation adjacent to the vineyard (20 m from the border). We placed six pots of grape and plantain in pairs at a distance of six meters between the pairs in each type of location. Therefore, 36 plants were placed in each vineyard during each experiment. Infested plants were left in the field for two weeks

The 2012 parasitism experiment

A field experiment was performed in eight vineyards, six were those visited in 2011 and two additional vineyards. The experiment was set up over four consecutive days at a rate of two vineyards per day on 12-15 November and 4-7 December for the first and second sampling periods, respectively. We changed the experimental design from the previous year to increase statistical power. The border location was eliminated and grape was the only host plant. At each sampling point, we placed pots with grape in quadruplets in the vineyard and adjacent vegetation. The pots were placed three meters apart in a square arrangement at each sampling point (across two rows of vines within vineyards), and four groups of four pots were spaced 12 m apart. Thus 16 plants were assessed at each type of location, with a total of 32 plants being assessed at each vineyard during each visit.

Statistical analysis

To analyse the factors affecting parasitism of the experimentally introduced *E. postvittana*, the data from the 2011 and 2012 field experiments were modelled with the orthogonal split-split-plot and a split-plot general linear models, respectively, with the GLM procedure in the statistical package GenStat for Windows, 15th Edition

(VSN International, Hemel Hempstead, UK.). The vineyards were considered random blocks. For the 2011 experiment, the three habitat locations within each site (the vineyard, the border and the adjacent vegetation) were the main plot factor, the type of host plant was the split-plot factor, and the two repeated visits during the experiment constituted the split-split-plot factor. For the 2012 experiment, the habitat locations were the main plot factor, and the repeated visits were the split-plot factor. For all experiments, the fractions of larvae that were parasitised by 1) any species (overall), 2) *D. tasmanica* and 3) *T. unimaculatus* were calculated from the pooled numbers; these numbers were treated as the dependent variables. A modified Freeman and Tukey transformation (Zar 1999) was used to analyse the parasitism data to conservatively deal with the heterogeneity of variance in the presence of zero-inflation. The level of significance was set at 5% ($P < 0.05$). To determine if vineyard and adjacent vegetation habitats have significant effects on the activity of parasitoids, a one-degree contrast was conducted in both years.

Parasitoid-host trophic links

To determine the trophic links between leaf rollers and parasitoids, we sampled leaf rollers in both the vineyards and adjacent vegetation every two weeks during the field experiments. In addition, to more broadly survey the naturally occurring leaf rollers and related parasitoids in the Adelaide Hills region, a regular monthly survey was carried out on plants in a non-agricultural area: the Waite Conservation Reserve, Adelaide, South Australia from February 2011 to July 2012. A scanning sampling method was used to maximise the number of plants and grape leaf shoots sampled. Damaged leaves were the main plant parts sampled. We searched for leaf roller larvae or pupae for two hours at each sampling time. The collected leaf rollers were reared in small plastic cups (100 ml) with host plant leaves, and parasitism rates

were recorded. The leaf roller cadavers remaining after parasitoids emerged and the adult moths that emerged were preserved in 95% ethanol for identification through DNA analysis.

To determine the trophic links between the naturally occurring leaf rollers and parasitoids, the specimens were characterised through partially sequencing the mitochondrial cytochrome oxidase 1 (MT-CO1) gene using a PCR-based protocol. The PCR amplifications were performed using an MJ Research PTC-200 Thermo Cycler PCR system (MJ Research, Inc.). Partial COI sequences for the leaf rollers were amplified using the Lepidoptera-specific primers LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') and MH-MR1 (5'-CCTGTTCCAGCTCC-ATTTTC-3') (Hajibabaei et al. 2006; Rougerie et al. 2011). PCR was carried out in a 50 µL-reaction volume, contained 10× buffer, 10 mM MgCl, 2.5 pM each primer, 200 µM dNTPs, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 10–20 ng (1–2 µL) of genomic DNA and 1 U of Taq DNA polymerase (Platinum Taq DNA polymerase; Invitrogen). The thermal profile was as follows: 1 min at 94 °C, followed by five cycles of 40 s at 94 °C, 40 s at 45 °C and 1 min at 72 °C, followed by 35 cycles of 40 s at 94 °C, 40 s at 51 °C and 1 min at 72 °C, with a final extension step at 72 °C for 5 min. The PCR products were visualised using 2% agarose. Purified samples showing weak-to-strong bands were purified with a PCR product purification kit (Promega, Madison, Wisconsin, USA) and sent to the Australian Genomic Research Facility, Adelaide, South Australia for sequencing. The purified PCR products were unidirectionally sequenced using the primer LepF1 in 10 µL reaction volumes.

Partial MT-CO1 DNA sequences were obtained from host tissue remains. These sequences were then matched with partial MT-CO1 sequences with a number of known species in the GenBank public database (accession numbers KF395763.1,

KF404142.1) and partial MT-CO1 sequences of *Epiphyas* species provided by Dr Roberta Hitchcock (sequence ID ww09288, ww09286, ww04414). These sequences were aligned using the program ClustalX (Thompson et al. 1997). To calculate the genetic distances between each sequence pair, we employed MEGA 5.2 (Tamura et al. 2011) with the Kimura 2-parameter model. A neighbour-joining (NJ) tree based on the K2P distances was constructed using MEGA 5.2, and 1,000 bootstrap replicates were employed to calculate branch support. Sequences assigned to the same node were considered to belong to the same MOTU.

Results

The 2011 parasitism experiment

A total of 2,112 and 2,076 larval *E. postvittana* were recovered from the first and second sampling periods, respectively. The number of larvae varied because eggs are laid in masses that naturally vary in size and not all larvae survived. Thus, the total number recovered was less than the number placed in the field. More larvae were recovered from plantain than grape ($df = 35$, $t = 2.03$, $P < 0.001$). Three parasitoid species were recovered from the larvae placed on plants: *D. tasmanica*, *T. unimaculatus*, and *Phytodietus celsissimus* (Turner) (Hymenoptera, Ichneumonidae).

Dolichogenidea tasmanica parasitised the most larvae in the vineyards and was either absent or parasitised only a few larvae in the native vegetation (Fig. 1). The contrast for parasitism by *D. tasmanica* between vineyard and adjacent vegetation was statistically significant in the split-split plot analysis ($F_{1, 10} = 6.67$, $P = 0.027$). Parasitism by *D. tasmanica* at the border did not differ significantly from that in either adjacent vegetation or vineyards (post-hoc Fisher test with the Bonferroni adjustment,

P > 0.05). Parasitism by *D. tasmanica* was significantly higher on grape than plantain ($F_{1, 15}$ = 6.38; P = 0.023).

Parasitism by *T. unimaculatus* was significantly higher in adjacent vegetation than in vineyards ($F_{1, 10}$ = 17.2, P = 0.002). At the border, the parasitism rate was lower than in adjacent vegetation (P < 0.05) but not different from that in the vineyards (post-hoc directional Fisher test with the Bonferroni adjustment). Parasitism by *T. unimaculatus* was significantly higher in December 2011 than in November 2011 ($F_{1, 15}$ = 5.26; P < 0.05). There was no significant statistical effect of host plant or any other interaction effects on parasitism by *T. unimaculatus*.

Total parasitism was only significantly higher in adjacent vegetation than vineyards and the borders (ANOVA, followed by multiple comparisons with a Bonferroni test, $F_{2, 10}$ = 4.33; P < 0.05). There was no significant statistical effect of host plant or any other interaction effects on total parasitism

The 2012 parasitism experiment

A total of 2,032 and 1,800 larval *E. postvittana* were recovered from the first and second sampling periods, respectively. Only two parasitoid species were recovered, *D. tasmanica* and *T. unimaculatus* (Fig. 2). *D. tasmanica* parasitised the most larvae in the vineyards at five out of the eight sites and was not found in the native vegetation. Parasitism by *D. tasmanica* was significantly higher in the vineyards than in the adjacent vegetation ($F_{1, 7}$ = 7.30; P < 0.05). There were no significant statistical interaction effects involving parasitism by *D. tasmanica*. Parasitism by *T. unimaculatus* was significantly higher in the adjacent vegetation than in vineyards ($F_{1, 7}$ = 13.70; P < 0.05). There were no significant statistical effects of the replications, sampling points or any other interaction effects involving parasitism by *T.*

unimaculatus. The total parasitism was significantly greater in the adjacent vegetation than in the vineyards ($F_{1, 7} = 11.40$; $P < 0.05$), and in December 2012 compared to November 2012 ($F_{1, 56} = 6.08$; $P < 0.05$).

Parasitoid-host trophic links

In 2011, 110 leaf rollers were collected from the vineyards, and 125 were collected from the adjacent vegetation. In 2012, 379 leaf rollers were collected from vineyards, and 106 leaf rollers were collected from the adjacent vegetation. *D. tasmanica* and *T. unimaculatus* were the most abundant parasitoids. Parasitism was greater in the adjacent vegetation than in the vineyards in both seasons (Fig. 3).

From February 2011 to January 2012, 834 leaf rollers were collected from the Waite Conservation Reserve. The leaf rollers collected were *E. postvittana*, *Merophyas divulsana* (Walker) (Tortricidae, Lepidoptera), *Acropolitis rudisana* (Walker) (Tortricidae, Lepidoptera), and other unidentified species. There were 365 parasitised leaf rollers collected. These leaf rollers were parasitised either by wasps (324) or other parasitoids (41). The hymenopteran parasitoids that were reared included *D. tasmanica*, *T. unimaculatus*, *P. celsissimus*, a species of Cheloninae (Braconidae), and a *Bracon* sp. (Braconidae). Among these parasitoids, *D. tasmanica* and *T. unimaculatus* were the two most common species (Fig. 4). In addition to parasitic wasps, parasitic Tachinidae (Diptera) and nematodes were collected.

MT-CO1 DNA was successfully sequenced from 61 samples of remnants left by parasitoids. The corresponding GenBank accession numbers for host remnants are KF146183-KF146214 and KM115588-KM115616. The results indicated that *D. tasmanica* and *T. unimaculatus* share a range of host species and are not specific to

E. postvittana (Fig. 5). At least five host species MOTUs were revealed by the NJ tree, including *Epiphyas* spp., *Acropolitis* spp., *Merophyas* spp. and two other unidentified species groups.

Discussion

The two key larval parasitoids, *D. tasmanica* and *T. unimaculatus*, differ in their habitat use in vineyard ecosystems. *D. tasmanica* is mainly active in vineyards, while *T. unimaculatus*dominates in adjacent native vegetation. The parasitism pattern was consistent between the years and was supported by results from both field experiments (Figs 1 and 2) and vineyard sampling (Fig. 3). Even within the vineyards, *T. unimaculatus* mainly attacked hosts on cover crop plants under vines, rather than grape hosts (Fig. 3). There was a discrepancy in the observed levels of parasitism by *D. tasmanica* between sentinel plants and naturally occurring hosts. It are consistently parasitised leafroller hosts in non-crop habitats adjacent to vineyards (Fig. 3), while it rarely parasitised *E. postvittana* on the sentinel plants in those habitats (Fig. 1 & 2). Foraging choice by parasitoids can be altered by their experience (Steinberg et al. 1992; Vet and Dick 1992; Vet et al. 1995). The sentinel species plantain is not common in non-crop habitats adjacent to vineyards and grapes are absent. Therefore, naturally occurring *D. tasmanica* would have limited opportunities to gain experience on those plants, and would be expected to prefer to forage for hosts on locally more common plants.

T. unimaculatus mainly attacked hosts on cover crop plants under vines, rather than grape hosts (Fig. 3). However, in the natural habitats, the two parasitoids co-existed throughout the year (Fig. 4). This indicates the vineyard ecosystem may

strongly influence the composition of the parasitoid communites that attack leafroller species.

The molecular barcode analysis confirmed that both parasitoids are not specific to *E. postvittana*, but attack a number of leaf roller species (Fig. 5). Moreover, the findings of the field experiment and survey of naturally occurring leaf rollers indicated that parasitism rates in the vineyards and adjacent vegetation are typically less than 30 % (Figs. 1, 2 and 3), which suggests that there are plenty of un-parasitised hosts available for both parasitoid species. Therefore, there must be factors other than strong competitive interactions that affect the habitat partitioning of these parasitoids in vineyard ecosystems.

For both parasitoid species, the parasitism level at the vineyard border was not higher than that in their preferred habitats. This indicates that there are no substantial edge effects for both parasitoid species, and reinforces the proposition that both species have habitat preferences. Studies have indicated that some predator species or groups show strong habitat preferences either for natural habitats (Baldissera et al. 2004; Martin and Major 2001), or cropping habitats (Duelli et al. 1990; Orr et al. 2000). However, these studies are confined to single predator species. In our study, two generalist parasitoids that share the same hosts were assessed in both vineyard and adjacent ecosystems in which species interactions and differences between habitats could all affect their activities.

Habitat partitioning may affect ecosystem services such as pest control by parasitoids. In a study focussing on multispecies parasitoid-host systems, which involve three parasitoids that share the same whitefly species on cotton, different parasitoids attacked this host on different parts of host plants, and prey suppression

was maximised when all three parasitoids were present (Bogran et al. 2002). Recently, Derocles et al. (2014) demonstrated that aphidiine primary parasitoids of aphids rarely share hosts between crop and non-crop field margins. Furthermore, their result indicated that the non-crop field margins are not a substantial source of natural enemies that are active in crops. This finding is congruent with ours, even though the host insects are from different orders of insects. Therefore, conservation biological control may not rely on natural enemies from adjacent natural habitats. In our study, vineyards and the adjacent vegetation are two distinguishable habitats that differ in both biotic and abiotic characteristics. In addition, vineyards typically occur in spatially heterogeneous agroecosystems, where a number of factors generate spatial differences between vineyards and adjacent vegetation that may facilitate the coexistence of parasitoid species that attack the same hosts. These factors include plant diversity, cyclical seasonal changes, landscape characteristics, alternative host insects, host density fluctuations and abiotic factors.

Plants play a role that affects the activity of natural enemies at both local and landscape scales. In this study, parasitism of *D. tasmanica* differed between grape and plantain (Fig. 1), which suggests that plant species in, or adjacent to, a vineyard should affect levels of parasitism. In another study, parasitism of *E. postvittana* was consistently and significantly higher on the grape variety Cabernet Sauvignon compared to Chardonnay (Paull et al. 2013). Many other studies have demonstrated that plants affect the foraging success of predators and parasitoids through their architecture (Carter et al. 1984; Cortesero et al. 2000; Geitzenauer and Bernays 1996; Grevstad and Klepetka 1992) and by emitting herbivore-induced plant volatiles at sites of damage (Geervliet et al. 1994; Vet and Dicke 1992). Plants that emit highly attractive herbivore-induced plant volatiles are associated with higher

parasitism rates under both field and laboratory conditions (Poelman et al. 2009). Parasitoids rely on innate mechanisms to locate their hosts during foraging, but can also learn cues associated with host availability (Vet et al. 1995). Foraging experience on host plants can affect subsequent plant preferences (Papaj and Vet 1990). In a vineyard ecosystem, both exotic and native plants grow in, or adjacent to, vineyards. Indigenous parasitoids and their leaf roller hosts have established novel interactions with introduced host plants. The two native parasitoids may have different abilities to perceive and learn host plant-associated cues. For example, *D. tasmanica* may learn host-associated cues on grape and establish a preference for grape, while *T. unimaculatus* may not have the same capacity to perceive and learn cues associated with host plants, such as grapes. This hypothesis must be tested. Studies have demonstrated that planting species that support alternative hosts for natural enemies within or adjacent to crops may enhance parasitoid activity in crops (Letourneau and Altieri 1999; Pfannenstiel et al. 2012; Thomas et al. 1991; Unruh et al. 2012). But simply providing host plants won't necessarily enhance the activity of parasitoids. The attractiveness of host plants to parasitoids and how this could affect the actual parasitism in crop and adjacent non-crop habitats needs to be thoroughly investigated.

Cyclical seasonal changes in vineyard must influence the activity of parasitoids. In South Australian vineyards, wine grapes are deciduous plants while the most native plants are evergreen. During winter dormancy, there are limited resources such as hosts and other foods in vineyards for both parasitoids and their host insects, while these resources are still available in adjacent natural habitats. Therefore, vineyards may undergo cyclical colonisation by leaf rollers and parasitoids (Wissinger 1997). In this study, the field experiments were carried out during the

early vine growing season. We hypothesise that the two parasitoids, *D. tasmanica* and *T. unimaculatus*, respond to the seasonal changes differently, possibly due to differences in their mobility. *D. tasmanica* may be more mobile than *T. unimaculatus*, which would lead it to move from vineyard to other habitats during vine dormancy and return in the growing season. On the other hand, *T. unimaculatus* may tend to remain in the natural habitats where it is found throughout the year. *D. tasmanica* is known to travel at least 30 m over 7 days (Scarratt et al. 2008), but nothing is known about the mobility of *T. unimaculatus.* The mobility of these species need to be investigated further.

At the landscape scale, the quality and connectivity of remnant vegetation in agro-ecosystems is critical for parasitoid survival and diversity (Holzschuh et al. 2010; Kruess and Tscharntke 1994; Zabel and Tscharntke 1998), especially near crop edges (Bianchi and Van der Werf 2003; Miliczky and Horton 2005; Miliczky and Horton 2007; Pfannenstiel and Unruh 2003; Williams and Martinson 2000). In this study, each experimental site included adjacent native vegetation, which may facilitate the survival of some parasitoids, such as *T. unimaculatus*, that may not adapt well to cultivated crops (Paull 2007).

Host density may also affect parasitoid activity. Natural enemies often respond to their hosts in a density-dependent manner. In a study involving both naturally occurring hosts and experimentally manipulated hosts, the response of *D. tasmanica* to different host densities was found to be inversely density dependent (Paull et al. 2013). But there is no published information about the response of *T. unimaculatus* to the density of *E. postvittana*. The numbers of both parasitoids are low in their preferred habitats. This may affect their functional and numerical responses to their hosts, including *E. postvittana*. In addition, host densities are often

higher in crops than natural habitats (Segoli and Rosenheim 2013). Therefore, it is necessary to further determine if *D. tasmanica* and *T. unimaculatus* have responses to varying host densities, and whether these could affect their habitat preferences.

The activity of natural enemies could be affected by differing abiotic conditions between crop and non-crop habitats, including temperature (Amat et al. 2006), light and moisture (Smith and Rutz 1991), or other environmental conditions (Fink and Volkl 1995). In this study, the woody native vegetation adjacent to the vineyards included more shaded areas, and the temperature may have been lower than in the vineyard during hot weather. Therefore, the two dominant parasitoid species may prefer different abiotic conditions, such as temperature and moisture. *T. unimaculatus* may prefer woody vegetation, while *D. tasmanica* may be more active in open canopy areas, such as vineyards. These effects can be critical for conservation biology and biological control and need to be tested.

A growing body of evidence has emphasised the importance of natural habitats in promoting and maintaining the natural enemy activity in agro-ecosystems (DeBach and Rosen 1991; Landis et al. 2000b; Wilby and Thomas 2002). But this study demonstrates that parasitoids that share the same host in agro-ecosystems respond to crop and non-crop habitats differently. This indicates that the adjacent vegetation is not necessarily the likely source of some parasitoids of *E. postvittana* in vineyards. It implies more generally that non-crop vegetation cannot be relied upon as a source of natural enemies in agricultural systems. Therefore, for the purpose of conservation biological control, attention should not only be paid to the management of natural habitats or cultivated cropping areas. It is necessary to thoroughly understand the ecology of key natural enemies and how they interact with their hosts

in both crop and non-crop habitats. This could help to functionally explain the tri-trophic interactions involving herbivores, parasitoids and plants.

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Data Accessibility

Sequences deposited in GenBank (accession numbers: KF146183-KF146214; KM115588-KM115616)

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Figure Legends

Fig. 1. Fraction of experimental *E. postvittana* parasitised by *D. tasmanica*, *T. unimaculatus* and all parasitoids (mean ± SE). Data collected from two plants (grape and plantain), three locations (the vineyard interior, the border between the vineyard and the vegetation, and the adjacent vegetation) and two different visits (November 2011 and December 2011). Standard errors are for descriptive purposes only; the analysis was done on transformed data.

Fig. 2. Fraction of the experimental *E. postvittana* parasitised by *D. tasmanica* and *T. unimaculatus* given as the mean (± SE), using pooled data from eight sites at two locations (the vineyard interior and the adjacent vegetation) and two different visits (November 2012 and December 2012). Standard errors are for descriptive purposes only; the analysis was done on transformed data.

Fig. 3. Fraction of leaf rollers parasitised by larval parasitoids of naturally occurring leaf rollers (November to December 2011 and August to March 2013), using pooled data from six sites for 2011 and eight sites for 2012.

Fig. 4. Fraction of leaf rollers parasitised by *D. tasmanica*, *T. unimaculatus* and other parasitoids collected monthly from February 2011 to January 2012 at the Waite Conservation Reserve, Urrbrae, South Australia. The numbers above each bar indicate the number of leaf rollers collected.

Fig. 5. Neighbour-joining tree constructed with partial MT-CO1 sequences from the leaf roller remains left by parasitoids (based on K2P genetic distances), which indicate four MOTUs (within brackets). The branches are labelled with the sample number of the host leaf roller. The symbols on the right side of the sequence name indicate the reared parasitoids (triangles represent *T. unimaculatus*, and squares

represent *D. tasmanica*). The symbols on the left side of the sequence correspond to the habitats from which the leaf rollers were collected (black circle, wine grapes in the vineyard; white circle, natural habitats not adjacent to vineyard; black diamond, cover plants in the vineyard; and white diamond, adjacent vegetation). For each sample, Accession numbers of sequences obtained from GenBank are presented. The numbers next to the branches represent the bootstrap values after 1,000 replications. Values lower than 50 are not represented. The scale bar indicates a 1 % sequence divergence.

Fig. 1

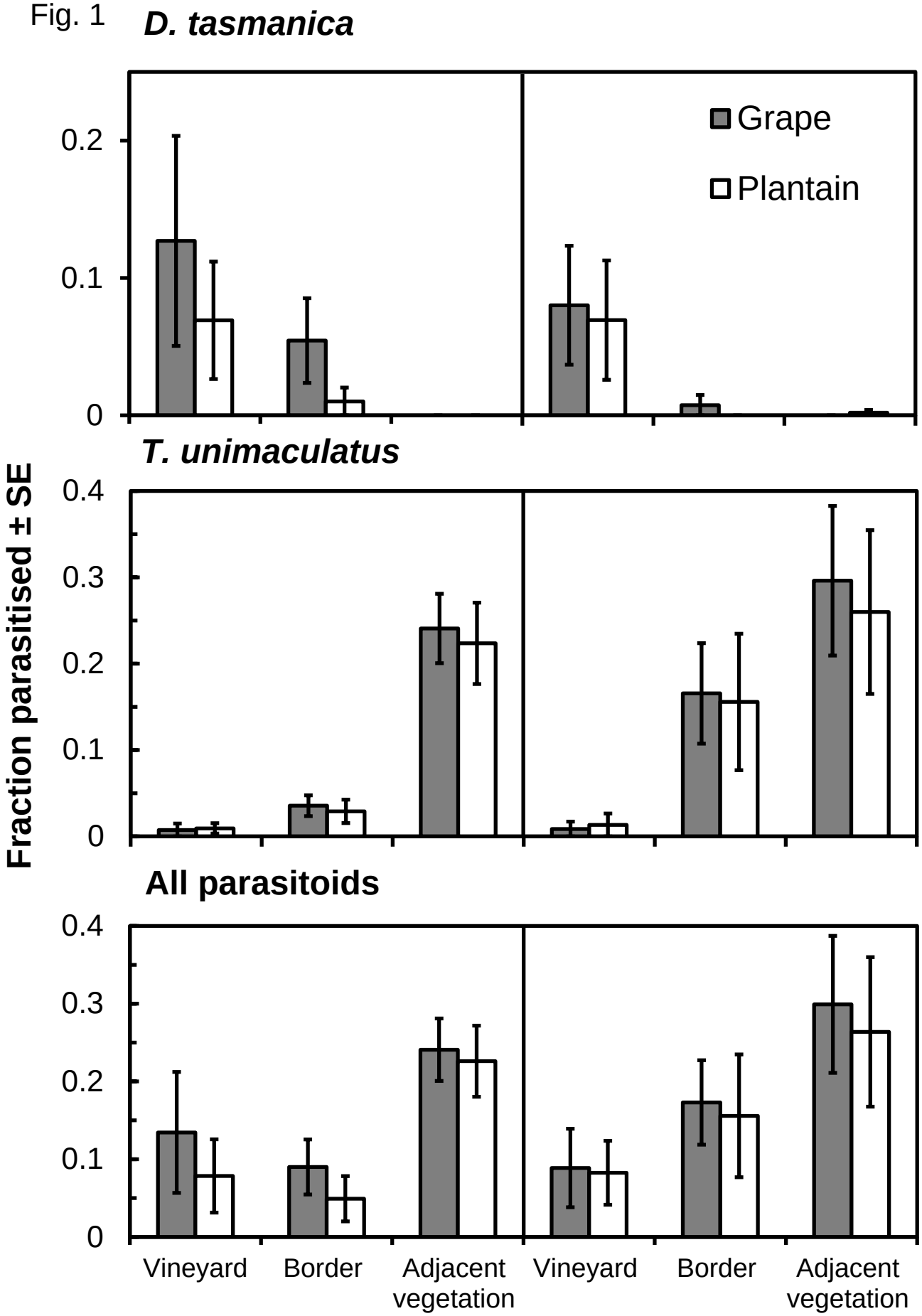
D. tasmanica
Grape
Plantain
0.2
0.1
0
T. unimaculatus
0.4
0.3
0.2
0.1
0
Fraction parasitised ± SE
All parasitoids
0.4
0.3
0.2
0.1
0
Vineyard
Border
Adjacent vegetation
Vineyard
Border
Adjacent vegetation
Nov. 2011
Dec. 2011

Fig. 2

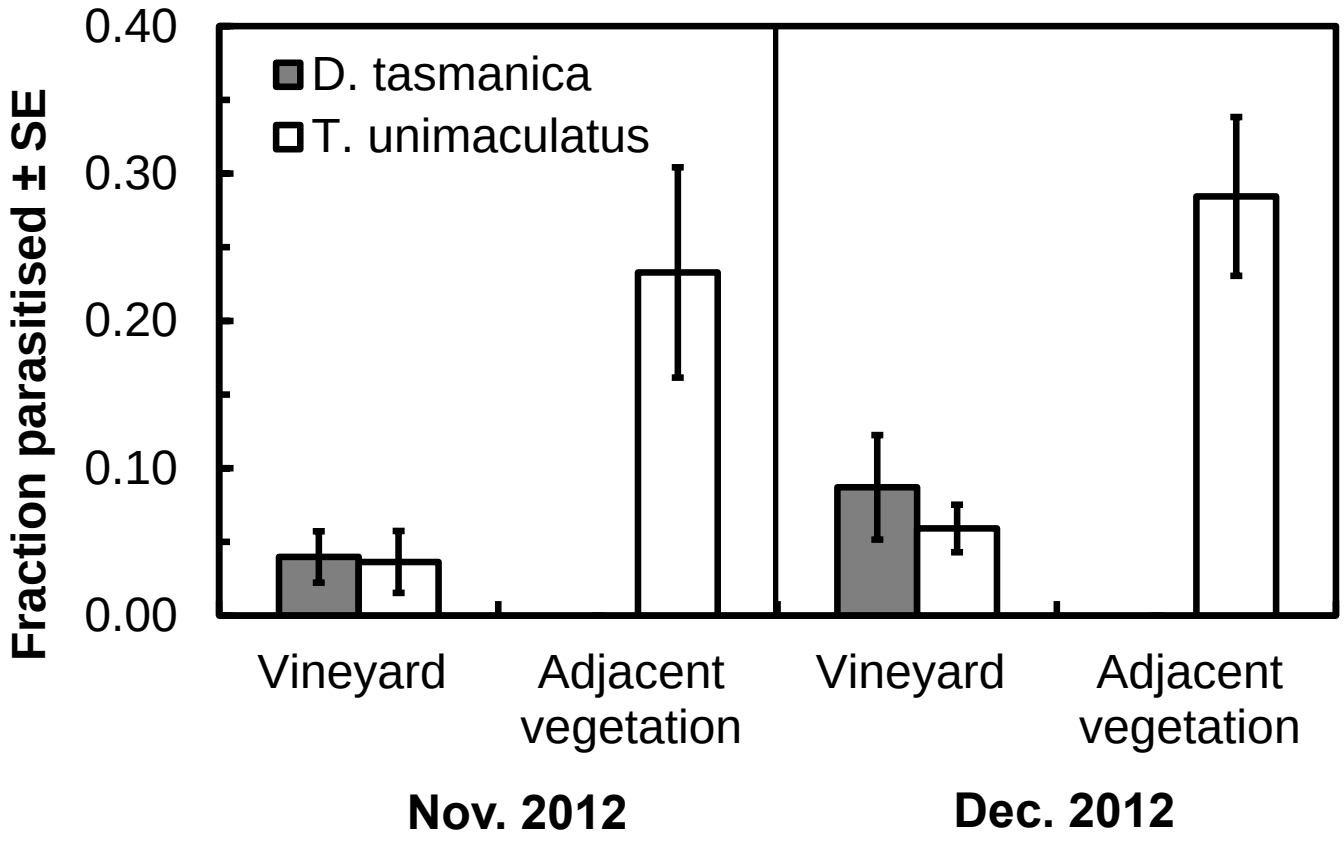
Fraction parasitised ± SE
0.40
0.30
0.20
0.10
0.00
D. tasmanica
T. unimaculatus
Vineyard
Adjacent vegetation
Vineyard
Adjacent vegetation
Nov. 2012
Dec. 2012

Fig. 3

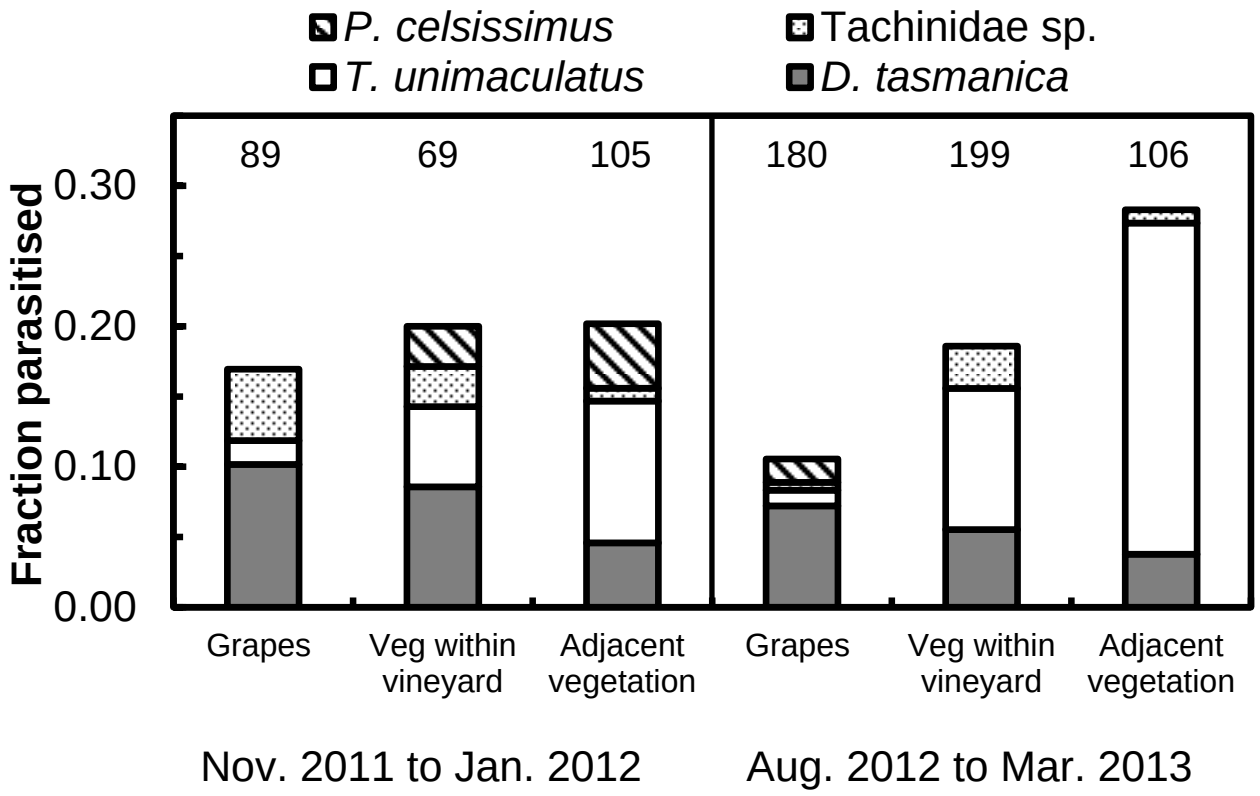
P. celsissimus
Tachinidae sp.
T. unimaculatus
D. tasmanica
Fraction parasitised
0.00
0.10
0.20
0.30
89
69
105
180
199
106
Grapes
Veg within vineyard
Adjacent vegetation
Grapes
Veg within vineyard
Adjacent vegetation
Nov. 2011 to Jan. 2012
Aug. 2012 to Mar. 2013

Fig. 4

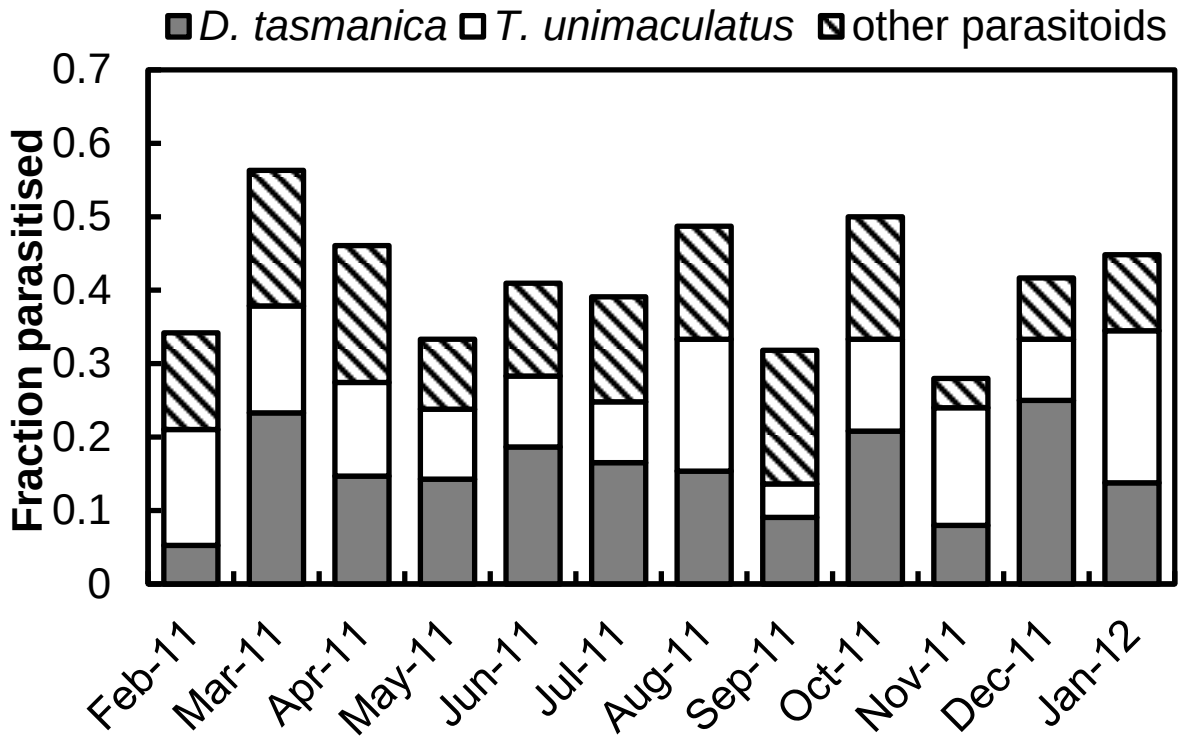
D. tasmanica
T. unimaculatus
other parasitoids
Fraction parasitised
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Feb-11
Mar-11
Apr-11
May-11
Jun-11
Jul-11
Aug-11
Sep-11
Oct-11
Nov-11
Dec-11
Jan-12

Fig. 5

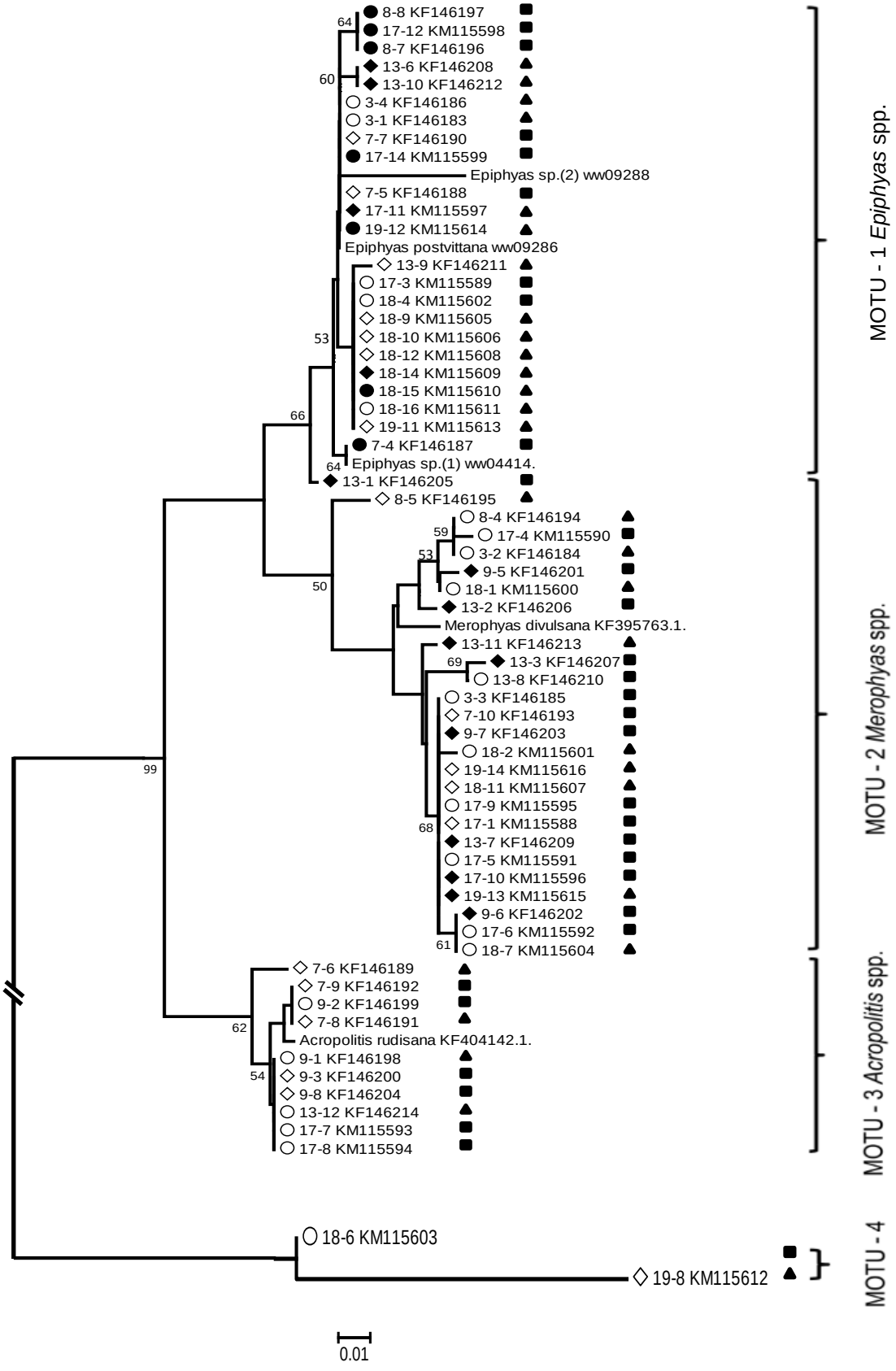
8-8 KF146197
17-12 KM115598
8-7 KF146196
13-6 KF146208
13-10 KF146212
3-4 KF146186
3-1 KF146183
7-7 KF146190
17-14 KM115599
Epiphyas sp.(2) ww09288
7-5 KF146188
17-11 KM115597
19-12 KM115614
Epiphyas postvittana ww09286
13-9 KF146211
17-3 KM115589
18-4 KM115602
18-9 KM115605
18-10 KM115606
18-12 KM115608
18-14 KM115609
18-15 KM115610
18-16 KM115611
19-11 KM115613
7-4 KF146187
Epiphyas sp.(1) ww04414.
13-1 KF146205
8-5 KF146195
8-4 KF146194
17-4 KM115590
3-2 KF146184
9-5 KF146201
18-1 KM115600
13-2 KF146206
Merophyas divulsana KF395763.1.
13-11 KF146213
13-3 KF146207
13-8 KF146210
3-3 KF146185
7-10 KF146193
9-7 KF146203
18-2 KM115601
19-14 KM115616
18-11 KM115607
17-9 KM115595
17-1 KM115588
13-7 KF146209
17-5 KM115591
17-10 KM115596
19-13 KM115615
9-6 KF146202
17-6 KM115592
18-7 KM115604
7-6 KF146189
7-9 KF146192
9-2 KF146199
7-8 KF146191
Acropolitis rudisana KF404142.1.
9-1 KF146198
9-3 KF146200
9-8 KF146204
13-12 KF146214
17-7 KM115593
17-8 KM115594
18-6 KM115603
19-8 KM115612
64
60
53
66
64
50
59
53
99
69
68
61
62
54
0.01
MOTU - 1 Epiphyas spp.
MOTU - 2 Merophyas spp.
MOTU - 3 Acropolitis spp.
MOTU - 4

Table 1. The location and description of eight experimental sites and one conservation park.

Vineyard or Conservation Park	Years	Latitude Longitude	Grape variety	Row width	Prominent cover plants with leaf rollers	Adjacent vegetation (vineyards)/vegetation (conservation reserve)
A	2011 2012	34°58'22"S 138°43'25"E	Pinot noir	3 m	*Plantago lanceolata, Arctotheca calendula*, *Trifolium* spp.	*Eucalyptus viminalis, Acacia pycnantha*, *Exocarpos cupressiformis*, *Calochlaenia dubia*, *Melaleuca armillaris*, *Plantago lanceolata*
B	2011 2012	34°56'36"S 138°50'21"E	Sauvignon-Blanc	2.1 m	*Plantago lanceolata., Arctotheca calendula*, *Trifolium* spp.	*Eucalyptus viminalis*, *Acacia pycnantha*, *Exocarpos cupressiformis*, *Calochlaenia dubia*, *Plantago lanceolata*
C	2011 2012	34°53'38"S 138°47'46"E	Chardonnay	2.5 m	*Plantago lanceolata., Arctotheca calendula*, *Trifolium* spp.	*Eucalyptus viminalis*, *Acacia pycnantha*, *Exocarpos cupressiformis*, *Calochlaenia dub*ia, *Melaleuca armillaris*, *Rosa* spp., *Plantago lanceolata*
D	2011 2012	35°4'13"S 138°34'0"E	Cabernet-Sauvignon	1.5 m	-	*Eucalyptus viminalis*, *Acacia pycnantha*, *Melaleuca armillaris*, *Rosa* spp.
E	2011 2012	34°50'39"S 138°50'43"E	Cabernet-Sauvignon	3 m	*Plantago lanceolata*	*Eucalyptus leucoxylon*, *Austrodanthonia setacea*, *Plantago lanceolata*
F	2011 2012	35°16'4"S 138°37'12"E	Cabernet-Sauvignon	3 m	*Plantago lanceolata*	*Eucalyptus leucoxylon*, *Acacia pycnantha*, *Calochlaenia dubia*, *Aristida behriana*, *Plantago lanceolata*
G	2012	35°13'S, 138°39'"E	Cabernet-Sauvignon	2.5 m	*Plantago lanceolata, Arctotheca calendula*	*Eucalyptus leucoxylon*, *Eucalyptus viminalis*, *Acacia pycnantha*, *Exocarpos cupressiformis*, *Calochlaenia dubia*, *Plantago lanceolata*
H	2012	35°00' 22" S 138°49'40" E	Pinot noir	2.5 m	*Plantago lanceolata*	*Eucalyptus leucoxylon*, *Plantago lanceolata*
Waite Conservation Reserve	2011 2012	34°58' 19" S 138°38'33" E	N/A	N/A	-	*Eucalyptus camaldulensis*, *Eucalyptus viminalis*,*Themeda triandra, Acacia melanoxylon*, *Exocarpos cupressiformis*, *Allocasuarina verticillata*, *Plantago lanceolata*, *Amyema miquelii*, *Allocasuarina verticillata*, *Rytidosperma* spp., *Arthropodium strictum*, *Bulbine bulbosa*, *Eryngium ovinum*, *Ranunculus lappaceus*

CHAPTER THREE

Interspecific competition between two generalist parasitoids that attack the leafroller *Epiphyas postvittana* (Lepidoptera: Tortricidae)

Statement of Authorship

Interspecific competition between two generalist parasitoids that attack the leafroller *Epiphyas postvittana* (Lepidoptera: Tortricidae)

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YF conceived and designed the experiments, carried out the experiments, analysed the data, and wrote the manuscript. SW provided conceptual and editorial advice throughout the project. HS provided conceptual and editorial advice throughout the project MK conceived and designed the experiments and analysed the data.

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Abstract

Two generalist parasitoids, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) and *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae) attack early instars of tortricid moths, including the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae). The two parasitoids co-exist, but *D. tasmanica* is dominant in vineyards and *T. unimaculatus* is mainly found in adjacent native vegetation. This difference suggests possible competition between the two species, mediated by habitat. Here, we report on the extent of interspecific differences in host discrimination and the outcome of interspecific competition between the two parasitoids. The parasitoids did not show different behavioural responses to un-parasitised hosts or those that were parasitised by the other species. Larvae of *D. tasmanica* out-competed those of *T. unimaculatus*, irrespective of the order or interval between attacks by the two species. The host larvae that were attacked by two parasitoids died more frequently before a parasitoid completed its larval development than those that were attacked by a single parasitoid. Dissection of host larvae parasitised by both species indicated that first instars of *D. tasmanica* attacked and killed larval *T. unimaculatus*.

Key words

Host discrimination, light brown apple moth, intrinsic competition, searching behaviour, parasitoid, *Dolichogenidea tasmanica*, *Therophilus unimaculatus*

Introduction

Understanding of the dynamics of competition among species of potential biological control agents, such as parasitoids, that share the same host species and habitat is important for evaluating their efficiency (Mackauer, 1990; Bogran et al., 2002; Gurr et al., 2004; De Moraes & Mescher, 2005; Harvey et al., 2013; Orre-Gordon et al., 2013). If individuals of two or more species of parasitoids attack the same single host at the same time, multiple parasitism may occur. Therefore, interspecific competition is expected to occur among these parasitoids (Godfray, 1994; Kato, 1996; Paull & Austin, 2006). Little is known about the extent and nature of such competition and its influence at the community level for parasitoids (Force, 1985; Godfray, 1994; Harvey et al., 2013). While competing parasitoid species can co-exist in the same environment (Hawkins, 2000; Van Nouhuys & Hanski, 2005; Aluja et al., 2013), certain circumstances, such as a lack of alternative host species, can lead to a situation where one parasitoid species dominates the parasitism of a host species (Pijls & Van Alphen, 1996). In some cases, an increase in the number of parasitoid species may result in a decline in the efficiency of biological control (Collier & Hunter, 2001; Collier et al., 2002). Therefore, it is important to understand how competing parasitoid species interact with each other and which species is superior in particular circumstances. Some studies have investigated competition between parasitoids with differing degrees of host specificity, and in most cases the generalists have a greater likelihood of outcompeting the more specialised species (Iwao & Ohsaki, 1996; De Moraes & Mescher, 2005). This study investigates competitive interactions that occur in Australian vineyards between two generalist larval parasitoids of a lepidopteran herbivore, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), the light brown apple moth (LBAM).

Interspecific competition between parasitoids can be classified as extrinsic competition, which includes all direct and indirect interactions between adult parasitoids while foraging for hosts, and intrinsic competition, which refers to indirect or direct competition among parasitoid larvae within an individual host (Harvey et al., 2013). Extrinsic competition usually occurs between adult conspecifics, and is not common inter-specifically (Boivin & Brodeur, 2006). It can involve aggressive behaviour between foraging females or the ability to discriminate against hosts already parasitised by other parasitoids. During intrinsic competition, larvae that share the same host could compete directly or indirectly through physical encounters or indirectly through physiological suppression of one species by another. In solitary parasitoids, intrinsic competition is severe, as it can results in the successful development of a single individual at the expense of others. Such competition has led to the evolution of entomological weaponry. For example, first instars of some parasitoids have large, sickle-like mandibles that are used to fight other larvae or destroy the eggs of competitors (Tian et al., 2008; Wang et al., 2008; Harvey et al., 2009; Paladino et al., 2010). In other cases, physiological suppression occurs when the parasitoid manipulates the physical and chemical environment of the host to create conditions that favour their own survival to the detriment of other parasitoids. This may involve venom, polydnavirus, teratocytes, or protein secretions that affect host development (De Moraes et al., 1999; Harvey et al., 2013).

The present study investigated extrinsic and intrinsic competition between two parasitoids, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) and *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae), that attack the light brown apple moth in Australia. *D. tasmanica* is reported to parasitise *Merophyas divulsana* (Walker) (Lepidoptera: Tortricidae) (Bishop & Mackenzie, 1991) and *E*.

postvittana (Paull & Austin, 2006). *T. unimaculatus* is known to parasitise hosts in at least three families and six genera of Lepidoptera. These include *Myrascia bracteatella* (Walker) (Lepidoptera: Oecophoridae), *Etiella behrii* (Zeller) (Lepidoptera: Pyralidae), and *M. divulsana*, *Phricanthes asperana* (Meyrick), *Acropolitis magnana* (Walker), and *E. postvittana* (Stevens et al., 2011). In addition, we have reared both species from the same species at least the same four genera of tortricids using DNA barcodes (Chapter 2). Therefore, we consider these species to be generalists in their host associations. The overlap in the host range of these parasitoids indicates that they are competitors, which is likely to influence their abundance in vineyards and in natural habitats. To study extrinsic competition between the two above parasitoid species, a wind tunnel was used to evaluate whether hosts already parasitised by the other species are equally attractive compared to un-parasitised larvae. Intrinsic competition was then studied by investigating the outcome of larval interactions between the two parasitoid species. We conclude by discussing how this information can help to understand co-existence of the two parasitoid species and its implications for their role in the biological control of *E. postvittana*.

Materials and methods

Study organisms

The light brown apple moth is a native Australian leafroller that attacks a wide range of host plants. It is the most destructive insect pest of wine grapes in Australia (Scholefield & Morison, 2010). This species has been introduced to and become a pest in New Zealand, the United Kingdom, Hawaii and California (Suckling & Brockerhoff, 2010). There are at least 25 parasitoid species associated with *E.*

postvittana in Australia (Paull & Austin, 2006). Some of these share a range of other host species (Yi Feng, un-published data). Among these, *D. tasmanica* is considered the predominant species in many habitats (Charles et al., 1996; Suckling et al., 1998; Paull, 2007; Suckling & Brockerhoff, 2010), while *T. unimaculatus* is found in natural habitats in which these two species coexist (*T. unimaculatus* is described as *Bassus* sp. in Paull and Austin 2006 and Paull 2007). In a survey conducted in eight vineyards in the Adelaide Hills in South Australia, *D. tasmanica* was most common in vineyards, while *T. unimaculatus* was most abundant in adjacent native vegetation (Chapter 2).

Insect rearing

E. postvittana was reared on an artificial diet (Yazdani et al., 2014) at 22 ± 2ºC, 12 L: 12 D. It has been kept in culture in an insect rearing room for nearly 200 generations, with the annual addition of field-collected individuals to maintain genetic diversity. The colonies of *D. tasmanica* and *T. unimaculatus* were originally established from parasitised leafrollers that were collected in vineyards near Adelaide (35° 16' S, 138° 37' E). Parasitoids were reared on larval *E. postvittana* that fed on narrow leaf plantain, *Plantago lanceolata* L. in cages at 23 ± 2 ºC, 14 L: 10 D. *E. postvittana* was collected at least once every two months from the field (35° 58' S, 138° 38' E), and adult parasitoids hatching from these larvae were added to the cultures, as was done for the leafroller. To obtain parasitoids for experiments, newly-formed parasitoid cocoons were kept in glass vials (18 mm × 50 mm) with a drop of 10% honey in water. All experiments were conducted with 2-3 day old mated females. Pilot observations showed that the two species were most active at oviposition at these ages.

Inter-specific host discrimination

An experiment was conducted to determine whether hosts already parasitised by the other species are equally attractive compared to un-parasitised larvae. Individual second-instar *E. postvittana* feeding on plantain leaves that had been parasitised by one parasitoid species were exposed to the other species in a random order in a variable speed wind tunnel at a wind speed of 20 cm/s at 21 ± 2°C (for details see Keller, 1990). For each parasitised larva, there was a control of an un-parasitised larva of the same age that was handled in the same way. Each parasitoid was observed twice, once with a parasitised larva and once with an un-parasitised control, which were presented to wasps in a random order. The experimental treatments were larval status (parasitised or not) and the time between ovipositions (< 10 min, 24 h and 48 h). Larval *E. postvittana* that were parasitised by either of the two parasitoids were periodically dissected. This revealed that eggs of *D. tasmanica* hatch after around 48 hours, while it took almost twice this time for eggs of *T. unimaculatus* to hatch. Therefore, 48 hours was selected as the maximum interval between ovipositions so neonates of the second parasitoid of either species would encounter hosts containing first instars of the first parasitoid to oviposit.

Larvae parasitised at different time-intervals were used randomly in the wind tunnel for each observation. The order of observation for each treatment and control was also randomized. There were 10 replicates for each treatment. Parasitoid behaviour was recorded using the *Observer* software, version XT11 (Noldus Information Technology, Wageningen, Netherlands). Stinging behaviour is directly related to oviposition and interspecific host discrimination (Vet et al., 1984), so the total duration of stinging, and total time the parasitoid spent on the leaf bearing the host larva were recorded and analysed. Behavioural data were log-transformed to

stabilise variances. Statistical analysis was performed using IBM *SPSS* Statistic 19. A two-way analysis of variance (ANOVA) was followed by a Bonferroni post-hoc test to analyse the influence of time interval and parasitism status on the duration of oviposition and the time spent on the infested leaf.

Intrinsic competition between T. unimaculatus and D. tasmanica

An experiment was conducted to determine whether intrinsic competition occurs within a parasitised host. Individual second-instar *E. postvittana* were maintained on single plantain leaves for 24 h before the experiment to allow the accumulation of host odour and potentially stimulate parasitoids to oviposit. An individual *E. postvittana* larva feeding on a plantain leaf was exposed to one mated female parasitoid in a glass vial (see above). Once stinging was observed, the parasitoid was removed and the second species of parasitoid was added at a predetermined time interval (< 10 min, 24 h and 48 h). Therefore, the variables in this experiment were 1) time interval between stinging and 2) parasitisation order. There were 30 host larvae for each treatment (180 larvae in total). Hosts parasitised by a single parasitoid species (30 for each species) were used as controls. After parasitism, all larvae were reared individually in 100 ml plastic containers with plantain leaves at 23 ± 2 ºC, 14 L: 10 D. Larvae were checked daily. The presence of parasitoid cocoons, mortality, the emergence of parasitoids, their sex, and developmental times from oviposition to cocoon formation and from cocoon formation to adult emergence were recorded.

To investigate the relative hatching times of larval parasitoids, 40 second-instar hosts, each parasitised by a single parasitoid species (80 in total), were dissected at various times. Hosts attacked by *D. tasmanica* were dissected after 40 to 60 h, while

those attacked by *T. unimaculatus* were dissected after 80 to 100 h. Each host larva was placed in water in a glass staining block and dissected with watchmaker's forceps under a 20 × magnification dissection microscope.

Although each parasitoid species can sting the host parasitised by the other species, it is not clear whether both parasitoid species actually lay eggs in host parasitised by the other. To investigate this and explore the possible mechanism of intrinsic competition between parasitoid larvae, 80 second-instar *E. postvittana* larvae parasitised by both species were prepared. The treatments for parasitisation were 1) time interval between stinging (< 10 min and 48 h) and 2) parasitisation order. Therefore, there were twenty larvae for each treatment. Twenty control larvae were parasitised by a single parasitoid species for each species. Parasitised hosts were then dissected using methods described above at a rate of several larvae/hour at 90 h following oviposition by *T. unimaculatus*. Hosts parasitised only by *D. tasmanica* were dissected 48 h after oviposition.

Differences in parasitoid emergence in this intrinsic competition experiment were analysed using a binominal test, with 0.5 as the null hypothesis. To analyse the significance of the differences in pre-emergent mortality and sex ratio of emerging parasitoids between single parasitised and multiple parasitised hosts, χ^2-tests were used. These tests were used to analyse whether the time-interval treatments and order of parasitism resulted in differences in parasitoid emergence rate. Parasitoid developmental times, from egg to cocoon and from cocoon to adult, were analysed using one-way ANOVA followed by an LSD post-hoc test for multiple comparisons (IBM SPSS Statistic 19).

Results

Inter-specific host discrimination

The presentation of parasitised and un-parasitised hosts did not elicit any differences in either mean sting duration (fig. 1, *D. tasmanica*: F = 0.065; df = 1,54; *P* = 0.800; *T. unimaculatus*: F = 0.074; df = 1,50; *P* = 0.786) or mean time the parasitoids spent on the leaf bearing the host larvae (*D. tasmanica*: F = 0.391; df = 1,54; *P* = 0.534; *T. unimaculatus*: F = 0.092; df = 1,50; *P* = 0.763; fig.1).

For *T. unimaculatus*, there were significant differences in the sting durations (F = 6.116; df = 2,50; *P* = 0.001) and searching times (F = 7.763; df = 2,50; *P* = 0.014) between time-interval treatments. Stinging duration was longest when the interval between first and second attack was 48 h. The time spent on the plant/host complex when the interval was less than 10 min was significantly shorter than at 24 h and 48 h. There was no detectable effect of the time interval between ovipositions on the stinging duration (F = 3.044; df = 2,54 ; P = 0.056) or time spent on the leaf bearing the host larvae by *D. tasmanica* (F = 0.839; df = 2,54; *P* = 0.438).

Intrinsic competition between T. unimaculatus and D. tasmanica

Irrespective of the order of parasitization or the interval between the two parasitisation events, the emergence rates of *D. tasmanica* were significantly higher than for *T. unimaculatus* (fig. 2). The pre-emergent mortality of multiple-parasitised hosts (31.1%) was significantly higher than for single-parasitised one (8.3%) (χ^2 = 12.32, df = 1, *P* < 0.001). For *D. tasmanica*, the sex ratio of multiple parasitised hosts (42.4% female) did not differ from that in single-parasitised hosts (46.5%) (χ^2 = 0.15, df = 1, *P* = 0.39). The sex ratio of *D. tasmanica* from hosts that had been parasitised by *D. tasmanica* first (45.6%) was not significantly different from hosts that had been parasitised by *T. unimaculatus* first (39.3%) (χ^2 = 0.47, df = 1, *P* =

0.98). In addition, for *D. tasmanica*, the sex ratio was not significantly different between time-interval treatments: within < 10 min (51.4%), 24 h (41.5%), and 48 h (35.7%) ($\chi^2 = 2.56$, df = 2, $P = 0.56$).

Dissections indicated that eggs of *D. tasmanica* hatched between 48 and 56 h after oviposition, while those of *T. unimaculatus* took around 90 - 96 h to hatch. However, eggs of *T. unimaculatus* could not be found until they hatched, in which case it was larvae that were detected. It is possible that the eggs of this species may be embedded within the tissues of the host. Both *D. tasmanica* and *T. unimaculatus* laid eggs in host parasitised by the other species (table 1). In those cases where first instars of both species were found within the same host, the larval *T. unimaculatus* were all dead, while all larval *D. tasmanica* were still alive (table 1). The developmental time of male and female larval *D. tasmanica* did not differ between any treatments. Therefore, data for parasitoid larval stage development for both sexes were pooled. Because in most cases, live *T. unimaculatus* did not emerge from hosts that had been parasitised by both parasitoids, data for larval-stage development of *T. unimaculatus* was from single parasitised hosts by *T. unimaculatus*. For *D. tasmanica*, the mean developmental time (order of oviposition and time interval between parasitism) did not differ among treatments for both oviposition to cocoon formation and for cocoon formation to adult emergence (table 2). The mean larval development time from oviposition to cocoon formation for *T. unimaculatus* was significantly longer than *D. tasmanica* (table 2).

Discussion

This study demonstrated that neither *D. tasmanica* nor *T. unimaculatus* responded differently to hosts parasitised by the other species compared to un-parasitised host (fig. 1). Since multiple parasitism is common among parasitoids (Van Alphen &

Visser, 1990), the lack of interspecific host discrimination leads to competition between larval parasitoids inside the same host. In this study system, there is no 'need' for the superior intrinsic competitor *D. tasmanica* to develop interspecific discrimination ability, because its offspring are likely to survive in instances of multiple parasitism (fig. 2). However, for the inferior intrinsic competitor, *T. unimaculatus*, selection should favour detection of factors that indicate parasitism by another superior competitor if selection pressure is strong. This would enable it to exploit hosts that are free from its superior competitor, *D. tasmanica*. The data indicate that the chance that its offspring will survive multiple parasitism are virtually nil.

Studies here suggested that coexistence of parasitoids that attack the same host species largely depends on their life history characteristics (Amarasekare, 2003; Harvey et al., 2013). For example, differences in dispersal ability may enable the co-existence of species that compete for the same hosts, if the relatively poor intrinsic competitor has an advantage in dispersal at the landscape scale (Hanski & Ranta, 1983; Yu et al., 2004). The dispersal ability of *D. tasmanica* is limited within vineyards (Scarratt et al., 2008), while the dispersal ability of *T. unimaculatus* in both natural habitats and agriculture settings is still unknown. It is necessary to further investigate the dispersal ability of these two competing species to determine how they respond to habitats at the local and regional scales.

Resource or niche partitioning is a main factor that facilitates the coexistence of competing species (Amarasekare, 2003). There are many possible differences in the characteristics of niches that could allow coexistence of competing parasitoids. *T. unimaculatus* and *D. tasmanica* may prefer different host plants, host species and/or stages of hosts. Furthermore, *T. unimaculatus* has a much bigger body and longer

ovipositor than *D. tasmanica*. Therefore, *T. unimaculatus* could probably attack hosts in shelters that are unreachable by *D. tasmanica*. On the other hand, the relatively smaller body size of *D. tasmanica* enables it to search in places that are not accessible to the relatively larger *T. unimaculatus*. Competing species with different competitive abilities that can co-exist through partitioning different niches have been studied in different multispecies parasitoids-host systems. For example, Bogran et al. (2002) found that niche partitioning occurred among three parasitoids that share the same whitefly species in cotton. In this instance, the different parasitoids attack hosts located on different parts of plants, and the host suppression reached a maximum when all three parasitoids occurred together. In another study, Van Nouhuys & Punju (2010) found that an inferior competitor can coexist with a superior one by exploiting the small fraction of un-parasitised hosts left by the superior competitor. Moreover, a recent study showed that a parasitoid with a longer ovipositor could attack hosts in larger fruits than those used by its competitors (Aluja et al., 2013).

Our results suggested substantial disparity when the two larval parasitoid species were present within a host larva. In this study, we found first-instar *D. tasmanica* has sickle-like mandibles. When both species occurred in the same host, the first instar *T. unimaculatus* was always dead while first instar *D. tasmanica* was always alive (table 1.). Therefore, one mechanism of intrinsic competition between early instars of the two parasitoids could be physical combat, which is common for first instar parasitoids (Tian et al., 2008; Wang et al., 2008; Harvey et al., 2009; Paladino et al., 2010; Harvey et al., 2013). In addition to this combat, the competitive superiority of *D. tasmanica* may be aided by the relatively shorter egg-hatching time compared to *T. unimaculatus*. Studies have demonstrated that species with shorter egg developmental times have a greater chance to win in intrinsic competition (Mills,

2003; De Moraes & Mescher, 2005). Multiple-parasitism can sometimes affect the development of larval parasitoids, which results in longer developmental times in the superior competitor (Pschorn-Walcher, 1971; Reitz, 1996). However, in this study, the development time of larval-stage *D. tasmanica* in multiple parasitised hosts were not different from single parasitised hosts. Our results indicate that in the presence of *D. tasmanica*, larvae of *T. unimaculatus* were killed very soon after hatching (table 1). This could explain why multiple-parasitism did not affect the developmental time of larval *D. tasmanica*. In this study, we found multiple-parasitised hosts have higher mortality than those single parasitised, which may be caused by physiological factors or physical injury (De Moraes & Mescher, 2005), or parasitised hosts are more vulnerable to infection (Brodeur & Boivin, 2004).

It is well known that there can be advantages and disadvantages of introducing multiple parasitoids for biological control (Turnbull & Chant, 1961). Since it is hard to determine the best candidate parasitoids from a number of species, many biological programmes introduce multiple species (Ehler, 1990). However, some studies have indicated that multiple natural enemies might disrupt each other, and hinder the suppression of a pest (Rosenheim et al., 1995; Murdoch et al., 1998; Collier & Hunter, 2001; Collier et al., 2002). Furthermore, evidence suggests that generalist parasitoids normally out-compete the more specialised parasitoids during intrinsic competition (Iwao & Ohsaki, 1996; De Moraes & Mescher, 2005). In this study, the complete host ranges of these two generalist parasitoid species in Australian vineyard are still unknown. We hypothesise that they could co-exist through exploiting different niches. Therefore, it is important to study the effects of factors such as host plant species, host species, and habitat characteristics on the coexistence of the two parasitoid species. More importantly, all of these factors need

to be investigated to determine how the competitive interactions between the two species here could influence their efficiency in supressing *E. postvittana*. Such knowledge would facilitate “ecological engineering” (Gurr et al., 2004) that could help to improve parasitism rates by *T. unimaculatus* in vineyards.

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Table.1. Numbers of parasitoid larvae found during dissection of single and multiple parasitised larvae of *Epiphyas postvittana* following exposure to *Dolichogenidea tasmanica* parasitised and/or *Therophilus unimaculatus* at two time intervals (< 10 mins or 48 h)

Order of parasitism		Interval between ovipositions	n	Parasitoid species found in dissected larvae			
First species	Second species			Both species	*D. tasmanica*	*T. unimaculatus*	None
D. tasmanica		-	20	-	18	-	2
D. tasmanica	*T. unimaculatus*	< 10 min	20	3*	13	1	3
D. tasmanica	*T. unimaculatus*	48 h	19	0	15	0	4
T. unimaculatus		-	20	-	-	16	4
T. unimaculatus	*D. tasmanica*	< 10 min	18	1*	13	0	4
T. unimaculatus	*D. tasmanica*	48 h	20	11*	5	1	3

*All larvae of *T. unimaculatus* dead

Table.2. Mean (± SE) development time of larval *Dolichogenidea tasmanica* and *Therophilus unimaculatus* from oviposition until cocoon formation, and from cocoon formation until adult emergence under different treatments.

Order of parasitism		Interval between ovipositions	Developmental time (d)		
First species	Second species		n (survived/ total No.)	Egg to cocoon	Cocoon to adult
A. Development of *D. tasmanica*					
D. tasmanica		-	28/30	13.18±0.25[a]	8.68±0.24 [a]
D. tasmanica	*T. unimaculatus*	< 10 min	20/30	13.20±0.24[a]	8.45±0.18 [a]
D. tasmanica	*T. unimaculatus*	24 h	20/30	13.05±0.26[a]	8.25±0.20 [a]
D. tasmanica	*T. unimaculatus*	48 h	17/30	13.24±0.29[a]	8.88±0.26[a]
T. unimaculatus	*D. tasmanica*	< 10 min	22/30	13.05±0.28[a]	8.77±0.20 [a]
T. unimaculatus	*D. tasmanica*	24 h	21/30	13.33±0.33[a]	8.33±0.17 [a]
T. unimaculatus	*D. tasmanica*	48 h	18/30	13.17±0.31[a]	8.67±0.23[a]
B. Development of *T. unimaculatus*					
T. unimaculatus		-	27/30	18.30±0.44[b]	8.74±0.28 [a]

Means followed by same letter in columns do not differ statistically (LSD test, $P<0.05$).

Figure legends

Fig. 1. Mean stinging duration and searching time of *T. unimaculatus* and *D. tasmanica* searching on a leaf infested with either un-parasitized hosts or hosts parasitized by the other species at three time intervals. Error bars represent standard errors. Different letters above bars indicate significant differences between intervals ($p<0.05$). No significant differences in behaviour were found between parasitized and un-parasitized hosts for all analyses.

Fig. 2. Percentage emergence of *D. tasmanica* and *T. unimaculatus* when (a) *D. tasmanica* parasitized *E. postvittana* first, and (b) *T. unimaculatus* parasitized first. The floating bars at the right indicate the percentage emergence of either wasp species and the percentage that died or pupated. Hours show the time between first and second oviposition. Asterisks indicate significant differences in parasitoid species emergence within each time interval (binomial test, $*P<0.0005$, $**P<0.0001$). Numbers between brackets indicate the fraction of female parasitoids that emerged (*D. tasmanica* on the left, *T. unimaculatus* on the right). There were no effects of number of parasitisations, time interval between oviposition, and order of oviposition on the proportion of female emergence for *D. tasmanica* (χ^2-tests, $P > 0.05$).

Fig.1

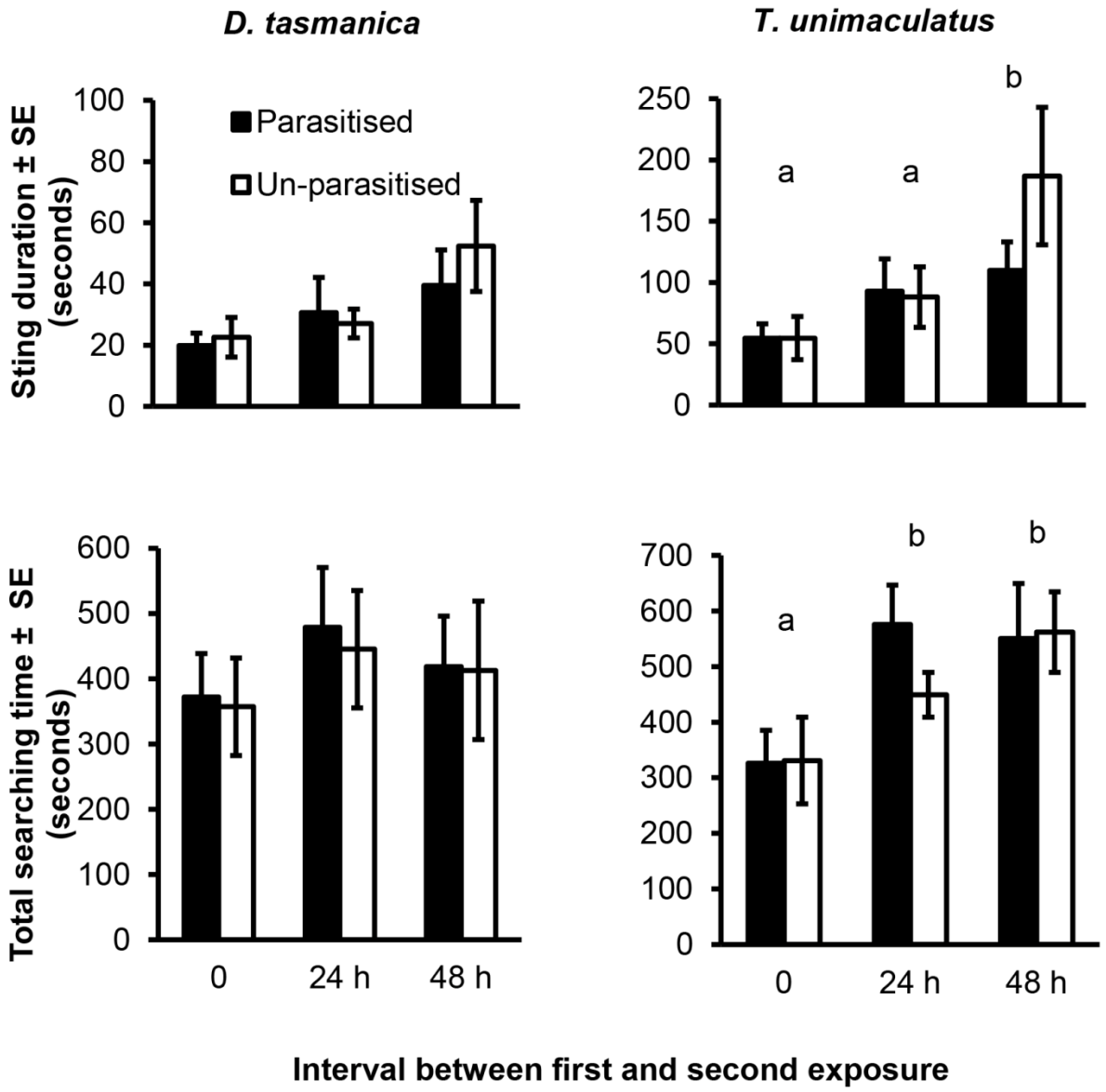
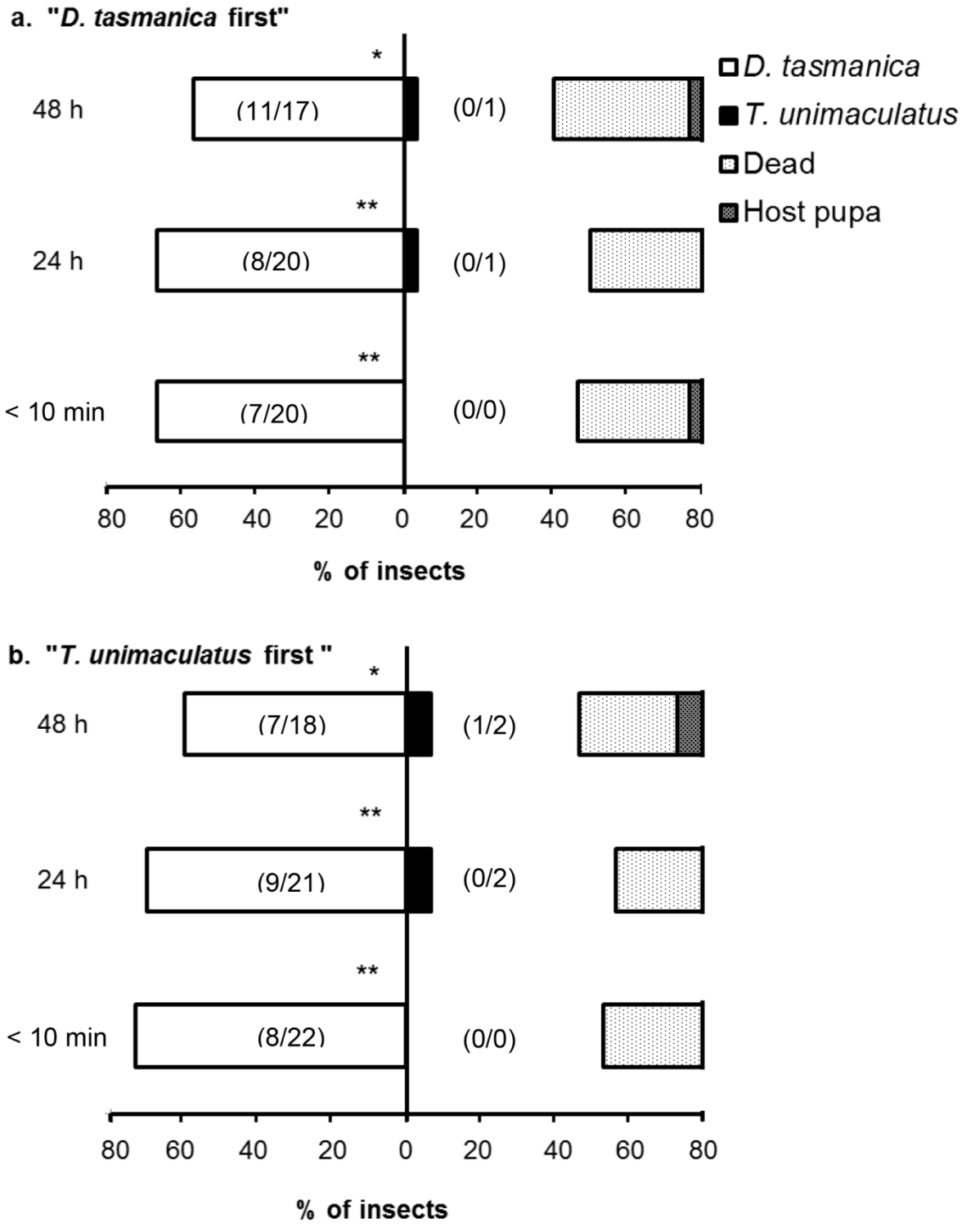

D. tasmanica
T. unimaculatus
Parasitised
Un-parasitised
Sting duration ± SE (seconds)
Total searching time ± SE (seconds)
100
80
60
40
20
0
250
200
150
100
50
600
500
400
300
700
a
b
0
24 h
48 h
Interval between first and second exposure

Fig. 2


a. "D. tasmanica first"
48 h
24 h
< 10 min
(11/17)
(8/20)
(7/20)
(0/1)
(0/1)
(0/0)
*
**
**
D. tasmanica
T. unimaculatus
Dead
Host pupa
80 60 40 20 0 20 40 60 80
% of insects
b. "T. unimaculatus first "
48 h
24 h
< 10 min
(7/18)
(9/21)
(8/22)
(1/2)
(0/2)
(0/0)
*
**
**
80 60 40 20 0 20 40 60 80
% of insects

CHAPTER FOUR

Host plants affect the foraging success of two parasitoids that attack light brown apple moth

Statement of Authorship

Host plants affect the foraging success of two parasitoids that attack light brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae)

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Abstract

The light brown apple moth, *Epiphyas postvittana* is a key pest of wine grapes in Australia. Two parasitoids, *Dolichogenidea tasmanica* and *Therophilus unimaculatus*, attack the larval stage of this pest. *D. tasmanica* is dominant in vineyards, whereas *T. unimaculatus* is mainly active in native vegetation. We sought to understand why they differ in their use of habitats. Plants are a major component of habitats of parasitoids, and herbivore-infested plants influence parasitoid foraging efficiency by their architecture and emission of volatile chemicals. While foraging, innate behavior affects the responses of parasitoids to volatiles released by herbivore-infested plants. We investigated how different plant species infested by *E. postvittana* could affect the foraging success of the two parasitoid species in both laboratory and field experiments. Four representative host-plant species that differ in their architecture and evolutionary relationships with the parasitoids and host species were selected for this study. In paired-choice experiments to determine the initial foraging preferences for plants, both parasitoid species showed differences in innate searching preferences among plant species. The plant preference of *D. tasmanica* was altered by oviposition experience with hosts that were feeding on other plant species. In a behavioral assay, the two parasitoid species allocated their times engaged in various types of behavior differently when foraging on different plant species. For both parasitoids, parasitism on *Hardenbergia violacea* was the highest of the four plant species. Significantly more larvae dropped from *Myoporum insulare* when attacked than from the other three host-plant species, which indicates that parasitism is also affected by interactions between plants and host insects. In vineyards, parasitism by *D. tasmanica* was significantly lower on *M. insulare* than on the other three host-plant species, but the parasitism rates were similar among the

other three plant species. Our results indicate that plants play a role in the habitat preferences of these two parasitoid species by influencing their foraging behavior, and are likely to contribute to their distributions among habitats.

Key words: tri-trophic interactions, foraging behavior, parasitism, light brown apple moth, learning behavior, wind tunnel

Introduction

Successful parasitism by parasitoids begins with a series of host-searching behaviors that lead females to locate their potential hosts. This process includes habitat location, host location, and host acceptance [1]. Parasitoid searching behavior is under strong natural selection pressure, because successful foraging is directly linked to reproduction [2,3]. Host-searching behavior determines the efficiency of parasitoids, and thus understanding it is a key element in evaluating their suitability as biological control agents [4].

Herbivore-infested plants influence the foraging efficiency of parasitoids in various ways [5,6]. For example, herbivore-induced volatiles emitted by the host plant are key signals that lead parasitoids to their hosts [7,8]. Herbivore-infested host plants can selectively attract natural enemies [9] and, in some cases when different plant species are infested with the same herbivore, some species may attract more parasitoids than the others, resulting in higher parasitism rates under natural conditions [10]. In addition to plant volatiles, plant architecture can influence the interactions between the parasitoids and their hosts [11-16]. Plant morphological characteristics such as plant-surface structural complexity [17], presence of dense trichomes [18,19], and leaf surface area [14,20,21] can significantly influence the success rates of parasitoids or predators in finding their hosts. To understand how plants affect parasitoid foraging efficiency, the effects of plant attributes including both plant volatiles and other characteristics like architecture should be considered.

Parasitoids may also rely on both innate mechanisms and learned cues associated with host availability during foraging [22]. Through learning, parasitoids can adaptively optimize their foraging efficiency by altering their innate preferences [23]. The effects of learning on the ability of parasitoids to locate hosts have been

documented in both laboratory [24,25] and field studies [26]. For instance, foraging experience on different herbivore-infested cabbage varieties can lead generalist parasitoids to have a preference for the herbivore-infested plant species that they have experienced [24]. However, when indigenous parasitoids forage for hosts on a wide range of plant species involving both native and exotic plants, it is not known whether previous oviposition experience on one plant species will influence their subsequent foraging preference in favor of the same species.

In agro-ecosystems, native plant species happen to grow within or adjacent to the crop plants, which are mostly non-native introduced species. To investigate the effect of plant species on the foraging efficiency of generalist parasitoids, both the plant attributes and the adaptive learning behavior of the parasitoids should be considered.

In this study, we investigated the foraging behavior of *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) and *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae). Both species are indigenous to Australia and are solitary, koinobiont, generalist endoparasitoids [27-29]. They both attack the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), which is a native Australian, leaf rolling, polyphagous, multivoltine moth. *Epiphyas postvittana* is the key insect pest of grapevines in Australia [30], and it also attacks plants from 123 genera in 55 plant families in this country. Among these plant species, 22 are from native genera, while 101 are from exotic genera [31]. Approximately 25 hymenopteran parasitoids are reported to be associated with *E. postvittana* in Australia [32], however, *D. tasmanica* and *T. unimaculatus* are the predominant parasitoids of *E. postvittana*. The former is the most abundant larval parasitoid in vineyards, while the latter most common in the adjacent vegetation [33]. It is not

known why one parasitoid species is more active in vineyards while the other is not. Plants could play a key role among a number of factors that affect the activity of these parasitoids in vineyard ecosystems. However, it is not known how different plant species in and around the vineyards affect their foraging behavior and habitat associations of these two parasitoids.

We investigated (1) whether female *D. tasmanica* and *T. unimaculatus* have innate searching preferences for different host plant species infested with larval *E. postvittana*; (2) whether previous oviposition experience alters the host-plant preferences of the parasitoids; (3) how different plant species infested with *E. postvittana* affect the behavior and foraging efficiency of the two parasitoids; and (4) whether parasitism is affected by different plant species in vineyards. We first tested the in-flight preference of both parasitoid species for host-infested plants with dual-choice wind tunnel assays. Four representative plants species, which included both native and exotic species, were selected. We then tested whether previous oviposition experience on one plant species could influence the in-flight preference of *D. tasmanica* for host plants. Next, we investigated the searching behavior of two parasitoids on four plant species. Finally, a field experiment was conducted to determine whether parasitism of larval *E. postvittana* was influenced by their host plants in vineyards. Overall, we gained insights into how plant species affect the foraging success of two generalist parasitoids that attack *E. postvittana*.

Materials and Methods

Ethics Statement: Permission to conduct the field experiment was granted by the vineyard managers Geoff Hardy and David Hamilton. No permit was required for the laboratory studies.

Insects and Plants

An artificial diet was used to rear *E. postvittana* [34] at 22 ± 2 ºC under a 12 L:12 D light: dark cycle in an insect-rearing room. This culture has been maintained for 200 generations, with the annual addition of field-collected individuals to maintain genetic diversity. The colonies of *D. tasmanica* and *T. unimaculatus* were originally established from parasitized leafrollers collected in a vineyard (35°16'05" S, 138°37'10" E) near Adelaide, Australia in November 2011. These parasitoids were reared on larval *E. postvittana* that fed on plantain (*Plantago lanceolata* L.) and were maintained in cages at 23 ± 2 ºC with a relative humidity of 60 ± 10% under a 14 L:10 D light: dark cycle. Naturally occurring larval *E. postvittana* were collected from the field (35°58'18" S, 138°38'32" E) every two months and the newly emerged adult parasitoids were added to the respective colonies.

We chose four plant species that have been reported to be common host-plants for leafrollers in Australia [31] representing three categories: (1) an introduced economic crop, wine grape, *Vitis vinifera* L., cv. Chardonnay, which is highly susceptible to attack by *E. postvittana* [35]; (2) two Australian native perennial plants, *Hardenbergia violacea* (Schneev.) Stearn and *Myoporum insulare* R. Br.; and (3) an exotic ground cover species, plantain, *Plantago lanceolata* L. These plant species differ in their architecture and the level of protection available to larval *E. postvittana*.

Experimental plants were grown in containers. *P. lanceolata* was grown from seed three months prior to the experiment. The Chardonnay grape vines were grown from pencil-sized cuttings collected from a vineyard during winter. Native plants (*H. violacea* and *M. insulare*) (~20 cm high) were purchased from a Nursery. For the laboratory experiments, all plants were grown individually in UC soil mix [36] in plastic pots (50 mm × 50 mm × 120 mm). For the field experiment, all plants were

grown individually in UC soil mix and cocopeat potting mix at a ratio of 1:1 in nursery bags (300 mm × 120 mm × 150 mm) in a glasshouse. Plants were placed in a field cage two weeks before the onset of field experiments to allow them to acclimatize to natural conditions.

Parasitoid Handling

We used two- or three-day old mated female parasitoids. Newly formed parasitoid cocoons were collected and held individually in 100 ml plastic cups, each with a drop of honey and a water-soaked cotton dental wick. The newly emerged female parasitoids were caged with five males for 24 hours, with a drop of honey and water-soaked cotton dental wick, to ensure mating. The mated females were then isolated in glass vials (18 mm diam × 50 mm) with a drop of honey. Immediately before release, the individual parasitoids were primed by exposing them to feces collected from *E. postvittana* reared on an artificial diet in a Petri dish (80 mm diam). This stimulated the parasitoids with host-related cues that were not from any of the experimental plants. Individual parasitoids were then transferred to a clean vial (same as above) for release in the wind tunnel. The bottom half of the vial was filled with cotton to ensure the parasitoid did not move to the bottom and stay there.

Choice Experiment

An experiment was conducted to test the initial in-flight preference of the parasitoids for volatiles from different plants that were damaged by *E. postvittana*. There were four plant species, and therefore six pairs of volatile sources that were tested for each parasitoid species. Both parasitoid species were tested in dual-choice situations in which two volatile sources were placed in pairs in a wind tunnel at a wind speed of 20 cm/s at 21 ± 2ºC (Figure 2; see [37] for wind tunnel details). To strengthen the volatile emissions and ensure continuous host-feeding damage on

the plant leaves during the experiment, leaves of two plant species were infested with 20 second-instar *E. postvittana*. To reduce variations in morphology, texture, color, and structure and to ensure each parasitoid had a free and equal choice to fly to either of the target plants, the leaves of both plant species were placed in a metal screen tea ball (5.5 cm diam). Pilot tests indicated that only *D. tasmanica* would fly to host infested leaves that were enclosed in a tea ball. Therefore, to test the inflight preference of *T. unimaculatus*, plants infested with 20 second instar *E. postvittana* 24 h before the test were used. While different methods were used to test the innate preferences for host infested plants by these parasitoid species, this difference did not compromise our overall aim, which was to determine how host infested plants might differentially attract these parasitoids. Care was taken that all volatile sources from the same plant pair were used on the same day. To avoid plant-position bias effects, the positions of the two tea balls or the intact plants were randomized and with equal numbers of each test plant in each position. A single parasitoid was released from a glass vial on a stand 25 cm downwind from the tea balls or plants at approximately the same height, which were 25 cm above the floor of the wind tunnel and separated by 5 cm. The observation for each parasitoid lasted a maximum of five minutes. A ‘choice’ was recorded when a female landed on a tea ball or an infested plant. Wasps that did not respond within five minutes or landed elsewhere were recorded as ‘no response’. The experiments were conducted between 9:00 am and 4:00 pm, and each parasitoid was tested only once. Thirty-six parasitoids of each species were tested for each plant pair.

Effects of Learning on Host-Plant Preference

An experiment was conducted with *D. tasmanica* to examine whether previous oviposition experience on host-infested plants alters the subsequent preference for

host-induced plant volatiles. To provide female parasitoids with multiple oviposition experiences, they were allowed five sequential ovipositions on leaves of grape or *P. lanceolata*. A choice experiment was then conducted with an experimental design that was similar to the previous choice test. The parasitoids were observed in the wind tunnel to determine their landing preference between two plant species, of which one was the species of their previous oviposition experience. The experiment was conducted with 36 experienced parasitoids for each pair of plants and 36 naive parasitoids tested with the same pair of plants as a control, which also served to validate the results of the previous experiment. Due to insufficient numbers of *T. unimaculatus*, this parasitoid species was not included in this part of our study.

Behavioral assay

To determine if parasitoid behavior varies among host plants, we also observed their foraging activities on the four plant species, which was influenced by the combined effects of all plant characteristics (plant chemistry and structure). This study was conducted in a wind tunnel (Figure 3). An individual plant infested with two second-instar *E. postvittana* was placed upwind of the parasitoid in the wind tunnel. To avoid the parasitoid spending excess time on the test plant, a second host plant of the same species and condition as the test plant was placed 40 cm upwind during each observation to provide an alternative landing place for the parasitoid. The second plant was also infested with two second-instar *E. postvittana*. Before the experiment, all of the leaves on the test plants were examined to check for the position and number of host larvae, host damage, and frass on the plants. A single parasitoid was released from a glass vial 25 cm downwind from the host infested plant. The foraging behavior of individual parasitoids on the downwind host-infested plant was then observed, with the observations lasting until the parasitoid left the plant to either

another location in the wind tunnel or the alternative plant. For each parasitoid species, twenty wasps were observed for each host-plant species, and each parasitoid was observed only once. The order of testing the plant species was randomized.

Based on preliminary observations, a catalogue of female behavior for *D. tasmanica* and *T. unimaculatus* searching was constructed (Table 1). The mean duration for each type of behavior was calculated from when a parasitoid first landed on the host plant. According to a pilot experiment, a common host defensive behavior to avoid parasitoid attack is to drop from the plant. Therefore, the dropping behavior of the host larvae was also recorded, as were the number of larvae that were stung. Each day, observations were conducted between 10:00 am and 4:00 pm. Parasitoid behavior was recorded with the Observer XT ver. 11 software package (Noldus Information Technology B.V., Wageningen, the Netherlands). The egg hatching time of *D. tasmanica* and *T. unimaculatus* are about two and four days after egg laying, respectively [38]. Therefore, the larvae stung by the *D. tasmanica* and *T. unimaculatus* were dissected two and four days after the experiment, respectively, to determine the parasitism rate.

Field Experiment – Parasitism of *E. postvittana* on Four Plant Species

To evaluate whether the four plant species influence the levels of parasitism by *D. tasmanica* and *T. unimaculatus* in vineyards, a field experiment was conducted at two sites (vineyard A: 35º13' S, 138º39' E; vineyard B: 35º16' S, 138º37' E) and repeated twice at each vineyard between February and March 2013. An orthogonal split-plot design was used where the vineyards were considered random blocks, the plant species were the main-plot factor, and the repeated visits were the split-plot factor. In each vineyard, the four different species of potted plants infested with first-

instar *E. postvittana* (around 20 larval *E. postvittana* on each plant) were placed in five sets of quadruplets (sample unit = 3 plants/species) inside the vineyard and 20 m from the border (Figure 4). At each site, 60 plants were placed in the field and left for two weeks of free access to wild parasitoids. The plants were placed in the two vineyards on consecutive days. After two weeks, the plants were removed and replaced with a fresh pair of plants. The larvae on each plant were collected and reared in 440 ml plastic containers at 22 ± 2 ºC under a 14 L:10 D light/dark cycle in an insect-rearing room. The parasitism rate and fate of the larvae (dead, parasitized, or pupated) were recorded.

Statistical Analysis

To determine which host plant was preferred by the parasitoids, the choice within an experiment was analyzed using a binomial test, with 0.5 as the null hypothesis. To examine if learning can alter the landing preference of the parasitoids, chi-squared tests with Cochran's correction for continuity [39] were used to compare the landing choices of the naive/control and experienced wasps.

The mean proportion of time devoted to each type of behavior after landing on a plant was calculated for each parasitoid species on each plant species. The proportion of time that wasps were engaged in each behavior was calculated for each individual, and differences among the plant species for each parasitoid were analyzed with Kruskal-Wallis tests (IBM SPSS Statistics v. 20, IBM-SPSS Inc., Chicago, IL).

In addition, Chi-square tests were also used to determine if differences in parasitism rates and host defensive behaviors (larvae dropping) among four plant species were statistically significant. If the null hypothesis was not rejected, a retrospective power analysis for Chi-square was carried out (Functions CHISQ_POWER and

CHISQ_SIZE, default power = 0.8, www.real-statistics.com) to determine the required sample size with the significance level of 0.05.

To analyse the factors affecting parasitism in the experimentally introduced *E. postvittana*, the data from field experiments were modelled with orthogonal split-plot general linear models with the GLM procedure in the statistical package GenStat for windows 15th Edition (VSN International, Hemel Hempstead, UK.). For all experiments, the fractions of larvae that were parasitised by all parasitoids on each plant species in each quadruplet were calculated from the pooled numbers; these numbers were treated as the dependent variables. The arcsine transformation [39] was used to analyse the parasitism data. The Bonferroni adjustment was used for post-hoc multiple comparisons of means. The level of significance was set at 0.05.

Results

Choice Experiment

Parasitoids had varied preferences for landing in the dual-choice experiments. Female *D. tasmanica* preferred to land on one species combination over another in four of the six plant-host-complex pairings (Figure 5a). *H. violacea* was preferred over *M. insulare*, grape and *P. lanceolata,* and *M. insulare* was preferred over grape. *T. unimaculatus* females showed preference in three of the six pairings (Figure 5b). *H. violacea was preferred* over grape and *P. lanceolata*, and *M. insulare* was preferred over grape.

Effects of Learning on Host Plant Preference

Oviposition experience on a plant affected subsequent plant preferences in *D. tasmanica*. In the experiments that involved experience on *P. lanceolata*, the change

in preference between control and experienced wasps was statistically significant only when the comparison between them involved the choice between *P. lanceolata* and grape (Figure 6a). However, there was a statistical change in the degree of preference between species in all paired groups of control and experienced wasps. For example, naive wasps displayed no preference between *M. insulare* and *P. lanceolata*, but wasps that had experience on *P. lanceolata* significantly preferred to land on it. Likewise, naive wasps that were presented with choice between *H. violacea* and *P. lanceolata* preferred to land on *H. violacea,* but this preference was not displayed by wasps that had experience on *P. lanceolata*. Oviposition experience on grapes led to more frequent landing on grapes by *D. tasmanica* in the presence of each of the other three plant species (Figure 6b). Interestingly, the preference of naive *D. tasmanica* for *H. violacea* over grape was reversed after females had oviposition experiences on host feeding on grapes.

Behavioral assay

The behavioral profiles of both parasitoids varied among the four host-plant species (Figure 7). The fractions of time *D. tasmanica* engaged in stinging, probing and grooming differed among plant species, while the fractions of time *T. unimaculatus* allocated to pulling, antennating and grooming differed among species. The parasitism rate of *T. unimaculatus* on *H. violacea* was higher than on the other three plant species. The parasitism rate by *D. tasmanica* on *H. violacea* was higher than on other plant species but this difference was not statistical significant, possibly due to low statistical power (Figure 8). The most common way that larval *E. postvittana* avoided the parasitoids' attacks was to drop from the plant on a silk thread when contacted by a parasitoid. In the laboratory observations, more larvae dropped from *M. insulare* than from the other plants (Figure 8), and the two parasitoids responded

differently toward the escaping hosts (Figure 9). Among the dropping hosts pooled among all plants, significantly more were parasitized by *D. tasmanica* than by *T. unimaculatus.* When encountering an escaping host hanging from a silk strand, *D. tasmanica* immediately followed the larva or attacked it on the thread, or searched for few seconds, flew to locate it, and then attacked the larvae on the thread. If the host dropped to the ground, some of the *D. tasmanica* flew or walked to the ground and searched for the host. In contrast, *T. unimaculatus* displayed hauling behavior when encountering a dropping larva, continuously reeling the silk thread with its legs up to pull the hanging larva back to the plant, even though in many cases the larva would drop to the ground. No *T. unimaculatus* was observed following the dropping host or searching for the larvae on the ground. *D. tasmanica* found and attacked the dropping hosts more successfully than did *T. unimaculatus* (Figure 9).

Field Experiment – Parasitism of *E. postvittana* on Four Plant Species

The number of larval *E. postvittana* that were found on each three-pot group of plant species differed significantly among the four plant species (Figure 10a; $F_{3,76} = 70.06$, $P < 0.05$). A low number of larval *E. postvittana* was recovered from *M. insulare* compared with other plant species in both replications.

Only two parasitoid species, *D. tasmanica* and *T. unimaculatus*, were recovered from the larval *E. postvittana* on plants placed in the vineyards. No *T. unimaculatus* were collected from larvae on *M. insulare*. The angular-transformed parasitism rates for *D. tasmanica* and overall parasitism differed among plant species (Figure 10b; *D. tasmanica*, $F_{3, 76} = 12.41$, $P < 0.05$; overall parasitism, $F_{3, 76} = 34.64$, $P < 0.05$). However, the angular-transformed parasitism rates for *T. unimaculatus* were not different among plant species (Figure 10b; *T. unimaculatus*, $F_{3, 76} = 2.52$, $P = 0.234$), which reflects the low and inconsistent appearance of this species between sites and

dates. Overall parasitism on *M. insulare* was significantly lower than on grape and *H. violacea*, but there were no detectable differences in parasitism by *D. tasmanica* among plant species when Bonferroni-test adjustments were made to multiple comparisons.

Discussion

Herbivore infested plants affect the foraging behavior and efficiency of both *D. tasmanica* and *T. unimaculatus*. Although we did not directly measure and compare the herbivore-induced plant volatile profiles of the plant species we tested, the effect of host induced plant volatiles were directly observed through comparing the responses of the parasitoids to paired host-infested plants. The results indicate both parasitoids have innate preferences for plant species. The Australian native plant, *H. violacea*, was the most attractive species to both parasitoids (Figure 1). Studies have demonstrated that among a range of plant species infested with the same herbivore, the host-induced volatiles differ in both specificity and quantity, and affect the natural enemies differently [9,40,41]. The native plant *H. violacea* was more attractive to the parasitoids, but the exotic plants *P. lanceolata* and grape also attracted the parasitoids. This suggests adaptability by these generalist parasitoids as well as innate responses to common characteristics of plants. In this study, the plant species on which parasitoids were reared could have potentially affected their behavior [42]. However, our main aim was to evaluate how plant species could generally affect the behavior of parasitoids that are foraging in a diverse landscape. We would not expect any potential bias from rearing on a selected plant species to affect this broader evaluation.

Previous oviposition experience on both the grape and *P. lanceolata* affected the subsequent plant preference of *D. tasmanica* in the dual-choice tests (Figure 6b). When all statistical analyses are considered together, the results indicate that *D. tasmanica* is more likely to fly to a particular plant species after it has experience with parasitizing a host on it. The magnitude of such shifts in preference is likely to depend on the degree of innate preference, the level of experience and the characteristics of the plants involved. From these results, we hypothesize that *T. unimaculatus* can also learn to associate hosts with some plants as a result of experience. Assuming this is the case, the preferences of both species should reinforce their experiences with hosts on innately preferred and common host plant species in or near vineyards.

D. tasmanica and *T. unimaculatus* allocate their times differently on the different plant species. In addition, *D. tasmanica* spends relatively more time antennating and probing and less time stinging than *T. unimaculatus* (Figure 7). These results indicate that a combination of plant characteristics influences the foraging behavior of these parasitoids. Many studies have demonstrated that plant structure can affect parasitoid foraging efficiency [43]. The size, heterogeneity and connectivity of plants have been shown to affect parasitoid foraging success, as confirmed both by modelling the impact of plant structure on parasitism rates based on artificial plants and by experiments with real plants [44]. The probability of parasitoids and predators encountering a host or prey generally decreases with an increase in plant structural complexity [11,14,16,20,45,46], plant size [47], and plant surface area or volume [48-50].

Our results also indicate that the plant species affect the defensive behavior of the host, and therefore indirectly affect the foraging efficiency of parasitoids. Host larvae

falling from plants may be at a higher risk of encountering other predators or parasitoids [51]. Because of this, plant species that facilitate escaping behavior in the field may not support large numbers of parasitoids.

D. tasmanica was found to be the dominant species that parasitizes larval *E. postvittana* in vineyards. Both *D. tasmanica* and *T. unimaculatus* were found in the field experiment, but *D. tasmanica* parasitised the majority of the sentinel larval *E. postvittana* in the vineyards. Parasitism by *D. tasmanica* was consistent between sites and dates, while parasitism by *T. unimaculatus* was inconsistent and low (Figure 10b). This is in line with a previous study that indicated the dominance of *D. tasmanica* in vineyards [33]. Under field conditions, host-related chemical cues are the main long-distance attractor for parasitoids, which could influence their foraging efficiency. Plants could be a key factor that affects the activity of parasitoids in certain habitats. Studies have suggested that plants which are attractive to parasitoids are associated with a higher parasitism rate under field conditions [10]. There is independent evidence that parasitism by *D. tasmanica* varies significantly among plant species [52]. There is independent evidence that parasitism varies significantly among plant species [50], which is consistent with the results of our field experiment. Moreover, our behavioural assays indicated that plants vary in their level of attraction to females, and this can be influenced by a wasp's experience. In one behavioural assay, parasitism rates did not differ among plant species, but this non-significant result is likely to be due to low statistical power (Figure 10). This indicates that differences among plant species may not be pronounced when some species combinations are compared.

Research has indicated that successful foraging on certain host plants can narrow a parasitoid’s foraging range through learning [53]. The field experiment was carried

out in vineyards, where the main background plants were grapes and *P. lanceolata* was a common ground cover plant. Although *H. violacea* could be the preferred host plant species of the naive wasp, the parasitoids may gain experiences more frequently after attacked hosts feeding on the more abundant and naturally occurring grape or *P. lanceolata* in vineyards and thus strengthen their habitat preference. This could happen for both *D. tasmanica* and *T. unimaculatus* that were attracted to vineyards. However, when both parasitoids are active in a vineyard, their competitive interactions would influence their foraging success and abundance [38]. In this case, the more abundant parasitoid species should be found to have a competitive advantage over its competitors.

Results from our field experiment showed the number of larval *E. postvittana* recovered from the four different plant species varies. It is noticeable that much smaller numbers of larval hosts were recovered from *M. insulare* compared to the other three plant species (Figure 10a). Studies have indicated that plants infested with a relatively high density of host larvae should attract more parasitoids [54]. Therefore, the smaller number of host larvae could have negatively affected the parasitism of *E. postvittana* on *M. insulare*. It is also possible that larvae could not defend themselves as effectively from parasitoids and predators on *M. insulare*. In the case of parasitoid attack, larvae that dropped from the plant may have failed to return to it more frequently than on other host plant species.

In conclusion, plants influence host availability and attract the two parasitoids differently. Different plant species provide different levels of protection for larval *E. postvittana* and thereby affect the foraging behavior and efficiency of parasitoids that attack it. Results of our field experiment indicate *D. tasmanica* is the dominant parasitoid in vineyards, and plant species affect parasitism by this parasitoid species.

Putting these effects together, we conclude plants are likely to affect the habitat preferences and distributions of these parasitoids that share the same hosts. In this study we investigated tri-trophic interactions involving two Australian native plants, *H. violacea* and *M. insulare*, as well as the introduced exotics, *P. lanceolata* and grape. This approach revealed how plants generally affect the parasitoid-host interactions in real vineyard ecosystems, in which numerous plant species are involved in the interactions between the herbivore and its associated parasitoid species.

Future research should address the question of whether adding specific plant species that are preferred by parasitoids could increase the suppression of *E. postvittana* in vineyards. To strengthen the activity of parasitoids that are already active in vineyards or attract more parasitoids into vineyards, it is necessary to evaluate a wider range of supplementary plant species, especially native and perennial species. Understanding the interactions at the tri-trophic level of plant, pest and natural enemies should help us “ecologically engineer” vineyards [55] to promote the activity of these parasitoids and therefore enhance the ecosystem service of biological control [56].

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Tables

Table1. A catalogue of behavior of *Dolichogenidea tasmanica* and *Therophilus unimaculatus.*

Event	Description
Antennating	Walking with antennae touching and sweeping along the substrate
Flying	Any airborne activity
Grooming	Preening any part of the body
Probing	Walking while drumming the substrate with antennae and jabbing with the ovipositor
Stationary	Standing still with moving antennae
Stinging	Stinging a host with the ovipositor
Walking	Walking with antennae not touching the substrate
***Pulling**	Pulling the thread of a hanging host larva and hoisting it up

* *T. unimaculatus* only

Figure Legends

Figure 1. Braconid parasitoids, *Dolichogenidea tasmanica* and *Therophilus unimaculatus*, stinging second-instar *Epiphyas postvittana*. (a) *D. tasmanica*; (b) *T. unimaculatus.* These actively foraging parasitoids are generalists that attack a range of leafrollers (Lepidoptera: Tortricidae). Photos by (a) Mike Keller, and (b) Yi Feng.

Figure 2. Experimental set-up of the wind tunnel for the choice test. There are three areas between the release point and the test plant: Area A has volatile cues from host plant one; Area B has volatile cues from host plant two; and Area C has volatile cues from both test plants.

Figure 3. Experimental set-up of the wind tunnel for the behavioral assay. An individual plant infested with two second-instar larval *E. postvittana* was placed upwind of the parasitoid in the wind tunnel. To avoid the parasitoid spending excess time on the test plant, a second host plant of the same species and condition as the test plant was placed further upwind during each observation to provide an alternative landing place for the parasitoid.

Figure 4. Scheme of the experimental set-up in vineyards. Three pots of each plant species were arranged together and three meters away from the other plants within the sampling point. The sampling points were 12 m apart. Dashes lines indicate the vine rows.

Figure 5. Distribution of choices made by *Dolichogenidea tasmanica* and *Therophilus unimaculatus* in response to plants infested with second instar *Epiphyas postvittana*. (a) *D. tasmanica*; (b) *T. unimaculatus.* Within each choice test, the per cent of parasitoids that made no choice is shown at the right. Host

plants: P, *Plantago lanceolata*; G, grape; H, *Hardenbergia violacea*; M, *Myoporum insulare*. Asterisks indicate a significant difference between the targets (binomial test; *P < 0.05, **P < 0.005). *NS*, not significant.

Figure 6. Distribution of choices made by *Dolichogenidea tasmanica* in response to different species of plants infested with *Epiphyas postvittana*. The female *D. tasmanica* had previous oviposition experience on (a) *Plantago lanceolata* or (b) grape. Host plants: P, *P. lanceolata*; G, Grape; H, *Hardenbergia violacea*; M, *Myoporum insulare*. Control groups were naive parasitoids. Within each choice test, the per cent of parasitoids that made no choice is shown at the right. Asterisks on the left side of the table indicate a significant difference between the targets (binomial test; *P < 0.05, **P < 0.005). Asterisks on the right side of the table indicate a significant difference between the experimental and control groups (χ^2 tests with Cochran's correction for continuity; *P < 0.05). NS, not significant.

Figure 7. Proportion of time spent by *Dolichogenidea tasmanica* and *Therophilus unimaculatus* while foraging on different plant species. (a) *D. tasmanica*; (b) *T. unimaculatus.* Behaviors (see Table 1 for definitions): An, antennating; Fl, flying; Gr, grooming; Pr, probing; Pu, pulling; Sta, stationary; Sti, stinging; Wa, walking. Host plants: P, *Plantago lanceolata*; G, Grape; H, *Hardenbergia violacea*; M, *Myoporum insulare*. Bold behavior characters indicate significant differences among plant species (Kruskal Wallis tests; P < 0.05)

Figure 8. Fraction of second instar *Epiphyas postvittana* that a) dropped from plants when approached by parasitoids and b) were parasitized by *Dolichogenidea tasmanica* and *Therophilus unimaculatus* among four host-plant species in the behavioral assay. The number of larvae that dropped/total differed significantly among four host plant species (*D. tasmanica*, χ^2=10.80,

P=0.013; *T. unimaculatus*, χ^2=18.15, P=0.0004). Number of larvae parasitised was not different among host plant species for *D. tasmanica* (χ^2=5.27, P=0.153). Chi-square power analysis (default power = 0.8) indicated that the sample size must be increased by 2.06 times before the experimental results would be likely to produce statistically significant differences. No. of larvae parasitised was higher on *H. violacea* than the other three host plant species for *T. unimaculatus* (χ^2=8.51, P=0.037). Numbers within each bar indicate the sample sizes.

Figure 9. Number of dropped larvae parasitized by *Dolichogenidea tasmanica* and *Therophilus unimaculatus* in behavioral assay. χ^2 test; *P < 0.05.

Figure 10. (a) Mean number of larval *Epiphyas postvittana* recovered and (b) parasitism of *E. postvittana* in the field experiment. The larvae were feeding on grape, *Hardenbergia violacea*, *Plantago lanceolata*, and *Myoporum insulare*. Data are expressed as overall means ± standard error.

Fig. 1

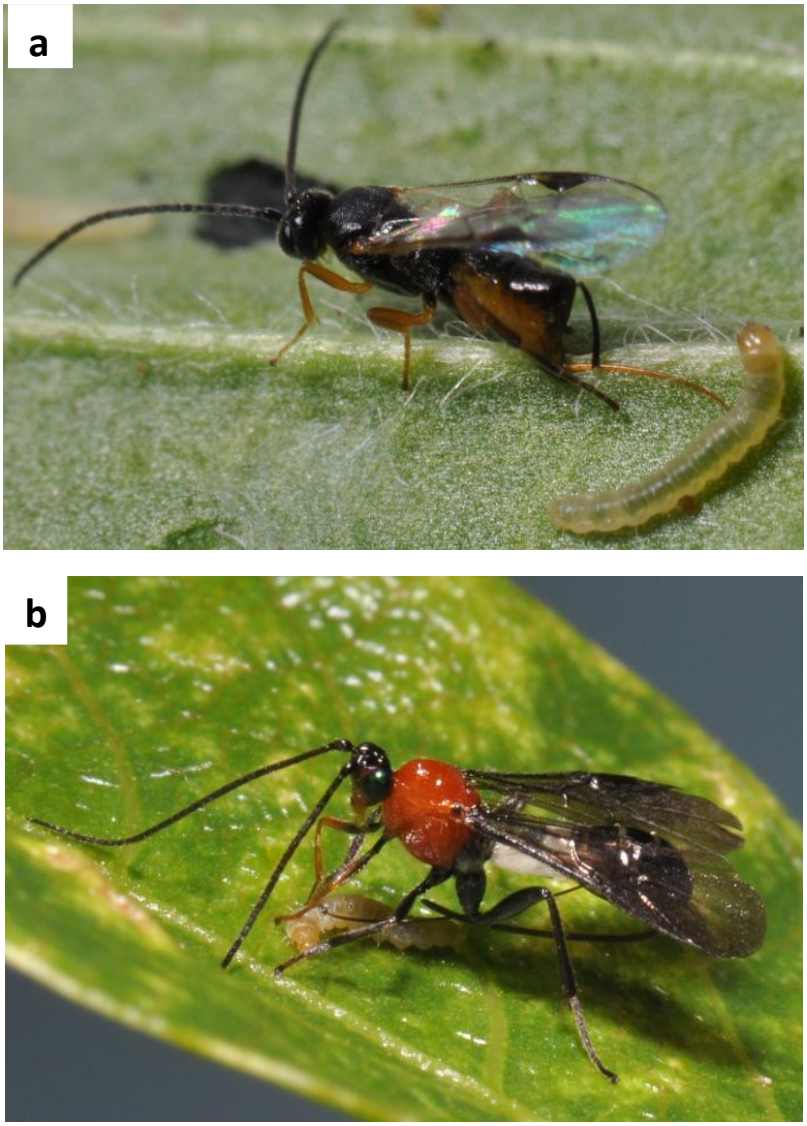


Fig. 2

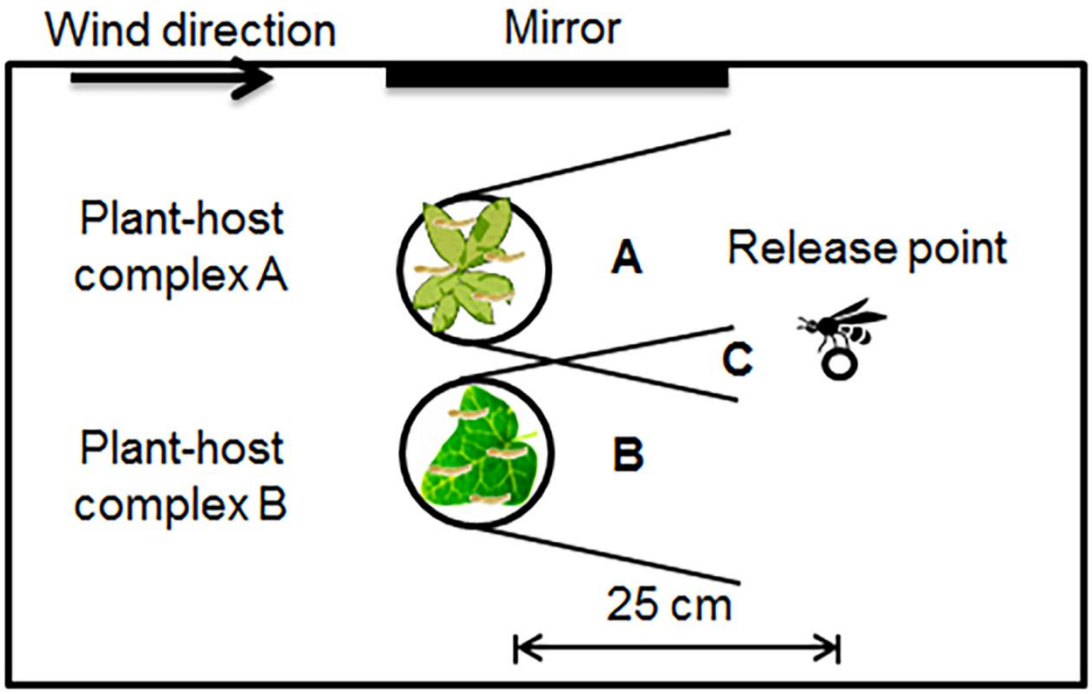

Wind direction
Mirror
Plant-host complex A
A
Release point
C
Plant-host complex B
B
25 cm

Fig. 3

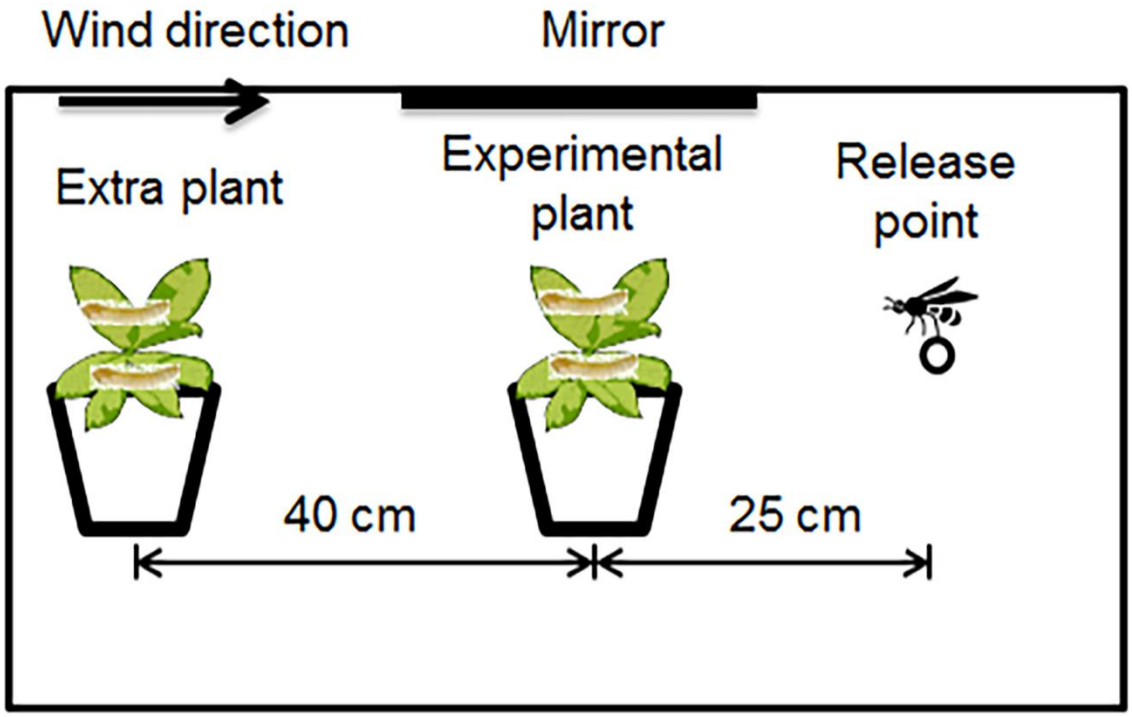

Wind direction
Mirror
Extra plant
Experimental plant
Release point
40 cm
25 cm

Fig. 4

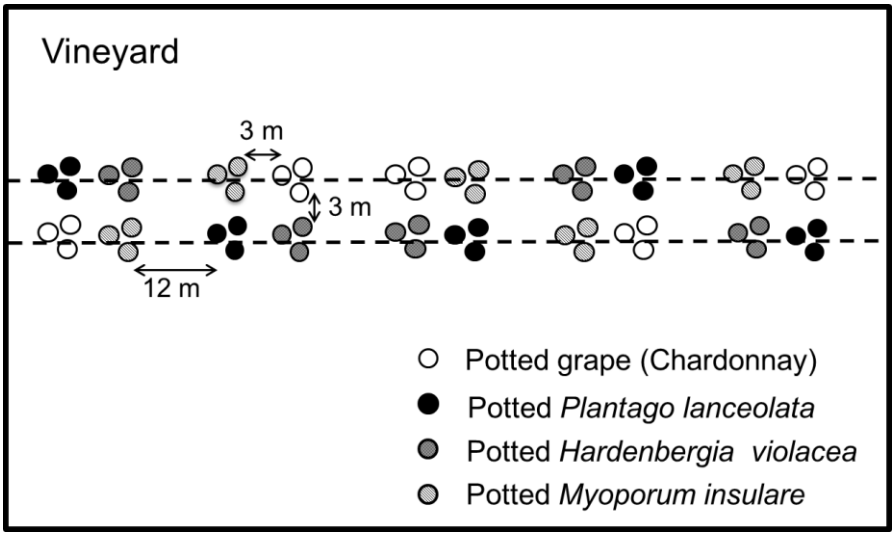

Vineyard
3 m
3 m
12 m
Potted grape (Chardonnay)
Potted *Plantago lanceolata*
Potted *Hardenbergia violacea*
Potted *Myoporum insulare*

Fig. 5

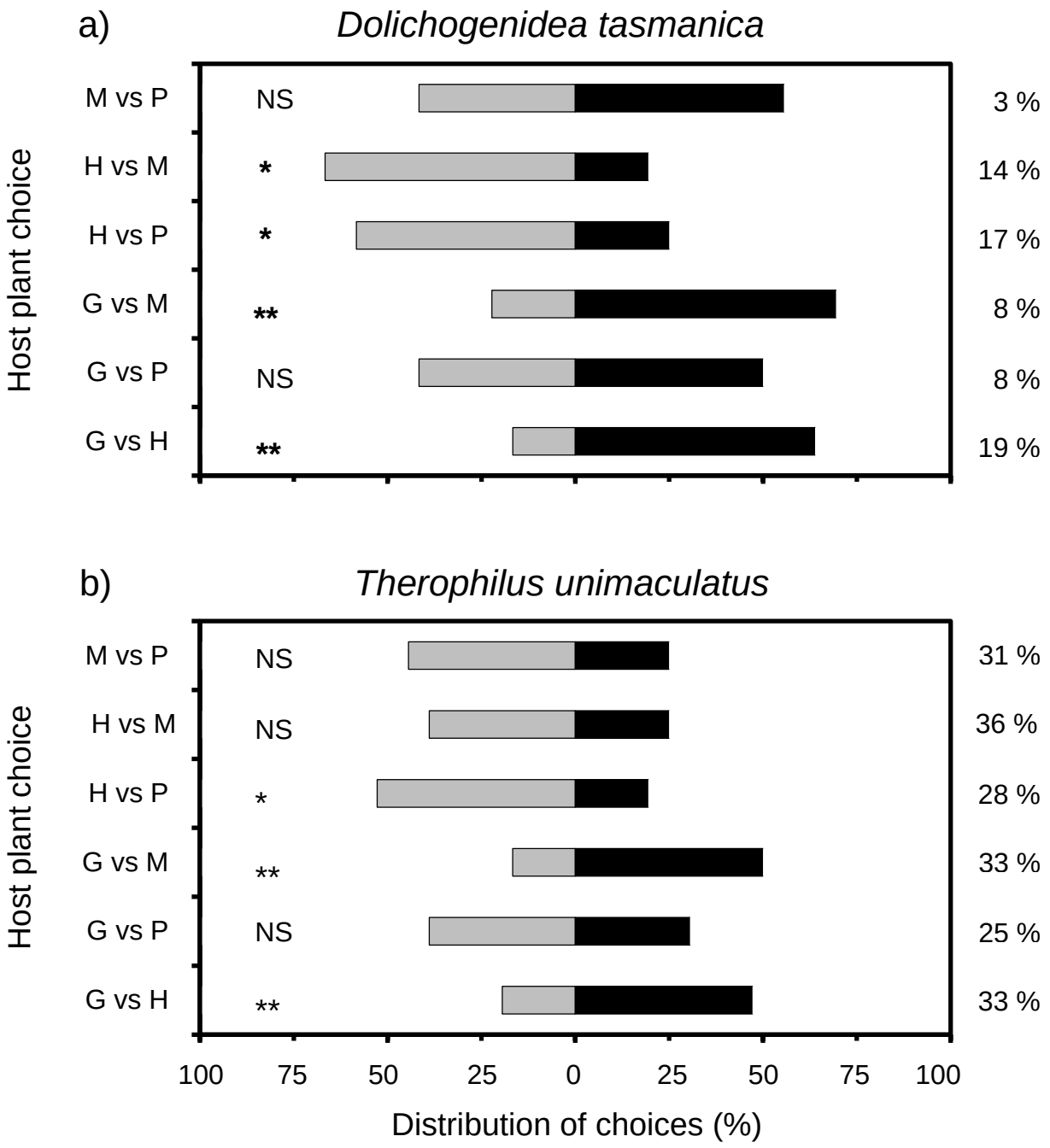

a)
Dolichogenidea tasmanica
Host plant choice
M vs P
H vs M
H vs P
G vs M
G vs P
G vs H
NS
*
*
**
NS
**
3 %
14 %
17 %
8 %
8 %
19 %
b)
Therophilus unimaculatus
Host plant choice
M vs P
H vs M
H vs P
G vs M
G vs P
G vs H
NS
NS
*
**
NS
**
31 %
36 %
28 %
33 %
25 %
33 %
100
75
50
25
0
25
50
75
100
Distribution of choices (%)

Fig. 6

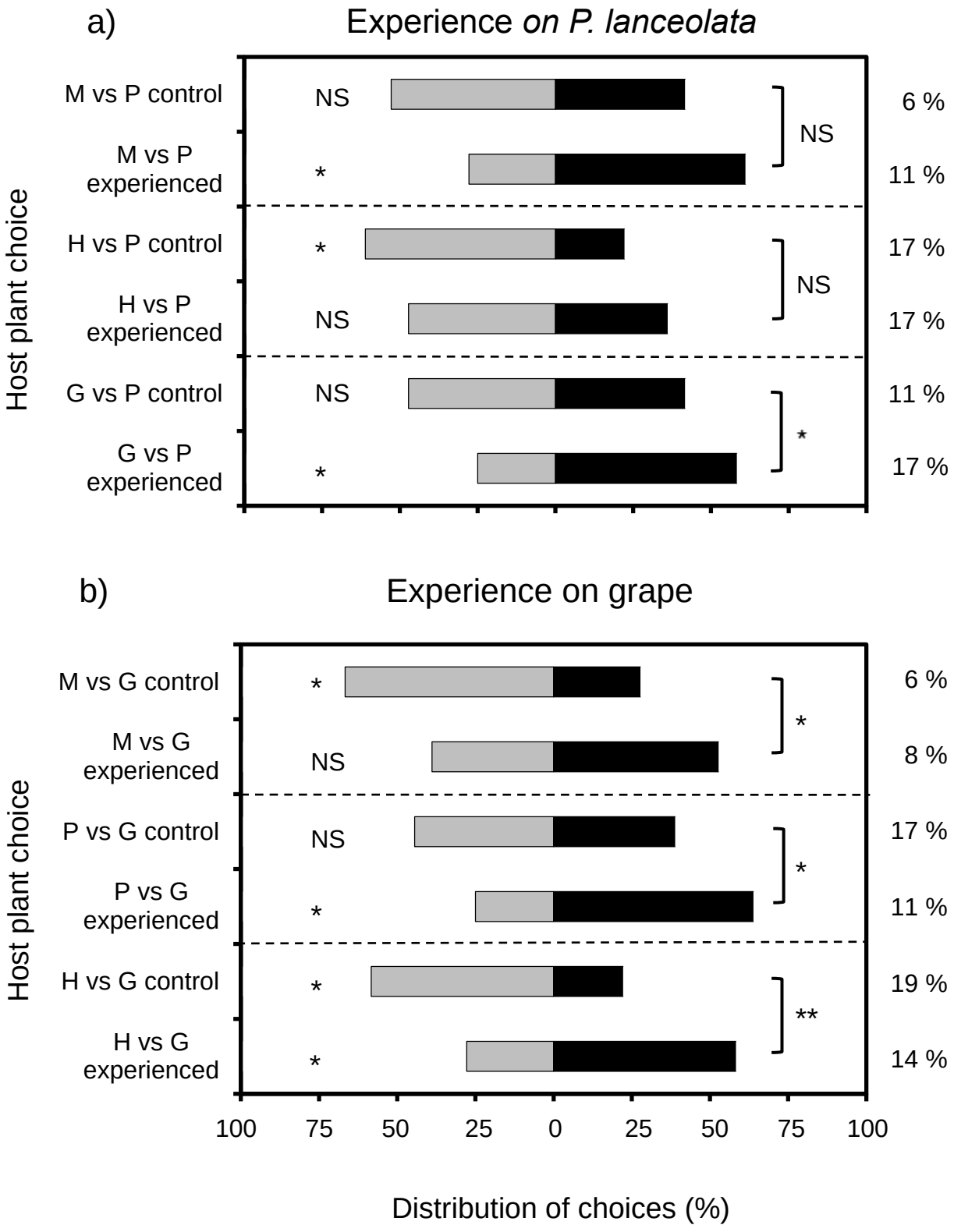
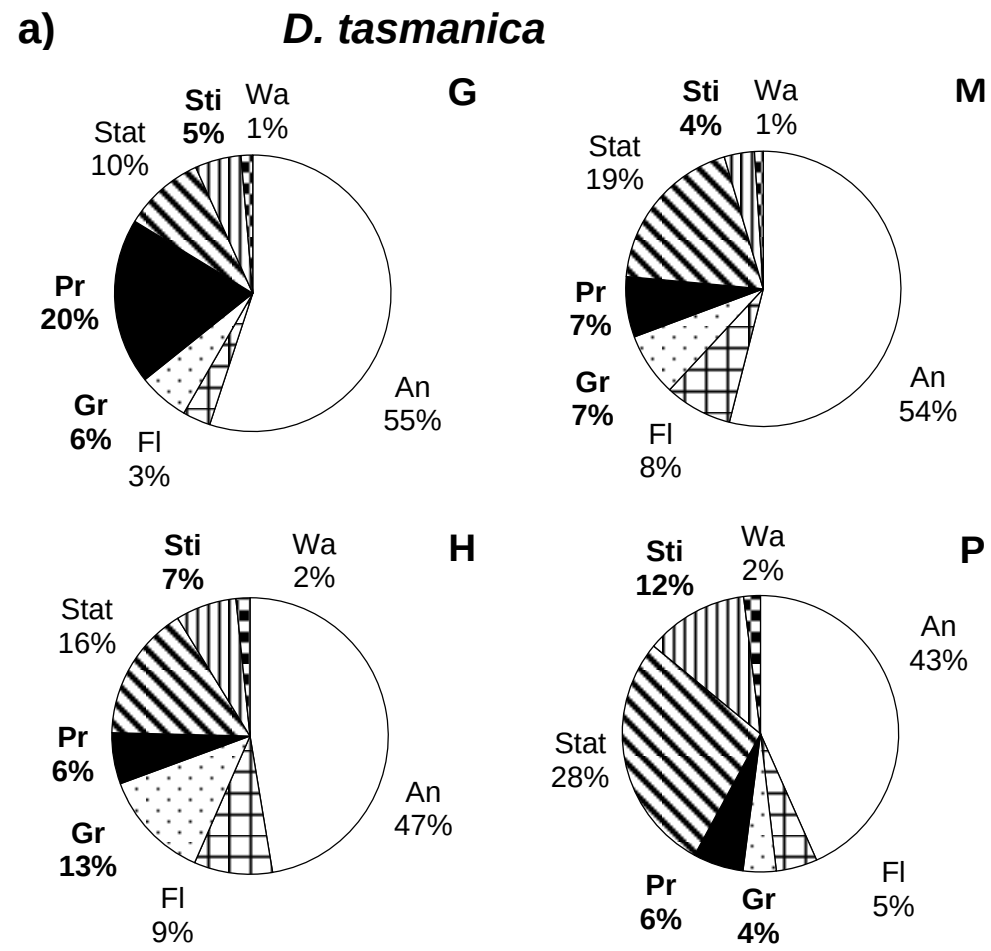

a)
Experience *on P. lanceolata*
M vs P control
M vs P experienced
H vs P control
H vs P experienced
G vs P control
G vs P experienced
NS
*
NS
*
NS
*
NS
NS
*
6 %
11 %
17 %
17 %
11 %
17 %
b)
Experience on grape
M vs G control
M vs G experienced
P vs G control
P vs G experienced
H vs G control
H vs G experienced
*
NS
NS
*
*
*
*
*
**
6 %
8 %
17 %
11 %
19 %
14 %
Host plant choice
100
75
50
25
0
25
50
75
100
Distribution of choices (%)

Fig. 7


a)
D. tasmanica
G
Sti 5%
Wa 1%
Stat 10%
Pr 20%
An 55%
Gr 6%
Fl 3%
M
Sti 4%
Wa 1%
Stat 19%
Pr 7%
An 54%
Gr 7%
Fl 8%
H
Sti 7%
Wa 2%
Stat 16%
Pr 6%
An 47%
Gr 13%
Fl 9%
P
Sti 12%
Wa 2%
An 43%
Stat 28%
Fl 5%
Pr 6%
Gr 4%

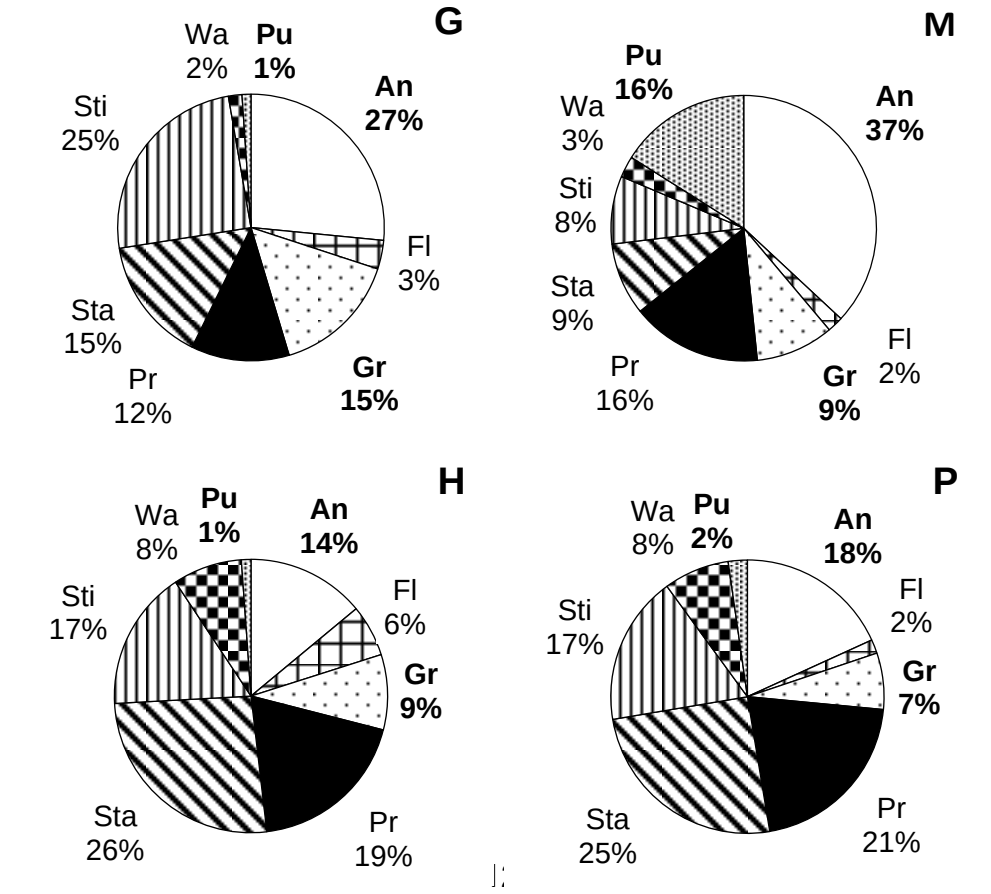

b)
T. unimaculatus
G
Wa 2%
Pu 1%
An 27%
Sti 25%
Fl 3%
Sta 15%
Pr 12%
Gr 15%
M
Pu 16%
An 37%
Wa 3%
Sti 8%
Sta 9%
Fl 2%
Pr 16%
Gr 9%
H
Wa 8%
Pu 1%
An 14%
Fl 6%
Sti 17%
Gr 9%
Sta 26%
Pr 19%
P
Wa 8%
Pu 2%
An 18%
Fl 2%
Sti 17%
Gr 7%
Pr 21%
Sta 25%

Fig. 8

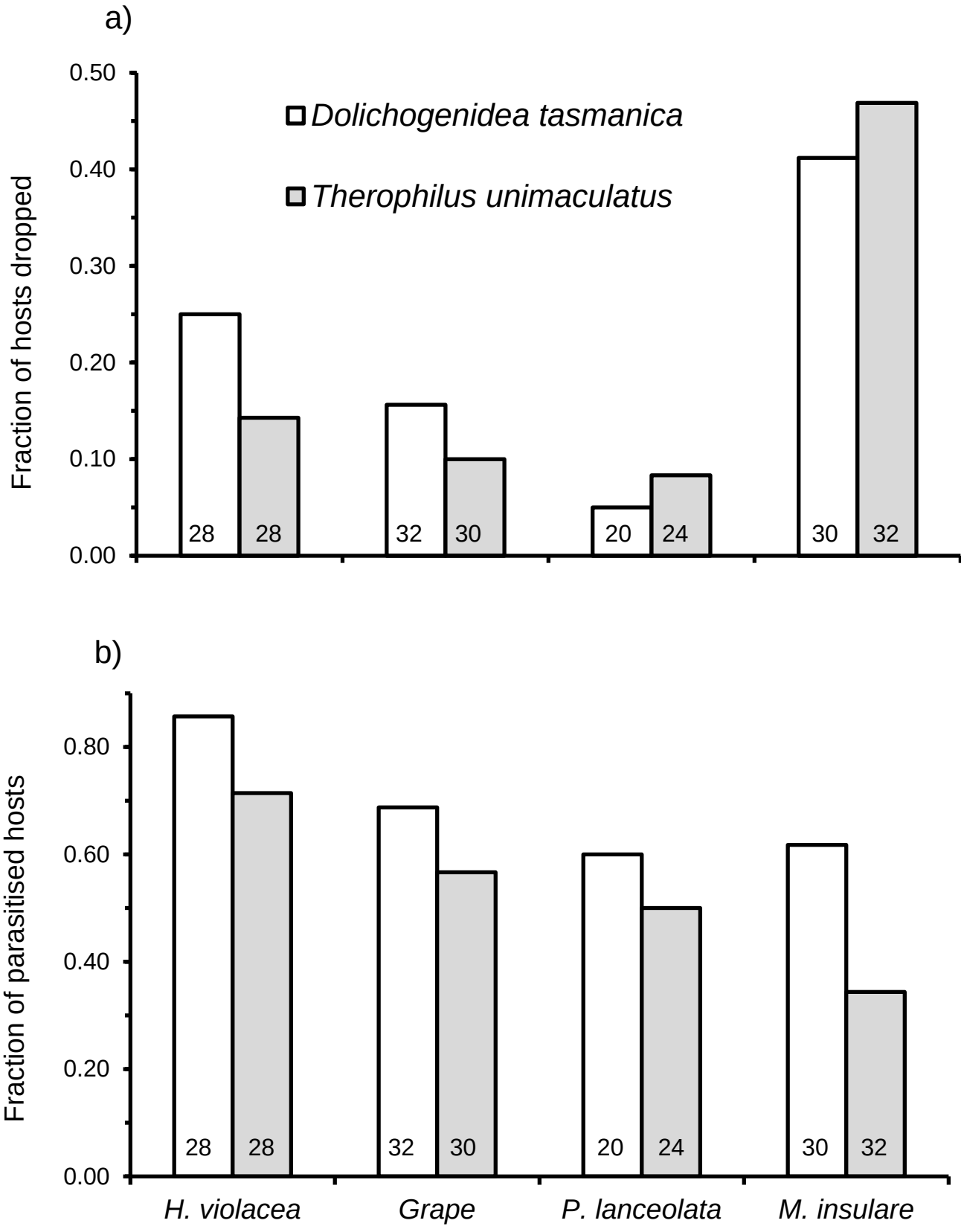

a)
0.50
0.40
0.30
0.20
0.10
0.00
Fraction of hosts dropped
Dolichogenidea tasmanica
Therophilus unimaculatus
28
28
32
30
20
24
30
32
b)
0.80
0.60
0.40
0.20
0.00
Fraction of parasitised hosts
28
28
32
30
20
24
30
32
H. violacea
Grape
P. lanceolata
M. insulare

Fig. 9

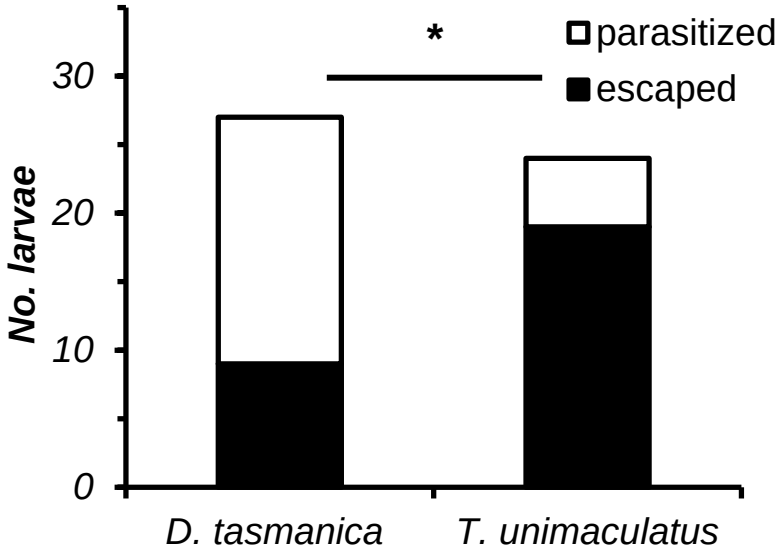
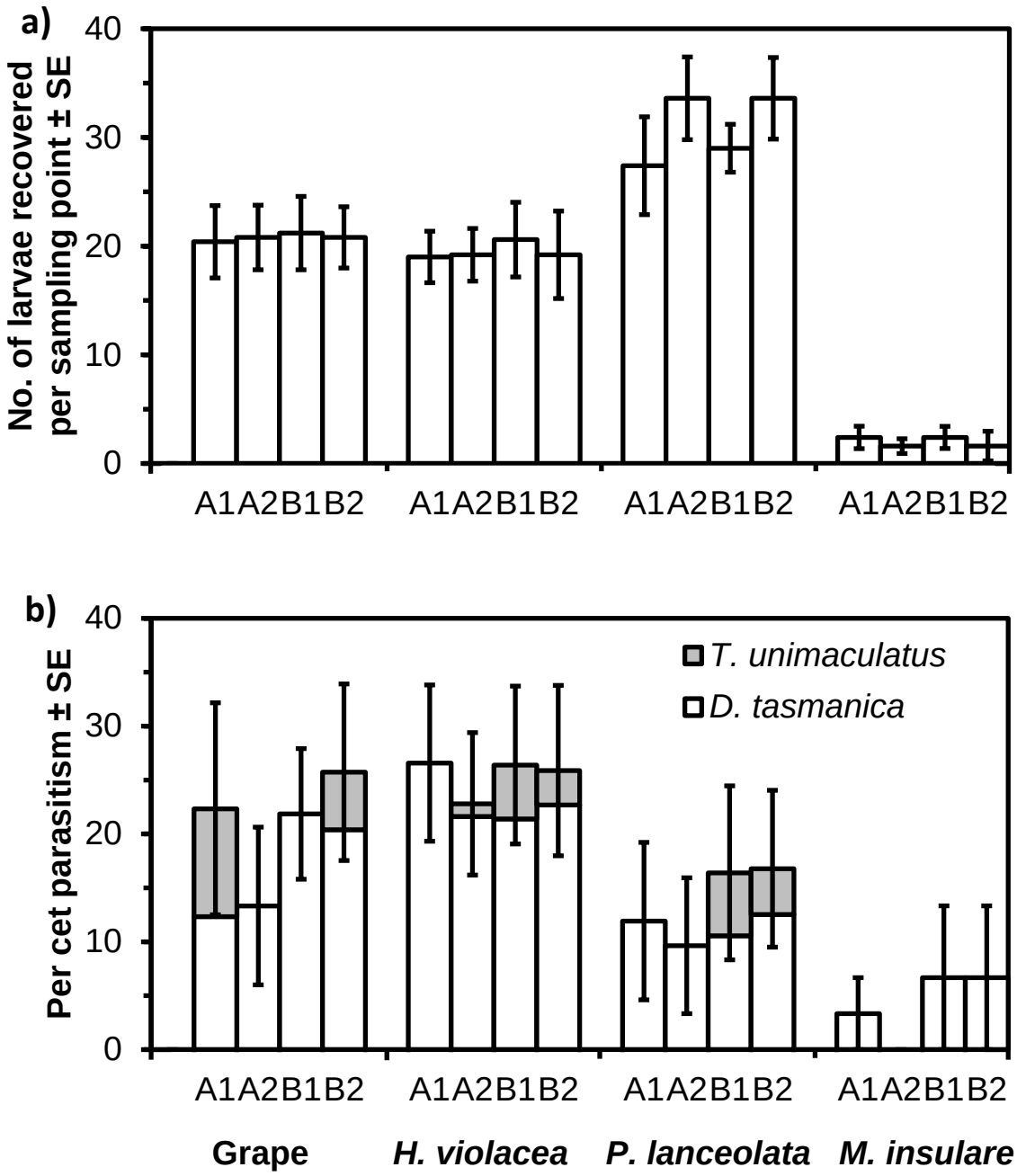

*
parasitized
escaped
No. larvae
30
20
10
0
D. tasmanica
T. unimaculatus

Fig. 10


a)
No. of larvae recovered per sampling point ± SE
40
30
20
10
0
A1A2B1B2
A1A2B1B2
A1A2B1B2
A1A2B1B2
b)
Per cet parasitism ± SE
T. unimaculatus
D. tasmanica
40
30
20
10
0
A1A2B1B2
A1A2B1B2
A1A2B1B2
A1A2B1B2
Grape
H. violacea
P. lanceolata
M. insulare

CHAPTER FIVE

Effect of temperature on the developmental rate of Therophilus unimaculatus (Hymenoptera: Braconidae)

Statement of Authorship

Effect of temperature on the developmental rate of *Therophilus unimaculatus* (Hymenoptera: Braconidae)

Yi Feng [1*], *Michael A. Keller* [1]

For submit to Austral Entomology

YF designed and performed the experiment, interpreted data and wrote the manuscript; MK help developing the idea and provide guidance throughout and assisted with statistical analysis and manuscript writing.

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Abstract

Therophilus unimaculatus (Turner) (Hymenoptera: Braconidae) parasitizes larvae of the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) in natural habitats and in the vicinity of vineyards. *E. postvittana* is an Australian native insect pest that causes damage to several economic crops, including wine grapes. The biology of *T. unimaculatus* is largely unknown. To evaluate its climatic adaptation and potential synchrony with its pest host, the temperature-dependent development of *T. unimaculatus* was studied under laboratory conditions at seven constant temperatures between 15 and 34.5 ℃. The lower thresholds for egg-larva, pupa and egg to adult development were 7.6, 11.5 and 8.8 ℃, respectively. Results show the mean developmental time from egg to adult emergence is shortest at 24.4 days at 28.9 ºC. The data were fitted to a non-linear model, which showed that the number of generations of *T. unimaculatus* is equal or greater than *E. postvittana* in three out of four locations in Southern Australia. This indicates that *T. unimaculatus* has the capacity to contribute to biological control of *E. postvittana*.

Keywords: *Therophilus unimaculatus*, *Epiphyas postvittana*, temperature, development time, thermal constants

Introduction

Therophilus unimaculatus (Turner) (Hymenoptera: Braconidae) is an Australian indigenous, solitary, koinobiont, endoparasitoid. This species has been recorded from the east coast of Australia from northern Queensland to Victoria and Tasmania, and as far west as South Australia (Stevens *et al.* 2010). It attacks a range of species, including two native pest lepidopteran species, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) and *Etiella behrii* Zeller (Lepidoptera: Pyralidae). *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) is a pest of grapes, pome fruit and other horticultural crops in Australia (Danthanarayana 1983; Scholefield and Morison 2010). It is an Australian native, leaf rolling, polyphagous, multivoltine moth. The range of host plants of *E. postvittana* is at least 123 genera in 55 families of plants (Suckling and Brockerhoff 2010). There are at least 26 parasitoid species associated with *E. postvittana* in Australia (Paull and Austin 2006). Among these parasitoids, *T. unimaculatus* is common in the vicinity of vineyards (Chapter 2). There is little known about the biology of *T. unimaculatus*. Temperature strongly affects all aspects of the biology of insects (Régnière *et al.* 2012), including the efficacy of parasitoids species that act as biological control agents (Shishehbor and Brennan 1996). In this study, we investigated the effect of temperature on the development of *T. unimaculatus* at seven constant temperatures to determine its response to temperature. We compared the predicted development of the parasitoid to one of its hosts, *E*. *postvittana*, to gain insight into how the two species might interact under Australian climatic conditions.

Materials and methods

Study species

Epiphyas postvittana was kept in a laboratory colony that was maintained on an artificial diet (Yazdani *et al.* 2014) at 22 ± 2 ºC under a 12:12 h light: dark cycle. This culture has been maintained for more than 200 generations. The colony of *T. unimaculatus* originated from parasitized larvae of *E. postvittana* that were collected from a vineyard (35°16'05'' S, 138°37'10'' E) and from the field (35°58'18'' S, 138°38'32'' E) near Adelaide, Australia in November 2011. *T. unimaculatus* was reared on larval *E. postvittana* that was reared on plantain, *Plantago lanceolata* L. (Plantaginaceae). The colony of *T. unimaculatus* was maintained in insect rearing cages (Bug-Dorm-2120F, 60 × 60 × 60 cm, MegaView, Taichung, Taiwan) at 23 ± 2 ºC with a 14:10 (light: dark) photoperiod.

Temperature studies

The effect of temperature on the development of *T. unimaculatus* was examined in incubators (Westinghous freestyle RP423T-R) with temperature controller (Scientific Equipment Manufacturers N759) at seven constant temperatures (15, 20, 25, 27.5, 30, 33, and 34.5 ºC) with a photoperiod of 14:10 (light: dark). The temperature in each incubator was monitored using a Tinytag® data logger to assure constancy (Gemini Data loggers UK Ltd, Chichester, West Sussex, UK). For each temperature treatment, two pots containing several plantain plants were placed in each incubator 24 hours before the experiment to stabilise the temperature of the plant before introducing parasitised larval *E. postvittana*. To begin each trial, parasitised *E. postvittana* were obtained by placing 20 second instar *E. postvittana*, which were feeding on plantain leaves, together with four adult female *T. unimaculatus* in 100 ml

plastic containers at room temperature until parasitization were observed. This process took less than 15 minutes. Ten parasitised larvae were then collected and placed on each pot of plantain in each incubator. To prevent escape, each pot was covered with a perforated plastic bread bag. The development of the immature parasitoids was checked daily. The times when parasitoid cocoons formed were recorded. Each cocoon of *T. unimaculatus* was individually transferred to a clean plastic cup (100 ml) with fresh plantain leaves and kept in the same incubator. Thereafter, the emergence times of adult parasitoids were recorded daily. Care was taken to make sure each plant was checked quickly to avoid the fluctuation of the temperature.

Nonlinear regression analyses were used to describe the relationship between temperature and the development rate of *T. unimaculatus* from egg to cocoon formation, from cocoon formation to adult emergence, and from egg to adult emergence, respectively. The inverse of the developmental times at each temperature were used in regression analysis. The general model developed by Briere *et al.* (1999) was fitted to the data using GenStat for Windows (15th Ed., VSN International, Hemel Hempstead, UK.):

$$\text{If } T < T_0 \qquad R(T) = 0$$

$$\text{If } T_0 < T < T_L \qquad R(T) = \alpha\, T(T-T_0)(T_L - T)^{1/m}$$

$$\text{If } T > T_L \qquad R(T) = 0$$

where R is the rate of development at a given temperature T in ºC, T_0 is the lower developmental threshold, T_L is the upper lethal threshold, and α and m are empirical constants. This model fits the developmental data of a wide range of insects.

The effect of temperature on the development of *E. postvittana* from egg to adult was also fitted to the nonlinear model. Temperature-dependent developmental times of *E. postvittana* feeding on dock, *Rumex obtusifolius* L. (Polygonaceae), were obtained from Danthanarayana (1975). Developmental rates were estimated from the reported mean developmental times before performing non-linear regression to fit the model.

Estimated number of generations of E. postvittana and T. unimaculatus

The numbers of generations of *E. postvittana* and *T. unimaculatus* were estimated at four Australian locations to explore the role of temperature in the population dynamics of *E. postvittana* and *T. unimaculatus*, and to estimate the potential for synchrony between the two species. The numbers of generations were calculated using cumulative developmental rates that were calculated using Briere-type non-linear regression models of development from egg to adult (Briere *et al.* 1999). We assume there was no pre-oviposition period for both species; therefore the number of generations was likely to be marginally overestimated. Temperature data recorded every 10 or 15 minutes from 1st January until 31st December 2012 at four locations in Australia were obtained from the South Australian Murray-Darling Basin Natural Resource Management Board. The locations were Langhorne Creek, Loxton and Mount Pleasant in South Australia, and Red Cliffs in New South Wales. Some of the temperature data were missing within short intervals of one hour or less. The missing data were estimated using linear interpolation over the duration of each gap.

Results

Therophilus unimaculatus completed development from egg to adult between 15 and 34.5 ºC. The developmental rates from oviposition to cocoon formation (egg-larva),

from cocoon formation to emergence of adult parasitoids (pupa) and from oviposition to emergence of adult parasitoids (egg-adult) at seven constant temperatures (Table 1) fit the Briere-type model well in each case (Fig. 1). The observed development time was shortest at 27.5 ºC (25.12 ± 0.83 SE days) and 30.0 ºC (25.25 ± 1.19 days), while the predicted shortest developmental time is 24.4 days at 28.9 ºC. The estimated lower developmental threshold varied between 7.6 and 11.5 ºC, and the estimated upper lethal threshold varied between 35.4 and 36.6 ºC, depending on the stage (Table 2). Temperature affected the survival of immature *T. unimaculatus*, with the minimum mortality recorded at 25 ºC (Fig. 2).

The numbers of generations of *E. postvittana* were smaller than those of its parasitoid, *T. unimaculatus*, at three of the four locations (Table 3).

Discussion

This study demonstrated the effect of constant temperatures on the developmental times, rates and survivorship of *T. unimaculatus* that developed within its host *E. postvittana*. The juvenile developmental rates of *T. unimaculatus* vary between 7.6 ºC and 35.4 ºC and follow the nonlinear Briere-type model (Fig. 1). The longest observed developmental time of larval stage was 48.22 ± 2.59 days at 15 ºC, and shortest was 16.58 ± 0.77 days at 27.5ºC (Table 1). The lower developmental threshold of *T. unimaculatus* estimated by Briere-type model was 7.57 ºC, which was higher than that of its host *E. postvittana* as estimated at 7.5 ºC by Danthanarayana (1975) or 4.44 ºC in the present study. High mortality of larval *T. unimaculatus* parasitising *E. postvittana* were observed at the highest (48% at 34.5 ºC), and lowest temperatures (30% at 15 ºC) (Fig. 2). This indicates that extreme temperatures strongly affect the survival of *T. unimaculatus.*

Temperature influences the development and survival of host and parasitoids, and therefore must affect their population dynamics. The relationship between the developmental rate of a parasitoid and that of its host maybe critical to the degree of natural pest suppression in a wild population, as parasitoids often exhibit developmental rates or temperature thresholds that are different from those of their hosts (Bernal and Gonzalez 1993). When a parasitoid develops faster than its host and there are more parasitoid generations than host generations, there can be a faster numerical response. However, if a host develops faster than its parasitoid, then the rate of population increase of the host may be greater than the capacity of the parasitoid population to respond numerically. The seasonal temperature profiles at different geographic locations can be quite different and therefore affect the synchrony between parasitoid and host. The number of estimated generations of larval *T. unimaculatus* was greater than or equal to its host, *E. postvittana*, at the four sites that were examined (Table 3), which indicates that populations of the parasitoid have the capacity to synchronise with those of *E. postvittana*. The higher and lower developmental thresholds of *T. unimaculatus* were both higher than those of *E. postvittana*. Therefore, *T. unimaculatus* can develop when temperature is above the upper threshold of *E. postvittana*, while *E. postvittana* can develop when temperature is below the lower threshold of *T. unimaculatus*. Thus, the number of generations of *T. unimaculatus* and *E. postvittana* and the potential for synchrony between their populations are affected by the seasonal pattern of extreme temperatures. In places like Mount Pleasant, the pattern of high and low temperatures leads both species to have virtually the same number of generations each year. While in the other three locations examined, where temperatures above the upper lethal threshold of *E. postvittana* occur more often, the number of generations for *T. unimaculatus* is

higher than *E. postvittana*. An analysis of the developmental rates of both species indicates that *T. unimaculatus* develops at a greater rate than *E. postvittana* whenever the temperature is greater than 16.0 °C (Fig. 3). Thus it is expected that *T. unimaculatus* could make the greatest contribution to suppression of *E. postvittana* in the warmer months of the year.

Overall, this study investigated the juvenile development of *T. unimaculatus* parasitizing *E. postvittana* at seven constant temperatures. The Briere-type model effectively predicts the developmental rate of *T. unimaculatus* and *E. postvittana*. In addition, a comparison of the developmental times and numbers of generations of both *T. unimaculatus* and its host *E. postvittana* at four locations indicated that this parasitoid can complete more generations than its host in some regions. These results should contribute to the understanding of the interactions between *T. unimaculatus* and *E. postvittana* under different climatic conditions.

Acknowledgments

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Figure legends

Fig. 1. The rate of development (± SE) of (a) Egg-larval stages (b) Pupae and (c) Egg-adult emergence of *T. unimaculatus* vs. temperature. Values are means ± standard errors. The data were fit to a nonlinear regression model of temperature-dependent development (Briere et al, 1999).

Fig. 2. Percentage survival of *Therophilus unimaculatus* parasitising larval *Epiphyas postvittana* at seven constant temperatures for egg and larval stages, pupae and egg through adult development (n = 40 for each temperature).

Fig. 3. The rate of development of from egg to adult emergence of *T. unimaculatus* and *E. postvittana* vs. temperature. Development of *E. postvittana* from egg to adult based on the data of Danthanarayana (1975). The data were fit to a nonlinear regression model of temperature-dependent development (Briere *et al.* 1999).

Table 1. Developmental times (mean ± SD) of immature stages of *T. unimaculatus* parasitising *E. postvittana* at seven constant temperatures

		Developmental time (days)					
Temperature (℃)	*n*	Egg-larva	*n*	Pupa	*n*	Egg-adult	*n*
15	40	48.22 ± 2.59	28	38.71 ± 3.60	14	86.21 ± 2.36	14
20	40	26.44 ± 0.83	28	12.43 ± 0.81	21	38.57 ± 1.48	21
25	40	18.41 ± 0.84	34	8.77 ± 0.50	30	27.40 ± 0.94	30
27.5	40	16.58 ± 0.77	30	7.92 ± 0.44	26	25.12 ± 0.83	26
30	40	16.74 ± 0.72	25	8.45 ± 0.54	20	25.25 ± 1.19	20
33	40	21.34 ± 0.94	22	9.29 ± 0.70	14	30.14 ± 1.65	14
34.5	40	32.04 ± 1.96	21	14.38 ± 1.06	13	46.23 ± 2.51	13

Table 2. Parameters estimates for the non-linear models (Briere et al 1999) describing the relationship between development rate (1/day) and temperature for *T. unimaculatus* and *E. postvittana*

Stages	a	T_0	T_L	M
	T. unimaculatus			
Egg-larva	3.57E-05	7.57	35.38	1.86
Pupa	5.30E-05	11.51	36.60	1.30
Egg to adult	2.42E-05	8.84	35.56	1.77
	E. postvittana			
Egg to adult	2.70E-05	4.44	33.04	2.60

Table 3. Estimated numbers of generations of *Epiphyas postvittana* and *Therophilus unimaculatus* at four locations in Australia during 2012

Site	Latitude / Longitude	Species	Number of Generations
Langhorne Crk., South Australia	35°17' S	*E. postvittana*	4.96
	139°02' E	*T. unimaculatus*	5.15
Loxton, South Australia	34°27' S	*E. postvittana*	5.20
	140°34' E	*T. unimaculatus*	5.66
Mt. Pleasant, South Australia	35°46' S	*E. postvittana*	4.40
	139°03' E	*T. unimaculatus*	4.42
Red Cliffs, New South Wales	34°18' S	*E. postvittana*	5.50
	142°11' E	*T. unimaculatus*	6.08

Fig. 1.

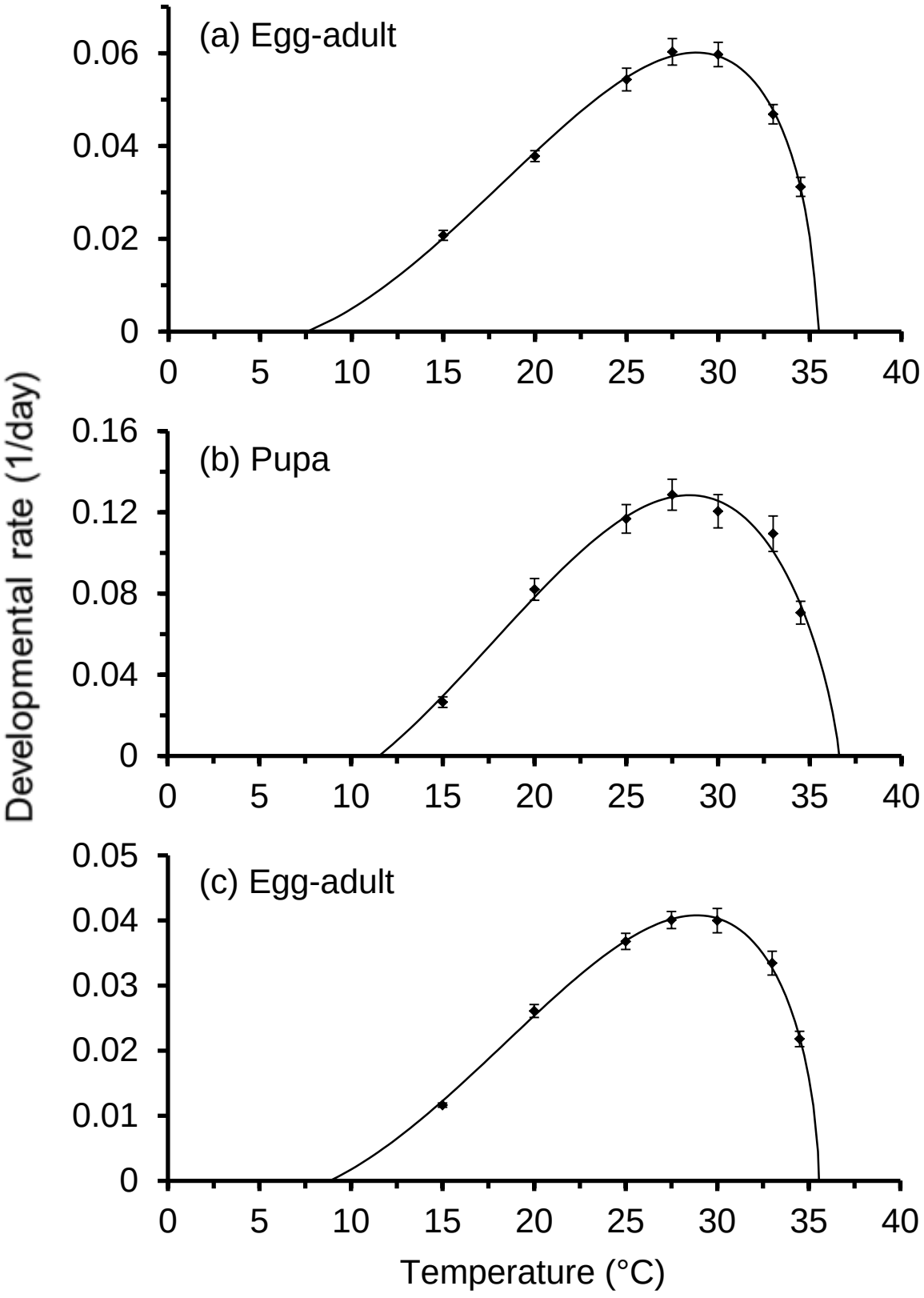

(a) Egg-adult
(b) Pupa
(c) Egg-adult
Developmental rate (1/day)
Temperature (°C)
0.06
0.04
0.02
0
0.16
0.12
0.08
0.05
0.03
0.01
0 5 10 15 20 25 30 35 40

Fig. 2.

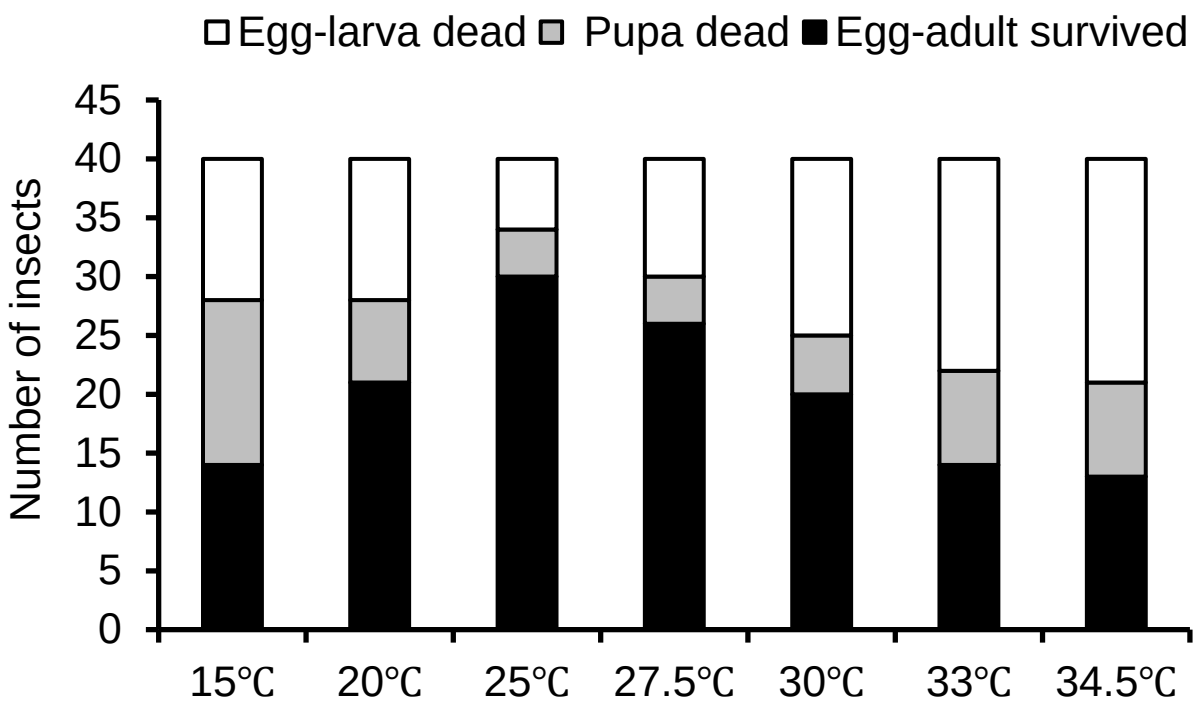
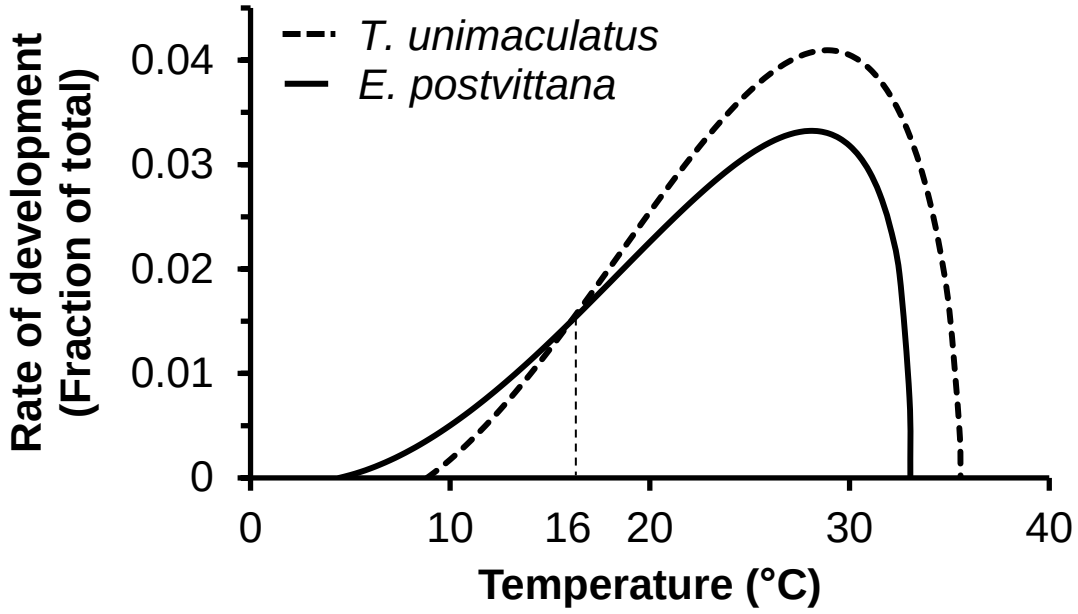

Egg-larva dead
Pupa dead
Egg-adult survived
Number of insects
45
40
35
30
25
20
15
10
5
0
15℃
20℃
25℃
27.5℃
30℃
33℃
34.5℃

Fig. 3.


T. unimaculatus
E. postvittana
Rate of development
(Fraction of total)
0.04
0.03
0.02
0.01
0
0
10
16
20
30
40
Temperature (°C)

CHAPTER SIX

General Discussion

The main aim of this thesis was to investigate why some parasitoids that attack *Epiphyas postvittana* are so uncommon in vineyards, and how competitive interactions and the behaviour of some parasitoids could influence their population levels and parasitism rates in vineyards. This was achieved by comparing the rates of parasitism between vineyards and adjacent natural habitats, together with investigations of interactions between parasitoids, and a number of factors that could affect interactions among *E. postvittana* and its associated parasitoids. This project provides insights for the biological control of *E. postvittana* and related insect pests, especially tortricids, and tri-trophic interactions among plants, pests and natural enemies in agro-ecosystems. Future research should investigate how factors such as the movement of parasitoids, alternative host species, learning by parasitoids, density dependent responses, landscape characteristics, seasonal changes in vineyards, and the fourth trophic level, i.e. hyperparasitoids, could affect the community of parasitoids and predators that attack *E. postvittana*.

Sampling of the parasitoids that attack larval *E. postvittana* indicated that *Dolichogenidea tasmanica* and *Therophilus unimaculatus* are the dominant parasitoid species in and around vineyards in the Adelaide Hills Region of South Australia. Other parasitoid species occasionally occur at lower frequencies in vineyards and nearby vegetation. Results of a survey of larval tortricids and molecular identification of larvae parasitised by *D. tasmanica* and *T. unimaculatus* indicated these two parasitoids attack an overlapping range of leaf roller species (Chapter 2). This result was consistent with the fact that natural enemy communities in agro-ecosystems are typically dominated by a few species as suggested by studies investigating the diversity and abundance of predatory groups such as spiders (Öberg et al., 2007), ground beetles (Ekbom and Wiktelius, 1985), and

parasitoids (Tylianakis et al., 2007). In these cases, the most common predators or parasitoids are thought to contribute to the stability of the pest-natural enemy community, and thus to contribute to the suppression of pests (Tscharntke et al., 2007). However, such studies have largely focused on isolating factors and specific mechanisms that influence the activity of natural enemies in crops (Andow, 1991, Corbett and Plant, 1993, Kennedy and Storer, 2000, Landis et al., 2000). Little is known about how those natural enemies that are active in cultivated habitats are related to those active in adjacent un-cultivated habitats. (Derocles et al., 2014)

One of the key findings in this study is populations of *D. tasmanica* and *T. unimaculatus* have different activity levels between vineyards and adjacent natural habitats in vineyard agro-ecosystems. *D. tasmanica* is mainly active in vineyards, whereas *T. unimaculatus* occurs mainly in adjacent native vegetation. This finding suggested competition between the two parasitoids species that is modulated by habitat characteristics. Studies indicate that understanding the competitive interactions between parasitoids that share the same host is important for determining their efficiency in pest control (Bogran et al., 2002, De Moraes and Mescher, 2005, Gurr et al., 2004, Harvey et al., 2013, Mackauer, 1990, Orre-Gordon et al., 2013).Therefore, aspects of the competitive interactions between *D. tasmanica* and *T. unimaculatus* that parasitise *E. postvittana* were investigated (Chapter 3). During extrinsic competition, both parasitoid species did not respond differently to larvae of *E. postvittana* that were parasitised by the other species, compared to un-parasitised *E. postvittana*. Both parasitoids have been observed to attack the same individual host in sequence. During subsequent intrinsic competition, *D. tasmanica*

out-competes *T. unimaculatus* regardless of the order or interval between parasitisation.

Herbivore-infested plants are known to affect the foraging efficiency of parasitoids in various ways (Price et al., 1980, Lill et al., 2002). In Australian vineyards, numerous native and introduced plants are found in vineyards and adjacent habitats. This project examined the effects of a sample of host infested plants on the foraging efficiency of *D. tasmanica* and *T. unimaculatus*. A series of laboratory experiments demonstrated that different species of plants attract the parasitoids differently. In addition, oviposition on and host feeding on one plant species was shown to affect the subsequent host plant preferences of parasitoids in some instances. Moreover, both parasitoid species allocate their foraging time differently while searching for hosts on different plant species. A field experiment also indicated that host infested plants affect the level of parasitism of *E. postvittana* in vineyards (Chapter 4). The results indicated that plants play a key role that affect the fitness of the two parasitoid species by influencing their foraging choices and efficiency, and therefore contribute to the distributions of parasitoids among habitats.

Beside the effects of host plants on host insects and the behaviour of parasitoids, differences in abiotic conditions between cultivated and uncultivated habitats are also likely to affect the activities of natural enemies. These include temperature (Amat et al., 2006), light and moisture (Smith and Rutz, 1991), and other environmental conditions (Fink and Volkl, 1995). In this project, the temperature dependent development of immature stage of *T. unimaculatus* that was reared on larval *E. postvittana* was experimentally measured. In addition, the developmental times of *T. unimaculatus* and *E. postvittana* were compared by fitting non-linear models of temperature-dependent development (Chapter 5). The results indicated

that *T. unimaculatus* develops faster than *E. postvittana* whenever the temperature is higher than 16 ℃. Therefore *T.* unimaculatus could potentially contribute to suppression of *E. postvittana* during warmer periods of the year. Currently, there has not been a thorough investigation of the thermal responses of other parasitoid species that share the same hosts with *T. unimaculatus*. In vineyard agro-ecosystems, temperatures in the woody vegetation adjacent to the vineyards are likely to be lower than those in vineyards, especially during summer. If parasitoid species have different temperature responses, one species may be more active in native vegetation, while other species may be more active in the open canopy of grapevines. Further studies are necessary to investigate and evaluate the effects of temperature on the interactions among parasitoid species that attack *E. postvittana* and their potential to supress it. In addition, abiotic factors such as wind and moisture should be investigated to understand their influence on the activities of these parasitoids in cultivated and uncultivated habitats.

In order to sustainably control an insect pest, it is important to evaluate the responses of the closely associated natural enemy species to different habitats and identify key factors that influence their efficiency to control the pest. The results of Chapter 2, 3, 4 and 5 of this thesis pointed out that various factors in and adjacent to vineyards could affect the activity of parasitoids associated with *E. postvittana*. Future research should first address the effects of alternative hosts, cyclical seasonal changes in vineyards, and the movements of parasitoids on interactions among parasitoids, *E. postvittana* and related species of tortricids in vineyards and adjacent vegetation. Other factors (Figure 1 in Chapter 1) also warrant further investigation.

Alternative hosts

Epiphyas postvittana is not the only tortricid species that occurs in Australian vineyards and natural habitats (Gillighan 2014). The results of this study indicated that both *D. tasmanica* and *T. unimaculatus* attack an overlapping range of tortricid species and there are probably complex associations among them. These interactions could strongly affect the activities of parasitoids across the landscape. Studies have suggested that providing plant species that support alternative hosts for natural enemies within or adjacent to crops could enhance parasitoid activity in crops (Unruh et al., 2012, Pfannenstiel et al., 2012, Letourneau and Altieri, 1999, Thomas et al., 1991). However, the role of alternative hosts on the activity and fitness of parasitoids that are associated with *E. postvittana* at Australian vineyards is not understood. It is necessary to further investigate whether *D. tasmanica* and *T. unimaculatus* discriminate among different host species and whether they have innate preferences for certain host species. Furthermore, in terms of biological control, it should be worthwhile to know how host species could affect the foraging efficiency and habitat preference of these parasitoids, and the extent to which these parasitoids shift between alternative hosts and *E. postvittana.*

Cyclical seasonal changes in vineyards

Cyclical seasonal changes in vineyards strongly affect the availability of resources and must influence the activity of parasitoids. During winter dormancy in Australian vineyards, there are usually limited resources available for parasitoids, such as alternative hosts and food sources and shelter plants. The alternative resources that support the activities of parasitoids in vineyards are mainly found in the understory vegetation. In contrast, natural habitats adjacent to vineyards are more heterogeneous and stable than vineyards. Natural habitats provide natural enemies with resources such as overwintering shelter, and refuges from disturbance

(Cortesero et al., 2000, Landis et al., 2000), alternative food (Wratten et al., 2004, Berndt and Wratten, 2005, Begum et al., 2006, Berndt et al., 2006, Lee and Heimpel, 2008, Gámez-Virués et al., 2009), and alternative hosts (Pfannenstiel and Unruh, 2003, Williams and Martinson, 2000). Therefore, grapevines in vineyards undergo cyclical colonisation by leaf rollers and parasitoids, either from the inter-row vegetation or from adjacent native vegetation (Wissinger, 1997). *D. tasmanica* and *T. unimaculatus* may have different responses to the seasonal changes in vineyards. *D. tasmanica* may be better adapted the environment in vineyards and be better able to colonise, maintain or rebuild its populations in this habitat, while *T. unimaculatus* may be better adapted to the environment found in natural habitats. To test this hypothesis, future studies are needed to quantitatively evaluate and compare parasitism by both parasitoid species in both vineyard and adjacent vegetation before, during and after vine dormancy.

Movement of parasitoids

Movement enables parasitoids to find resources such as alternative food and hosts, and to escape from adverse conditions. This is critical for the persistence of populations of parasitoids in agro-ecosystems, where resources may be isolated and dynamically distributed in many cases (Schellhorn et al., 2014). Studies have indicated that the abundance and diversity of insect natural enemies in crops can be associated with other habitats at a larger landscape scale (Schmidt and Tscharntke, 2005, Schmidt-Entling and Döbeli, 2009, Schweiger et al., 2005).Therefore, it is necessary to understand how movement affects the population dynamics of natural enemies and their ability to supress pests at both local and landscape scales in agro-ecosystems. The movement abilities of *D. tasmanica* and *T. unimaculatus* may be quite different, which would influence their ability to seasonally colonise grapevines

in vineyards. Although it is difficult to quantify the movement of parasitoids partly due to their relatively small size, novel methods and tools have been applied to quantify the movement of insects (Ovaskainen et al., 2008, Patterson et al., 2008, Urban et al., 2009). A previous study indicated that *D. tasmanica* can travel at least 30 m over 7 days in vineyards (Scarratt et al. 2008), while the movement ability of *T. unimaculatus* is still unknown. Therefore, differential mobility of these species may influence their relative activities in vineyards and adjacent vegetation. This needs to be investigated to understand the relationships among species, habitat characteristics and the level of pest suppression in agro-ecosystems.

Experience of parasitoids

Parasitoids rely on innate behavioural mechanisms as they forage for hosts, but in addition they can adaptively optimise their foraging efficiency by learning cues that are associated with host availability (Vet et al., 1995, Wackers and Lewis, 1994, Vet and Dicke, 1992). Studies have indicated that learning influences the ability of parasitoids to find their hosts in both laboratory (Geervliet et al., 1998, Lewis and Takasu, 1990) and field experiments (Papaj and Vet, 1990). However, in dynamic agro-ecosystems, the link between parasitoid learning and pest suppression is not well understood. It is necessary to further investigate, for example, whether the effects of host-associated cues, such as feeding damage to host plants and exposure to alternative hosts, can reinforce the preferences of parasitoids for selected pest hosts on crops in agro-ecosystems. Alternative host plants and host species will be selected and added into vineyard to evaluate if parasitism of *E. postvittana* would be enhanced on grape vine.

Density dependent responses of parasitoids

One of the requirements for effective pest control by natural enemies is their ability to express a rapid numerical response to increasing pest density. Effective natural enemies should be present within cultivated habitats or easily re-colonise them. In a study involving both a naturally occurring hosts and an experimentally manipulated hosts, the response of *D. tasmanica* to different host densities was found to be inversely density dependent (Paull et al., 2013). To evaluate the potential of *T. unimaculatus* to suppress *E. postvittana* and compare with the responses exhibited by *D. tasmanica*, it is necessary to further investigate their functional and numerical responses to different host densities, especially densities above the economic threshold.

Landscape effects

Natural pest suppression relies on the activities of natural enemies that already exist in agro-ecosystems (Barbosa, 1998, Landis et al., 2000). However, agricultural intensification reduces the size and integrity of natural habitats, as well as landscape complexity. This results in a mosaic of cultivated and uncultivated regions in a landscape (Tscharntke et al., 2002, Tscharntke et al., 2005, Daily et al., 2001). This fragmentation threatens the diversity and abundance of natural enemies (Harrison and Bruna, 1999, Tscharntke and Brandl, 2004). Remnant habitat islands may not be able to support populations of specific natural enemies. In addition, the reduced connectivity of fragmented habitats may cause local and even regional species extinction (Hanski and Ovaskainen, 2000, Hanski and Beverton, 1994). Fragmentation in agro-ecosystems reduces resource availability for natural enemies which are less adaptable than their herbivore hosts in both cultivated and uncultivated habitats (van Nouhyus and Group, 2005, Shaw, 2006, Elzinga et al., 2007, Tscharntke and Kruess, 1999). This may be even stronger for more

specialised parasitoids. In Australia, natural and seminal natural habitats close to vineyards could strongly affect natural enemy activity (Thomson and Hoffmann, 2010). However, the effects of landscape factors such size and connectivity of natural habitats near vineyards on the activity of natural enemies associated with *E. postvittana* are still largely unknown, and it is necessary to measure population levels of *E. postvittana* and other host species in relation to landscape parameters.

Effects of fourth trophic level-hyperparasitoids

In this study, only three trophic levels were investigated. Hyperparasitoids at the fourth trophic level could also affect the fitness and efficiency of parasitoids (Sullivan and Volkl, 1999). Studies have indicated that herbivore-induced plant volatiles attract not only parasitoids, but are also utilised by hyperparasitoids that are searching for parasitoid hosts (Poelman et al., 2012). Since there are at least five recorded hyperparasitoid species associated with *E. postvittana* (Paull and Austin, 2006), it is necessary to further investigate the impact of the fourth trophic level on the habitat preference of the two key parasitoids that attack *E. postvittana* and its effect on biological control of *E. postvittana*.

This project investigated the distribution and activity of parasitoids that attack *E. postvittana* in different habitats associated with vineyard agro-ecosystems. Factors such as the presence of alternative hosts, the characteristics of various host plant species, competition and abiotic factors were shown to influence populations of *E. postvittana* and its parasitoids. The underlying ecological mechanisms associated with these factors are still not thoroughly understood. However, it is clear that each of the identified biotic and abiotic factors plays a role in driving the population dynamics and spatial distributions of these natural enemies and their tortricid hosts.

It is likely that these factors are common drivers of the tri-trophic interactions among other tortricid species, their associated natural enemies and host plants in crops and adjacent semi-natural habitats. One key message to emerge from this work is that one cannot easily predict how the activities of the parasitoids that attack *E. postvittana* contribute to host insect suppression in various habitats. Future studies should be conducted to evaluate the effects of host density on parasitism quantitatively in both vineyards and adjacent habitats. This would support the sentinel plant studies and give clear indication of the degree to which those parasitoids contribute to the actual pest suppression. In particular, the adjacent natural habitats may not always be the source of natural enemies that are active in crops. This suggests that attention should not exclusively be paid to restoration or enrichment of adjacent natural habitats for enhancement of biological control. Factors that affect natural enemy activities in both cultivated and uncultivated habitats should also be investigated in the development of sustainable biological control.

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