Enhanced conservation biological control of light brown apple moth in vineyards

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ABSTRACT

The light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is the most damaging insect pest of wine grapes in Australia. Biological control contributes to the management of LBAM. This project aims to enhance the conservation biological control of LBAM by examining how the provision of alternative hosts and native flowering plants can sustain parasitoids like *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae). Native plants and alternative host insects that could support conservation biological control of LBAM in South Australian vineyards are evaluated. The contribution that *D. tasmanica* makes to biological control of LBAM would be more reliable if this wasp parasitises common alternative hosts, maintains populations on them when the grapevines are dormant, and then moves to attack LBAM when it reinvades vineyards.

This study examined the foraging behaviour of *D. tasmanica* on LBAM and two tortricid species that are associated with vineyards, *Acropolitis rudisana* (Walker) and *Merophyas divulsana* (Walker). The reciprocal responses of these insect hosts were also studied to determine their susceptibility to wasp attack. During behavioural assays, all hosts were accepted by *D. tasmanica* with high parasitism rates. The parasitoid responded differently to different host species. These experiments indicate that populations of *D. tasmanica* should be conserved, and LBAM more reliably suppressed, if the alternative hosts, *M. divulsana* and *A. rudisana*, are present.

Host choice between *E. postvittana* and *M. dilvusana* by *D. tasmanica* was also studied in a wind tunnel, where the wasp could express its natural searching behaviour. Choices tests were conducted to examine how natal hosts, host stages and the wasp's experiences could affect the landing selections of *D. tasmanica*. Developmental outcomes of parasitoids on different host species were also examined. *D. tasmanica* exhibited no clear preference for either host. Host species did not affect the body size of the wasp, but did influence its developmental time, probably as a result of differences in the host's body sizes. The sex ratio of the wasp did not vary between these host species. These results suggest that *M. divulsana* is a promising alternative host species to support parasitoid populations in vineyards.

A field study was conducted to evaluate the potential impacts of candidate plants on biological control of leafrollers, especially LBAM. Five species were planted beside vineyards to provide shelter, nectar and alternative hosts for beneficial insects. The plants were *Bursaria spinosa*, *Leptospermum lanigerum*, *Hakea mitchellii*, *Melaleuca lanceolata* and *Myoporum petiolatum*. Similar leafroller abundances and parasitism rates were found between vineyard rows adjacent to the native plants compared to rows furthest from them. The absence of a difference was possibly due to the proximity of the experimental treatment areas and the movement of parasitoids. The results, including increasing parasitoid diversity over time, imply potential benefits of the plants for better leafroller management.

The results from my studies suggest that selected supplementary resources can benefit parasitoids and thereby stabilise or enhance biological control of LBAM in vineyards. This research provides a foundation to develop strategies to better suppress LBAM by facilitating more stable biological control.

DECLARATION

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

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed Date 8 June 2018

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CHAPTER ONE

General introduction and literature review

1. General introduction

Regulation of crop insect pests by their natural enemies is an important ecosystem service that can support sustainable crop production while reducing the need for costly agrochemicals and associated environmental and human health concerns (Bianchi et al., 2006; Wyckhuys et al., 2013). Providing limiting resources to natural enemies through habitat manipulation as a form of conservation biological control can enhance both diversity and the ecosystem service they provide. At the field-scale, provision of floral resource plants is a common tactic to enhance local beneficial arthropods by providing them with plant-provided food supplements such as nectar (Baggen et al., 1998; Berndt et al., 2002; Begum et al., 2004; Berndt & Wratten, 2005; Berndt et al., 2006; Tompkins et al., 2010; Sigsgaard et al., 2013) and pollen (Hickman & Wratten, 1996; Wong & Frank, 2013); alternative hosts or prey (Perrin, 1975; Viggiani, 2003) and physical refugia (Thomas et al., 1991; Halaj et al., 2000; Collins et al., 2002; Collins et al., 2003)

While increasing plant diversity may increase the opportunities for enhancing natural enemies and regulating of insect pests, this such approach requires not plant diversity per se, but the right kind of biodiversity (Begum et al., 2006; Bianchi et al., 2006; Wäckers & van Rijn, 2012; Tschumi et al., 2015; Begg et al., 2017). For example, the provision of flowering plants may be risky if they benefit pests (Begum et al., 2006) or hyperparasitoids (Araj et al., 2008), reduce host foraging efficacy of parasitoids (Lavandero et al., 2006) by “masking of host-induced plant odors” (Tahvanainen & Root, 1972) or introduced new pest species. Careful selection of supplementary plant species for habitat management can reduce this possibility. The selection of supplement resources may be difficult, especially with highly polyphagous insect pests, but it can be guided through an understanding of the biology of what resources are needed by natural enemies (Landis et al., 2000; Tschumi et al., 2015) and the levels and the spatial and temporal context of these resource requirements (Tschumi et al., 2015; Gillespie et al., 2016). Thus, fundamental research into the non-pest resource requirements such as plant food, alternative hosts or prey and refugia under laboratory is the first step of the selection process. Then the risks and benefits of the such added resources should be assessed under the field conditions.

The light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is identified as the key insect pest of wine grapes in Australia and New Zealand (Charles et al., 1996; Suckling et al., 1998; Scholefield & Morison, 2010). Parasitism of LBAM contribute to the suppression of LBAM in Australian vineyards where there are at least 25 parasitoids and hyperparasitoids asscociated with LBAM (Paull & Austin, 2006). Of these species, the braconid *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) is the most abundance species attacking first to third instar larvae of LBAM (Yazdani et al., 2015a) and other leafrollers that found in landscapes where vineyards are planted, such as *Acropolitis rudisana* (Walker) and the lucerne moth, *Merophyas divulsana* (Walker) (Feng et al., 2017).

The biological control of leafrollers in Australian vineyards critically relies on the seasonal colonisation by natural enemies from overwintering habitats adjacent to vineyards. However, the lack of resources needed by natural enemies within vineyards has long been suspected to be an impediment to the success of this approach. The provision of floral plants could be a promising solution. However, the efforts of this approach have met with mixed achievements. Under laboratory conditions, access to flowering plants has contributed to an improvement of longevity, fecundity and the sex ratio of parasitoids (Berndt &

Wratten, 2005; Begum et al., 2006; Berndt et al., 2006). However, results testing the effects of flowering plants on biological control of LBAM under field conditions are inconsistent (Berndt et al., 2006). In addition, the difficulty of selecting suitable floral resources for a such highly polyphagous species like LBAM may be an impediment to the viability of this approach. Until recently, the selection of flowering plant species for use in conservation biological control of LBAM in Australian vineyards has been mainly focussed on a limited number of non-indigenous annual cover crop species, such as alyssum *Lobularia maritima* (L.) (Brassicaceae) and buckwheat *Fagopyrum esculentum* (Moench) (Ploygonceae) (Orre-Gordon et al., 2013). However, under Mediterranean conditions (i.e. South Australia) plant species that are not adapted do not establish successfully (Danne et al., 2010). In addition, these annual cover crops are typically removed annually to prevent competition for moisture when vines are growing, over the summer months. They do not meet the requirement in terms of providing limiting resources for natural enemies during the entire year that is critical in a conservation biological control strategy. To our knowledge, there has been no study on the use of alternative hosts to sustain parasitoids of LBAM in vineyards. Thus, selection of suitable alternative hosts and floral plants that provide sustainable limiting resources for natural enemy communities in vineyards is needed.

Therefore, this thesis aims to enhance conservation biological control of LBAM by parasitic wasps in vineyards. The experiments involved native flowering plants and alternative host insects to: (a) evaluate suitable native floral plant species and alternative hosts for conservation biological control of LBAM in South Australian vineyards, and (b) determine if the population density of *D. tasmanica* and other beneficial insects in vineyards was affected by the presence of selective native flowering plants and alternative hosts, especially in winter and spring.

2. Literature review

2.1. Fundamental concepts of conservation biological control

Conservation biological control (CBC) is one out of three forms of biological control - conservation, classical and inundation. CBC is defined as "*modification of the environment or existing practices to protect and enhance specific natural enemies of other organisms to reduce the effects of pests*" (Eilenberg et al., 2001). It is assumed that resident natural enemies have the potential to regulate pests when resources needed for their survival and development are available. In the context of CBC, these resources can be provided to natural enemies through habitat manipulations, including shelter, nectar, alternative prey/hosts, and pollen (Landis et al., 2000) that often summarised as SNAP (Gurr et al., 2017).

Providing shelter

The adults of many natural enemies require shelter for their survival and performance, which can protect them from unfavourable weather, desiccation, predation, parasitism and pathogens (Jervis et al., 2004). The shelter can be in the form of crop residues, vegetation in adjacent areas, and other plant debris.

The provision of shelter for natural enemies is one of the major practices in CBC. It can be achieved in a number of ways including using cover crops, leaving some crop parts relatively unmanaged or conserving non-crop habitats i.e. hedgerows bordering fields (Jervis et al., 2004). Shelter habitats can be either external to or lie within a main crop. The most common types of the external shelter are hedgerows, ditches, shelter belts, and field margins, while within crop shelter habitats are designed not to obstruct farming activities (Griffiths et al., 2008). Perhaps, one of the most successful examples of shelter habitat provided within crop is the establishment of "beetle banks" to provide long-term shelter for predators. Beetle banks were proposed by Thomas et al. (1991) when they investigated how to provide overwintering refuge sites for invertebrate predators in cereal fields in England. The beetle banks are areas of raised earth ridges sown with several grass species located in the centres of cereal fields (Thomas et al., 1991). The beetle banks provide a refuge for predators in the winter and enable them to quickly colonise the crop during spring (Thomas et al., 1991; Collins et al., 2003).

The effects of providing shelter for beneficial insects on CBC projects can be evaluated using criteria proposed by Jervis et al. (2004). These criteria are evaluated by answering a series of questions about a particular target natural enemy whether it has a clear need for a refuge and whether the natural enemy can commute between refuge and crop habitat. The authors suggested methods to answer these questions. For example, the need for a refuge can be examined through surveying beneficial insect densities in potential refuge sites during overwintering and summer aestivation and breeding time. The potential sites can be grassy field margins, hedgerows, nearby natural areas, cover crops and other adjacent sites.

Providing alternative hosts or prey

CBC through habitat modification can also provide alternative hosts or prey for natural enemies so as to maintain them during periods of pest scarcity or absence; e.g. in winter months. These alternative hosts, or prey, preserve reservoir populations of polyphagous natural enemies and enable them to “lay in wait” (Murdoch et al., 1985) until the target pest becomes available (Jervis et al., 2004). The provision of alternative hosts or prey for natural enemies in crop systems can be achieved by establishing or conserving field margin vegetation or by planting other crops that harbour them such as cover crops. One of the most successful examples of this technique is the use of sown wildflower strips or weed strips (Nentwig et al., 1998) to provide a host reservoir for natural enemies of aphids. Today, the application of flower strips has been widely accepted and encouraged in many countries (Moonen & Marshall, 2001; Pfiffner & Wyss, 2004).

Although the provision of alternative hosts or prey into agricultural systems is recognised as an important practice in CBC, there is very little research on this, except for research on parasitoids of aphids (Powell & Wright, 1988; Chow & Mackauer, 1991; Nentwig et al., 1998) and egg parasitoids of leafhoppers (Viggiani, 2003). Moreover, there is also a need for more fundamental research on parasitoid foraging behaviour, particularly behaviour of generalist species. Little is known about their innate preferences for hosts, and the effects of experiences on host preferences. In addition, the effects of density on choice of host species is not well understood.

In order to achieve success in using alternative hosts and prey in a CBC program, it is important to identify which alternative species are amenable to habitat manipulation. The suitable hosts or prey must not be harmful to the crop and must benefit natural enemies. Moreover, the natural enemy has to demonstrate a need for alternative hosts and prey. According to Jervis et al. (2004), when using alternative hosts or prey, it is critical to examine if the resource is limiting for the beneficial insects and it is really used by them under field conditions. This can be determined by posing two main questions (Jervis et al., 2004):

(1) Does the natural enemy actually need an alternative host or prey species?

This question can be answered by observing natural enemies in the field or studying them in the laboratory, as well as from the scientific literature.

Parasitoids must develop on or in alternative hosts. This can be examined by collecting and rearing a variety of potential host species from the field to exam if any target parasitoid emerges from the host. In the laboratory, the parasitoid must oviposit into or onto an alternative host and successfully develop from it. In some cases, a parasitoid may accept a species for oviposit but this does not necessarily mean that the parasitoid can successfully complete their development on it. The host's physiological defences may kill the parasitoid (Jervis et al., 2005). Therefore, under laboratory conditions, it is important to determine that the parasitoid can oviposit and successfully develop on an alternative host species.

With respect to predators, it is necessary to examine if they consume any other types of prey using feeding tests in the laboratory or dissection, serological or molecular techniques to detect the presence of alternative prey materials in the guts of field-collected predators (Jervis et al., 2004).

(2) Do natural enemies take advantage of alternative hosts or prey when they are provided?

The benefits from providing alternative hosts or prey are only gained if natural enemies can transfer from the alternative species to the pest, and they are capable of travelling from the sites inhabited by alternative hosts or prey to the crop.

The likelihood of movement between different hosts may depend on factors such as innate preferences and experience/learning of the natural enemies, host size and quality, and host behaviour. Comparative studies of the host selection behaviour of parasitoids in response to different host species may provide the answer.

Providing food plants

Many insect predators and parasitoids are omnivorous, so they need non-host food for their survival and performance. Food plants may provide nectar, pollen, extra-floral nectar or honeydew. However, in modern agriculture (monocultures), these sources of food are often scarce or even absent. Therefore, provision of food plants, particularly flowering plants, into crop habitats is a common practice in CBC. This technique provides plant food (i.e. nectar and pollen) to sustain natural enemies in agroecosystems and may also provide a physical refuge for natural enemies or a site that harbours alternative hosts or prey (Landis et al., 2000).

Laboratory and semi-field feeding studies have produced strong evidence that the provision of non-host food or prey can increase the longevity and fecundity of natural enemies (Baggen et al., 1998; Begum et al., 2004; Begum et al., 2006; Berndt & Wratten, 2005; Irvin et al., 2006; Vattala et al., 2006; Sigsgaard et al., 2013). However, field studies on the effects of non-host food on the fitness of natural enemies and pest regulation are still underrepresented in the scientific literature (Bell et al., 2006; Berndt et al., 2006; Winkler et al., 2006; Lee & Heimpel, 2008)

The selection of suitable plant candidates is vital for the success of a CBC program. The provision of flowering plants may be risky if they provide resources to pests (Begum et al., 2006) or hyperparasitoids (Araj et al., 2008). Thus, enhancing CBC is not "*a function of increased botanical diversity per se*", but relies critically on the selection of the "right" supplementary plants (Wäckers & van Rijn, 2012). Various criteria and methods have been used in previous studies to select suitable flowering plant candidates. Fiedler et al. (2008) reviewed past research on habitat manipulation for CBC, they listed selection criteria that are used to choose plants in CBC programs. These include attractiveness to natural enemies, plentiful production of pollen or nectar, appropriate flowering phenology, accessibility of plant resources and seed availability. Plant species that are already present in or adapted to crop areas are preferred. It is important to use plants that support natural enemies but do not benefit pests or hyperparasitoids and predators of natural enemies. Ideally, floral resource plants should provide not only plant foods but also other limiting resources for natural enemies such as alternative hosts or prey and refugia. In addition, to provide year-round limiting resources for natural enemies, a mix of selective plant species should be considered.

In a review of plant species use for habitat manipulation, Fiedler et al. (2008) found that most of plants used in CBC are annuals or biennials and most are not native to the test areas (56% of total reviewed studies). While the use of native plants are rare exceptions such as Taltarni Vineyards, Victoria, Australia (Bailey, 2012), the Waipara wine-growing area of North Canterbury Region of New Zealand (Meurk et al., 2006), Revegetation by design in Queensland, Australia and Reincorporation prairies in Midwestern, USA (Landis et al., 2012). Regarding native or exotic plants, in addition to supporting natural enemy populations, the incorporation of native plants may provide more diverse benefits. For example, native plants are well-adapted to local conditions, so they will likely have lower water, nutrient and pest control requirements compared to exotic plants. Moreover, native species also provide additional ecosystem services by enhancing wildlife habitat, stabilising soils and easing agricultural runoff (Landis et al. 2012). Although the annual plants have some advantages, the disadvantages seem to be overwhelming. The most profound limitations of annual plants are probably that they do not provide overwintering sites if they are removed following crop harvest and do not enhance native biodiversity (Landis et al. 2012). Thus, the provision of permanent habitats that sustain natural enemies is more easily achieved with perennial plants. Finally, while there are more than 20,000 flowering plant species in the world, only four common annual plant species have been tested in the majority of field studies: *Phacelia tanacetifolia* Benth (phacelia), *Fagopyrum esculentum* Moench (buckwheat), *Lobularia maritime* (L.) Desv. (alyssum) and *Coriandrum sativum* L. (coriander) (Landis et al. 2012). This suggests the need of further investigation on the selection of native plants for use in conservation biological control of crop insect pests.

2.2. Host searching behaviours in context of CBC

The path to successful parasitism of a host by a parasitoid includes a series of behavioural steps from habitat location, host location and host acceptance (Vinson, 1976). In all steps, parasitoids used cues that originate from the host, from the host plant on which the herbivore is feeding, from organisms related to the presence of the host, or from interactions between these sources to locate hosts (Vinson, 1976; Vet & Dicke, 1992; Godfray, 1994). For braconid parasitoids, visual, tactile and chemical cues could be potentially used to locate hosts (Vinson, 1976; Wäckers, 1994; Segura et al., 2007).

Parasitoids may rely on both innate mechanisms and the ability of learning of cues associated with hosts to locate them. Learning can occur during natal and/or adult experience (Vet & Dicke, 1992; Turlings et al., 1993). Positive impacts of learning may include optimisation of foraging efficiency (Vet & Groenewold, 1990; Vinson et al., 1998) and increase in the likelihood of encountering more suitable hosts (Papaj & Vet, 1990; Dutton et al., 2000).

A prospective host is often accepted if it is able to provide suitable nutritional and physiological conditions for the development of parasitoid offspring (Jervis & Kidd, 1996; Heimpel & Casas, 2008). However, the acceptance does not always lead to successful oviposition because of avoidance, physical and chemical defences by the host (Jervis & Kidd, 1996; Hopkinson et al., 2013). An understanding of host selection behaviour and related host defences can help in evaluating host candidates for use in conservation biological control.

2.3. Dispersal of natural enemies

Understanding the dispersal ability of biological agents is a key factor when commencing a biological control program. In classical and inundation biological control, estimates of dispersal ability of any introduced or released biological control agent will help to understand its relative searching capacity and to predict the area that will be covered in a release event (Avila et al., 2013). Regarding conservation biological control, the dispersal of natural enemies is vital for designing spatial distribution of floral resource subsidies in an agroecosystem used to enhance these beneficial agents (Scarratt et al., 2008). This knowledge will also help in elucidating the ability of natural enemies to suppress pests at both local and landscape scales.

Various techniques are available to quantify the dispersal of insects in the field, such as the use of rubidium (Corbett et al., 1996; Pickett et al., 2004; Scarratt et al., 2008) or dyes (Verhulst et al., 2013) to mark natural enemies. However, the accurate measurement of insect movements in the field is still a challenge due to their relative small size. Several studies have measured the dispersal ability of recently introduced biological control agents such as *Lysiphlebus cardui* Marshall (Hymenoptera: Aphidiidae) (Weisser & Völkl, 1997), *Cotesia flavipes* Cameron (Sallam et al., 2001), and *C. urabae* Austin (Hymenoptera: Braconidae) (Avilla et al., 2013). However, there is little work investigating on dispersal behaviour of local beneficial insects in the context of conservation biological control of insect pests. In vineyards, only movements of *Dolichogenidae spp.* (Bell et al., 2006) and *D. tasmanica* (Scarratt et al., 2008) have been investigated, while the dispersal patterns of other natural enemies are still unknown.

2.4. Leafrollers and associated parasitoids in Australian vineyards

2.4.1. Leafroller complex

Three leafroller species (Tortricidae) are commonly found in landscapes of South Australia where vineyards are planted, including *E. postvittana*, *Acropolitis rudisana* (Walker) and *Merophyas divulsana* (Walker) (Feng et al., 2017) and *Crocidosema plebejana* Zeller has also been recorded on grape vines (Retallack et al. 2018). Of these species, LBAM is the major insect pest of grapevines in Australia and New Zealand (Charles et al., 1996; Suckling et al., 1998). *Acropolitis rudisana* has been found on vine canopy but is less abundant. These species also feed on some weeds in and around vineyards, such as *Plantago lanceolate* L. (Plantaginaceae) (Feng et al., 2017).

The biology and ecology of LBAM is well studied (reviewed in Suckling and Brockerhoff (2010). However, there is not much information about the other species, *A. rudisana* and *M. divulsana,* except for a study on the development of *M. divulsana* under constant temperature and on several diets (Allsopp et al., 1983). Keys for larval indentification of *A. rudisana*, *E. postivittana* and *M. divulsana* feeding on capeweed were also developed (Cordingley & Danthanarayana, 1976). These species exihitied similar habits such as they shelter, feed and pupate within their nests made by rolling or webbing leaves and larvae often wriggle violently and may drop hanging suspended by silken threads when they are disturbed. Larvae of *A. rudisana* and *E. postivittana* closely resemble each other in terms of appearance, feeding habits, life-history and host-plant range. The length of final instars differs with larvae of *M. divulsana* being the smallest (11-12 mm), followed by *E. postvittana* (13-15 mm) and *A. rudisana*

(16-18 mm) (Cordingley & Danthanarayana, 1976). However, there is little information on the biology and ecology of both *A. rudisan* and *M. divulsana* and their association with vineyards.

2.4.2. LBAM

Life cycle and development

LBAM can produce 2-4 generations annually, depending on temperature and latitude. In Australia, there are usually three annual generations of LBAM, one summer generation(s) (from January to April), an autumn-winter generation (May-September); and a spring generation (October-December) (Danthanarayana, 1975). During summer, overlapping of stages is observed (Danthanarayana, 1975; 1983).

The summer generation develops from eggs laid in spring at the end of December and the beginning of January. The larvae grow rapidly and cause damage to bunches of grapes from January. The summer generation moths produce fewer eggs, in contrast to those of autumn-winter and spring generations due to unfavourable conditions such as hot weather, variety and quality of food plants available (Danthanarayana, 1983). The second generation, autumn-winter generation, is initiated from eggs laid during April and the larvae overwinter to pupate in September. The larvae overwinter in bunch residues, on weeds and cover crop plants, or in nearby vegetation. The larvae that overwinter in nearby vegetation can move into vineyards at budburst. The adults of this generation emerge and lay eggs in October and develop into the spring generation. The spring generation is responsible for extensive loss of newly set grape flowers (reviewed in Suckling and Brockerhoff (2010)).

Key aspects of the life cycle and development of LBAM must be considered in developing management strategies against this pest. (1) LBAM is active throughout the year as it has no diapause. (2) During winter, when some host plants have no foliage, LBAM will live on non-crop plants before migrating onto the crop. (3) In vineyards, the most adverse damage caused by LBAM larvae occurs when larvae of spring and summer generations migrate from foliage to grape bunches.

Host range and damage

LBAM is native to Australia, but has been introduced into New Zealand, Hawaii, England, California and Sweden (Suckling & Brockerhoff, 2010; Suckling et al., 2012). The insect has a wide host range including horticultural crops, vegetables, ornamental plants, forests and non-crop plants. In Australia, it has been recorded from 123 genera in 55 families, which involves 22 native and 101 exotic genera (Suckling & Brockerhoff, 2010). Worldwide there are more than 500 host plant species in 363 genera and 121 families recorded as its host. LBAM feeds mostly on dicotyledonous plants (Suckling & Brockerhoff, 2010).

LBAM is a polyphagous leafroller that can cause extensive damage as it may spread rapidly under warm and wet conditions. All larval feeding activities on foliage, buds, shoots and fruits can cause damage, however, damage to fruit brings the greatest economic impacts (Wearing et al., 1991). It is estimated that without insecticide applications, the damage caused by larval LBAM to fruits in Australia usually ranges from 5 to 20% and may exceed 30% (Wearing et al., 1991). In New Zealand, if crops are left unsprayed, damage levels may reach as high as 70% (Wearing et al., 1991).

In vineyards, total crop loss may occur as a result of severe infestation around flowering. Only spring and summer generations affect winegrapes while the winter generation lives on non-host plants in or around vineyards. Moreover, the crop loss in grapes can be increased due to the transmission of bunch rot caused by *Botrytis* *cinerea* (Pers.) (Helotiales: Sclerotiniaceae). Damage to berries can provide entry points for fungal infection, while the movement and webbing together of fruits or leaves can enhance conditions for rot expansion. *Botrytis* rot is associated with both summer and spring generations of LBAM (Bailey et al., 1997).

2.4.3. Natural enemies of LBAM

LBAM is attacked by a wide range of predators and parasitoids. The most important predators of LBAM larvae and pupae are various spiders and earwigs (i.e. *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae)) (Danthanarayana, 1983).

Parasitism of LBAM is an important mortality factor in Australian vineyards and orchards. There are 25 parasitoids and hyperparasitoids asscociated with LBAM in Australia (Paull & Austin, 2006). The egg parasitoid, *Trichogramma funiculatum,* is one of the important natural enemies of LBAM, although it is absent in the eggs of the winter generation. Among larval parastioids, the braconid *D. tasmanica* has been recorded as the most abundant parasitoid that attacks LBAM (Suckling et al., 1998; Paull & Austin, 2006; Feng et al., 2017).

2.4.4. *Dolichogenidea tasmanica*

The braconid *D. tasmanica* is indigenous to Australia and is a solitary, koinobiont, generalist endoparasitoid (Dumbleton, 1935). The wasp is recognised as an effective candidate for biological control of LBAM, and other leafrollers, as it can parasitise the first to third instar larve of LBAM (Yazdani et al., 2015a) and other leafrollers, *A. rudisana* and *M. divulsana* (Feng et al., 2017). The female of *D. tasmanica* also exhibits the ability to distingush unparasitised hosts from parasitised ones (Yazdani et al., 2015b).

Successful parasitism of *D. tasmanica* was influenced by host larval stages (Yazdani et al., 2015a), host plants (Feng et al., 2015; Suckling et al., 2001) and adaptive learning (Feng et al., 2015; Yazdani & Keller, 2016). Further, an empirical study in New Zealand also revealed that host larval species may be an important variable affecting parasitism level (Suckling et al., 2001). When three tortricid species, LBAM and two native species, were inoculated on potted apple trees, parasitism rates by the wasp were significantly different among host larval species, ranging from 26% to 83% (highest found in LBAM). The host defensive behaviour that observed in the laboratory was used as a potential explanation for this variable in parasitism rates. However, under field conditions, there was no differences in parasitism level among them (Suckling et al., 2001). Thus, the effects of host species on parasitism by *D. tasmanica* requires further study in order to identify which species could best contribute to conservation biological control.

2.4.5. Management of LBAM

Various methods have been used to manage LBAM including the application of insecticides, the use of pheromone-based mating disruption, and biological control (reviewed in Suckling & Brockerhoff, 2010). However, insecticide sprays are still widely used to control LBAM (Orre-Gordon et al., 2013).

Biological control of LBAM is now recognised as an important solution for sustainable viticulture production. Classical biological control was only applied in New Zealand where 19 parasitoids and 3 predators were imported from Australia as potential candidates for control of LBAM. Among them, 10 parasitoids were realeased between 1967 and 1972. And in 1969, about 250 specimens of *Dolichogenidea tasmanica* were released in New Zealand to control LBAM (reviewed in Suckling & Brockerhoff, 2010).

Two decades ago, the egg parastitic wasp, *Trichogramma carverae* Oatman & Pinto (Hymenoptera: Trichogrammatidae), was identified as one of the most important parasitoids that could contribute to suppressing LBAM (Glenn et al. 1997). The species was released in Australian vineyards at a cost of Aus$ 45/ha for each of the two to three releases needed per season (Gurr et al., 1998). The parasitism rates of LBAM eggs by *T. carvarae* are often not very high unless the wasp is regularly released in a larger number, which can be costly (Yazdani & Keller, 2017). In addtion, the control effectiveness of the wasp critically depends on the availability of a sugar source. Sugar-starved females have short lives and parasitise fewer hosts than satiated ones (Begum et al., 2004).

Conservation biological control of LBAM has mainly focused on providing flowering plants to sustain natural enemies. However, under field conditions, the effects of flowering resources on biological control of LBAM are not consistent (Bell et al., 2006; Berndt et al., 2006).

2.5. Level of success reached using CBC in control of LBAM in vineyards

Leafrollers, especially LBAM, are the main target of CBC in vineyards. To date, provision of flowering plants has been the only practice applied in CBC in vineyards. To our knowledge, no study on providing alternative hosts has been published. The provision of flowering plants has reached some success in management of LBAM. Under laboratory conditions, access to flowering plants has contributed to improvement of longevity, fecundity and the sex ratio of parasitoids (Begum et al., 2004; Berndt & Wratten, 2005; Scarratt, 2005). For example, the longevity of *D. tasmanica* increased seven-fold from 2.2±0.17 days to 15.7±2.77 days when it had access to alyssum flowers (Berndt & Wratten, 2005). However, one important problem related to the provision of flowering plants is that LBAM may also gain benefits from them in terms of increasing longevity (Begum et al., 2006).

Under field conditions, the use of non-indigenous cover crops such as alyssum *Lobularia maritima* (L.) (Brassicaceae) and buckwheat *Fagopyrum esculentum* (Moench) (Ploygonceae) is the most common practice in vineyards to enhance natural enemies of insect pests (Orre-Gordon et al., 2013). However, the effects of these plants on parasitism rates of LBAM by parasitoids are inconsistent and vary across year. For example, Berndt et al. (2006) reported that flowering buckwheat may increase the parasitism rate of LBAM by more than 50% in one of three vineyards studied. At the other two vineyards, the flowering buckwheat had no effect on parasitism rates.

In addition, under Mediterranean conditions like South Australia, non-indigenous plant species that are not adapted do not establish successfully (Danne et al., 2010). Another disavantage of annual cover crops is that they are typically removed anually to prevent competion for moisture when vines are growing, over the summer months. Therefore, there is a need to select native species that are more sustainable alternatives to the currently recommended species based on overseas studies. The use of perennial and indigenous plant species is suggested (Fiedler et al., 2008; Landis et al., 2000, Isaacs et al., 2009; Landis et al., 2012). As discussed before, native plants are often well adapted to local conditons, cover a larger flowering period and can provide shelter in the entire year, and especially serve as overwintering sites to support natural enemies. In addition, they can provide greater multiple ecosystem service than non-native and annual species in addition to improve biological control (Isaacs et al., 2009; Danne et al., 2010, Landis et al., 2012). In Australia, indigenous plants often harbour low densities of pests and high densities of their natural enemies, while weeds support more pests (Gagic et al., 2018).

Further research is necessary to develop effective CBC. A wider range of supplementary plant species particularly perennial and native species must be found. Alternative hosts that can sustain key natural enemies like *D. tasmanica* must be identified. The effects of these supplementary resources on the management of LBAM under field conditions, particularly influences on the searching efficiency and abundance and diversity of natural enemies must be elucidated.

2.6. Significance of the thesis

Consideration of the results from this study suggests practical ways to enhance biological control of LBAM and other leafroller species by parasitic wasps in vineyards through habitat management, so as to make biological control more reliable. The study also suggests further studies that could inform conservation biological control of LBAM and other leafrollers in vineyards and in other agroecosystems where these insect pests are present. Finally, the study also provides better understanding of behaviour of generalist parasitoids of lepidopteran larvae, particularly host foraging behaviours in response to hosts of different species that differ in physical conditions.

2.7. Scope and structure of thesis

The main body of this thesis is written as a series of manuscripts for publication. The main findings of the thesis are synthesised and integrated in Chapter 5. It includes the conclusions from the body of work presented here along with suggestions for further studies that could inform conservation biological control of LBAM and other tortricid pests.

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CHAPTER TWO

Behavioural responses of *Dolichogenidea tasmanica* to alternative hosts are aligned with a conservation biological strategy against light brown apple moth

Statement of Authorship

Title of Paper	Behavioural responses of *Dolichogenidea tasmanica* to alternative hosts are aligned with a conservation biological control strategy against light brown apple moth
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Principal Author

Name of Principal Author (Candidate)	Hieu Trung Bui		
Contribution to the Paper	Conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript.		
Overall percentage (%)	80%		
Signature		Date	01 June 2018

Co-Author Contributions

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

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

Name of Co-Author	Maryam Yazdani		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
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Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	1 June 2018

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Hieu Trung Bui		
Contribution to the Paper	Conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript.		
Overall percentage (%)	80%		
Signature		Date	

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Maryam Yazdani		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	5/06/2018

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	

Behavioural responses of *Dolichogenidea tasmanica* to alternative hosts are aligned with a conservation biological control strategy against light brown apple moth

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ABSTRACT

The braconid, *Dolichonenidea tasmanica,* is the most abundant parasitoid attacking the light brown apple moth (LBAM) *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), the major insect pest of grapevines in Australia and New Zealand. Enhancing populations of alternative hosts for the parasitoid is one way to enhance biological control of this insect pest. The host selection behaviour of this parasitoid in response to *E. postvittana* and two related alternative hosts, *Acropolitis rudisana* and *Merophyas divulsana*, was investigated to evaluate the viability of this approach. All hosts were accepted by the wasp with above 70% parasitism rate. During behavioural assays, the parasitoid responded differently to the host species, possibly due to differences in their physical characteristics, and their feeding and defensive behaviours. These experiments indicate that populations of *D. tasmanica* should be conserved if the alternative hosts, *A. rudisana* and *M. divulsana,* are present. Conservation of *D. tasmanica* in agricultural landscapes should promote biological control of LBAM.

Keywords: Biological control, Alternative hosts, Host searching behaviour, Vineyards

1. Introduction

Enhancing populations of alternative hosts for parasitoids is one way to enhance biological control of insect pests (Murdoch et al., 1985; Jervis et al., 2004). Providing the resources alternative hosts need in agroecosystems can lead to an increase in the abundance of a local parasitoid population (Altieri and Letourneau, 1982; Landis et al., 2000). However, prior to undertaking such a management approach, it is important to select appropriate alternative host candidates to enhance biological control yet minimize undesirable outcomes such as greater pest problems (for references, see Jervis et al., 2004). Comparative studies of the host selection behaviour of parasitoids in response to different host species can provide insights into the viability of this approach.

The control of herbivorous insect pests by parasitoids critically relies on their behaviours in searching and handling hosts (Mills and Wajnberg, 2008). The path to successful parasitism starts with scanning of environmental cues to locate a host, followed by physical contact to determine host suitability and ultimately oviposition in or on a suitable host (Vinson, 1976; Vinson, 1998). The cues that influence foraging behaviour can originate from the host, from the host plant on which the herbivore is feeding, from organisms related to the presence of the host, or from interactions between these sources (Vinson, 1976; Vet and Dicke, 1992; Godfray, 1994).

Generalist parasitoids attack a range of host species that are associated with similar cues that originate from their hosts and the niches they occupy (Rukmowati Brotodjojo and Walter, 2006). A prospective host is often accepted if it is able to provide suitable nutritional and physiological conditions for the development of parasitoid offspring (Jervis and Kidd, 1996, Heimpel and Casas, 2008). However, the attacks do not always lead to successful oviposition due to avoidance, and physical and chemical defences (Jervis and Kidd, 1996; Hopkinson et al., 2013). An understanding of host selection behaviour and related host defences can help in evaluating alternative host candidates in order to make conservation biological control by parasitoids more reliable.

The braconid *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) is indigenous to Australia and is a solitary, koinobiont, generalist endoparasitoid (Dumbleton, 1935). It has been reported as the most abundant parasitoid attacking the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), the major insect pest of grapevines in Australia and New Zealand (Charles et al., 1996; Suckling et al., 1998; Paull and Austin, 2006). Successful parasitism by this wasp is influenced by host larval stage (Yazdani et al., 2015), host plants (Suckling et al., 2001; Feng et al., 2015), and adaptive learning (Feng et al., 2015; Yazdani and Keller, 2016).

The generalist nature of *D. tasmanica* is a potential benefit for biological control of LBAM in vineyards as LBAM is a polyphagous pest that does not enter diapause in winter (Suckling and Brockerhoff, 2010). The contribution that *D. tasmanica* makes to biological control of LBAM in vineyards would be more reliable if this wasp is able to effectively search and parasitise locally available alternative hosts, maintain populations on them during winter and spring when the grapevines are dormant, and then move to attack LBAM when it reinvades vineyards in spring. Empirical studies in New Zealand indicated that host larval species may be an important variable affecting the level of parasitism (Suckling et al., 2001). When LBAM and two native tortricid species were inoculated on potted apple trees, parasitism rates by *D. tasmanica* were significantly different among host larval species. Differences in host defensive behaviour were considered to be a potential explanation for the observed variation in parasitism rates (Suckling et al., 2001). Thus, the effects of host species on parasitism by *D. tasmanica* requires further study in order to determine which species could best contribute to conservation biological control.

Acropolitis rudisana (Walker) and the lucerne moth, *Merophyas divulsana* (Walker) (Lepidoptera: Tortricidae), are two species that are found in landscapes of Australia where vineyards are planted. These species have the potential to be promoted as alternative hosts for *D. tasmanica* and other beneficial insects. Both are known to be parasitised by *D. tasmanica* and other parasitoids of leafrollers (Feng et al., 2017). Secondly, our observations indicate that they are active throughout the year on plants such as on *Plantago lanceolata L*. (Plantaginaceae), so they may support populations of *D. tasmanica* in winter and spring before LBAM reinvades grapevines. Finally, very few *A. rudisana* and no *M. divulsana* were found feeding on grapevines in our field collections (unpublished data). Therefore, it appears that these insects are unlikely to be grapevine pest if they are promoted as alternative hosts in vineyards.

The aim of this study is to investigate the host selection behaviour of *D. tasmanica* in response to LBAM, *A. rudisana* and *M. divulsana*. The defensive responses of these insect hosts are also studied to determine their suceptibility to wasp attack. Conclusions will inform their potential role in conservation biological control of LBAM.

2. Materials and Methods

2.1. Insect cultures

A laboratory population of *E. postvittana* has been maintained for more than 200 generations (Yazdani et al., 2015). Colonies of *M. divulsana, A. rudisana* and *D. tasmanica* were established from specimens collected from *Plantago lanceolata L*. (Plantaginaceae) at the Waite Conservation Reserve, Urrbrae, South Australia in September 2014. For all insect cultures, field-collected individuals from the Waite Conservation Reserve and McLaren Vale, South Australia were added to the respective colonies at least every two months to maintain genetic diversity. Tortricid cultures were reared following a method adapted from Cunningham (2007).

Field collections of tortricid larvae at Waite Conservation Reserve indicated that *M. divulsana* was the most abundant species and was parasitised by *D. tasmanica* and other parasitoids. Thus, the culture of *D. tasmanica* was reared on larvae of *M. divulsana* that fed on *P. lanceolata* in screened insect cages (600 x 600 x 600 mm) at 23 ± 2 ˚C under a 14 L: 10 D photoperiod. When cocoons formed, they were transferred to insect rearing cages (245 x 245 x 245 mm) in which emerging adults were provided with water and honey.

2.2. Behavioural assays

No-choice tests were conducted to elucidate the foraging behaviors of *D. tasmanica* to different tortricid host species as well as host susceptibility to parasitoid attack. To allow the wasp express its full range of behaviour, this study was conducted in a wind tunnel (Keller, 1990). Forty-eight hours prior to recording an observation, an individual leaf of *P. lanceolata* was infested with a second-instar of either *E. postvittana*, *M. divulsana*, or *A. rudisana* to enable the larva to produce feeding damage and deposit silk. The base of each leaf was wrapped in cotton wool and placed in an 18 mm diam. x 50 mm glass vial filled with fresh water. All wasps used in this experiment were two- or three-day old mated females. Each individual wasp was seperately kept in a glass vial (18 mm diam. x 50 mm) with a drop of honey.

The experimental design for this test was adapted from Feng et al. (2015). During a test, each individual leaf (test leaf) was placed upwind of the parasitoid in the wind tunnel (Figure 1). A second leaf (extra leaf) also infested with the same host species as the test leaf was placed 40 cm upwind during each test to provide alternative landing location for the parasitoid, so as to reduce the tendency of wasps to spend excessive time on the test leaf. In order to stimulate the wasp, each individual was exposed to a leaf of *P. lanceolata* that was damaged by the same test larval species prior to observation. A single parasitoid was then released from a glass vial 25 cm downwind from the host-infested leaf. The wind speed was 20 cm/s and the temperature was 23 ± 2 °C.

The foraging behavior of individual parasitoids and reciprocal host responses were recorded by a camcorder (HC-V550M, Panasonic, Australia). An observation ended when the parasitoid moved off the leaf to either another location in the wind tunnel or to the extra leaf, or 10 minutes had elapsed. During a test, if, after a second trial, the wasp was inactive within 5 minutes or did not fly to the test leaf but elsewhere in wind tunnel, that trial was identified as “no response”. Behaviour was classified using a catalogue for *D. tasmanica* that was developed by Yazdani et al. (2015). The sequence and duration of behaviour were transcribed from video recordings with the Observer XT ver. 11.5 (Noldus Information Technology B.V., Wageningen, The Netherlands).

Pilot observations revealed that hosts can avoid wasp attack by dropping from the plant. The number of larvae that avoided attack following an encounter with a wasp was recorded. Forty wasps were observed for each tortricid species. The order of testing the species was randomised.

Experimental larvae were kept individually in 100 ml round containers with fresh leaves of *P. lanceolata* for 72 h after exposure to *D. tasmanica*. Then the larvae were dissected to determine whether they were parasitised or not.

2.3. Leaf damage area and perimeter calculation

As leaf damage is a primary source of semiochemicals that attract parasitic waps, the amount of leaf damage caused by each host species was measured. After behavioral observations, twenty-five test leaves were randomly selected and individualy scanned into a digital format (JPG file) at 600 dpi using an all-in-one printer (Canon MG2560 PIXMA, Canon Australia). The leaf was scanned on a white paper printed with a 10x10 mm square, which was used as a standard for calibrating the pixel conversion. The area and perimeter of leaf damage in each image was estimated using ImageJ for Windows 64 bit ver. 1.51n (Wayne Rasband, National Institutes of Health, USA, https://imagej.net/Fiji/Downloads) following the methods of Pascau (2013).

2.4. Statistical analyses

The elapsed time in an observation was divided into three periods: pre-flight time, flying time and time after first landing on a host-infested leaf. Mean durations, frequencies and proportions of occurrence for each type of behaviour after first landing were analysed and the differences among them were detected by using the Kruskal-Wallis test (IBM SPSS Statistics v. 22, IBM-SPSS Inc., Chicago, IL). Values are shown as means ± SE. Kaplan-Meier survival analysis (Kaplan and Meier, 1958) was used to construct the survival curves for behaviours of *D. tasmanica* in searching each host species. The survival curves were compared via a Log rank (Mantel – Cox) test. If significant differences were found, pairwise log rank comparisons were conducted with an adjusted level of alpha = 0.05 using the sequential Bonferroni correction. Number of wasps that did not respond during the test, parasitism rates and host susceptibility to parasitoid attack were compared among these host species by using a chi-square test of homogeneity (IBM SPSS Statistics v. 22, IBM-SPSS Inc., Chicago, IL).

Differences were considered significant at $P < 0.05$. However, if the data did not meet the sample size requirement in SPSS statistics, Fisher's exact tests were used instead of the chi-square test.

The differences among leaf areas and perimeters damaged by each leaf-roller species were statically analysed using one-way ANOVA (IBM SPSS Statistics v. 22, IBM-SPSS Inc., Chicago, IL) (n=25). Differences were considered significant at $P < 0.05$.

3. Results

3.1. Host selection behaviour of D. tasmanica on three tortricid species

3.1.1. Pre-flying behaviour

When *D. tasmanica* was released downwind of the larvae of any of the three tortricid species, it spent a period of pre-flight time exhibiting a range of behaviours, such as antennating, walking, stationary, grooming and pointing. The proportions of wasps that did not take flight to reach the host-infested leaf did not differ among *A. rudisana* (12.5%), *M. divulsana* (10%) and *E. postvittana* (2.5%) (χ^2 (n=40, $df = 2$) = 2.95, $P = 0.34$).

The median time to initiate first flight toward *A. rudisana*, *M. divulsana* and *E. postvittana* were 31.94 ± 8.55 s, 43.78 ± 3.67 s and 22.70 ± 2.73 s, respectively. These times were significantly variable among the host species ($\chi^2 = 9.48$, $df = 2$, $P = 0.009$). It took longer time for the wasp to initiate flight toward either *M. divulsana* or *A. rudisana* than toward *E. postvittana* ($\chi^2 = 8.17$, $df = 1$, $P = 0.004$ and $\chi^2 = 5.24$, $df = 1$, $P = 0.022$, respectively). However, no statistical difference was found between *M. divulsana* and *A. rudisana* (χ^2) = 0.11, $df = 1$, $P = 0.744$) (Figure 1).

3.1.2. Flying behaviour

After leaving the releasing point, the duration of flight by responding females that flew to the infested leaf did not differ among these host species ($\chi^2 = 1.85$, *df* = 2, *P* = 0.396), with median ranks of 14.47 ± 3.96 s, 9.85 ± 1.60 s and 8.97 ± 1.62 s) for *A. rudisana, M. divulsana and E. postvittana,* respectively.

3.1.3. Behaviour after first landing

Total 87.5% (35 out of 40), 90% (36) and 97.5 % (39) of parasitoids landed on the leaf infested with either *A. rudisana, M. divulsana* or *E. postvittana,* respectively. For all host species, upon arrival at an infested leaf, females often quickly responded to the cues originated from damaged leaf and larva by antennating, probing the leaf surface and usually subsequently stinging. The fractions of time the wasps engaged in these three behaviour classes accounted for more than 70% of total time they spent on the leaves after landing (Figure 2). However, *D. tasmanica* spent a greater proportion and duration of time pointing and probing when searching for *M. divulsana* than *A. rudisana* and *E. postvittana* ($P<0.05$, Table 2 & Figure 2). Frequency of pointing was also greater in *M. divulsana*, compared to the other species (Table 2). In addition, duration of first sting were significantly variable among species ($\chi^2 = 8.162$, *df* = 2, *P* = 0.017; Figure 4). The wasp spent significantly less time to complete its first sting in *E. postvittana* than in *M. divulsana* (*P*=0.007) and in *A. rudisana* (*P* = 0.034) (Figure 3). There were no detectable differences among species in the proportion, duration and frequency of time devoted to other behaviours (Table 2 & Figure 2).

The interval of time between when the wasps landed on the leaf until the first host was stung varied significantly among species ($\chi^2 = 6.97$, *df* = 2, *P* = 0.031) (Figure 4). The time spent searching until the first sting was observed was statistically significant longer in *M. divulsana* than in *A. rudisana* ($\chi^2 = 3.93$, *df* =

1, *P* = 0.047) and in *E. postvittana* (χ^2 = 5.62, *df* = 1, *P* = 0.018). Statistical analysis did not detect a difference between *A. rudisana* and *E. postvittana* (χ^2 = 0.69, *df* = 1, *P* = 0.406).

During this experiment it was observed that some wasps stung host larvae more than one time (Figure 5). An analysis of proportions of wasps that did not sting, stung one time and more than one time did not conclusively show any differences among host species (χ^2 = 5.94, *df* = 2, *P*=0.051).

3.2. Larval feeding and defensive behaviours and wasp responses to host defences

It was observed that these host species deployed different feeding behaviours. Second instar larvae of *A*. *rudisana* and *E. postvittana* fed on the leaf surface of *P. lanceolata*. They covered their feeding sites with silk, that inhibited attack by wasps. However, second instar *M. divulsana* often mined the leaf.

When disturbed by wasp attacks, larvae of all species often responded by wriggling violently, moving within their shelters or occasionally dropping from the leaf and sometimes hanging on silk. After that, if they were not chased by the wasp, they would usually return to their feeding sites. Only four larvae of *A*. *rudisana*, two of *M. divulsana* and two of *E. postvittana* dropped from the leaf in this experiment, with the proportions of 11.4% (n=35), 5.6% (n=36) and 5.1% (n=39) respectively, which were not statistically significantly different among species (χ^2 = 1.25, *df* = 2, *P* = 0.59). When *D. tasmanica* encountered a dropping host, some of them immediately either walked or flew to chase and attack the host, but others did not immediately recognise the host's dropping and continued to search on the leaf. During observations, only two larvae of *A*. *rudisana* (50%), one of *E. postvittana* (50%), and no *M. divulsana* were chased and stung by the wasp. Larvae that remaind within silk shelters or leaf mines were often stung.

3.3. Parasitism rates

Overall parasitism rates by *D. tasmanica* were calculated in two ways. When wasps that responded and did not respond to odours from *A. rudisana*, *M. divulsana* and *E. postvittana* were included in the rates, 70%, 55% and 72.5% of larvae were parasitised, respectively (n=40). These parasitism rates were not statistically significantly different ($\chi^2 = 3.19$, *df* = 2, *P* = 0.20). When only wasps those landed on the infested leaf were considered, 30/35 (85.7%) of *A. rudisana,* 26/36 (72.2%) of *M. divulsana* and 33/39 (84.6%) of *E. postvittana* were parasitised, which was not significantly difference among species ($\chi^2 = 2.63$, *df* =2, *P*=0.27). Dissections revealed that there were no significant differences among species ($\chi^2 = 1.10$, *df* = 2, *P*=0.58) in the proportion of hosts that were stung in which oviposition was verified (*A. rudisana* 93.33%, *M. divulsana* 84.62%, *E. postvittana* 87.88%), without substantial differences among them.

3.4. Leaf damage calculation

There were no detectable differences among species in the area of leaf damage (F (2, 72) = 1.44, *P* = 0.24) and perimeter length of leaf damage (F (2, 72) = 2.37, *P* = 0.10) caused by feeding activities of larvae (Table 3).

4. Discussion

The results showed that overall patterns of host searching behaviours of *D. tasmanica* were similar among host species. The wasp responded to the odours of hosts of all species by flying towards the infested leaf. After landing, *D. tasmanica* usually searched on the feeding damage first. It spent most of its time antennating and probing, often subsequent stinging a host.

Despite broad similarities in the overall patterns of host searching behaviour*,* the study revealed that host species identity does affect the foraging behaviour and efficiency of *D. tasmanica*. When all statistical analyses are considered together, the results indicate that *D. tasmanica* more quickly responded to *E. postvittana* than the other hosts, especially in comparison to *M. divulsana*. However, experiments did not detect any differences in parasitism rates among these species*.* All host species are susceptible to attacks by *D. tasmanica* with high parasitism rates (above 70%), that were similar to the rate observed when it searched for *E. postvittana* in a previous study (Suckling et al., 2001). In winter, populations of LBAM in vineyards are low, so the presence of alternative hosts, *A. rusiana* and *M. divulsana*, can maintain populations of *D. tasmanica* and that should promote biological control of LBAM.

In order to understand these differences in host searching behaviour of *D. tasmanica*, all factors involved in the host searching behaviours of the parasitoid must be considered. It has been shown that chemical, tactile and visual cues are typically used by braconid parasitoids to locate hosts (Wäckers, 1994; Segura et al., 2007). The most attractant chemicals used by parasitoids during searching for their hosts are produced when the hosts eat or defecate (Godfray, 1994). Female *D. tasmanica* is attracted by odours from feeding sites (Suckling et al., 2012) and its response to hosts are positively link to the amount leaf damage, faeces and silk created by *E. postvittana* (Yazdani et al., 2015). Among these sources of cues, leaf damage is a primary source of semiochemicals that attract parasitic waps (Suckling et al., 2012). In our study, the leaf damage areas and lengths were not significantly different among species (Table 3), suggesting that the amount of plant volatiles does not vary substantially among species. Second instar *M. divulsana* often fed inside leaf mines while the other species fed on the leaf surfaces. Feeding inside plant tissues by host larvae may reduce cues and thereby minimize the risks of discovery by their parasitoids (Godfray, 1994). This may have affected the responses of *D. tasmanica* to *M. divulsana*.

Different host species may quantitatively and qualitatively induce the release of different plant volatile emissions that could differently affect the parasitoid behaviour (Turlings et al., 1990; Du et al., 1996; Mumm and Dicke, 2010). Such qualitative changes were not investigated in this study. But our host system involved closely related tortricid species feeding on the same host plant, suggesting that they may create similar cues. In addition, it has been shown that generalist parasitoids often innately use general cues, which are common to all hosts and their food plants when host species are initially encountered (Steidle et al., 2001; Gols et al., 2012;). Thus, it appears here that amount of cues is likely the main factor driving the response by *D. tasmanica* to different host species. However, to confirm this, further study on the volatile profiles among host species, and their effects on host searching behaviours by the parasitoid is required.

Host physical characteristics and active defensive behaviour are possibly the most important factors that determine successful parasitism after a host is located. *D. tasmanica* spent a longer time stinging *M. divulsana* and *A. rudisana* compared to *E. postvittana,* which was visibly more active in its behavioural defence. This is consistent with observations of *D. tasmanica* when it attacks different instars of *E. postvittana*. The first instar, which has a weak defensive behaviour, is stung longer than the second and the third instars (Yazdani et al., 2015). Similar results were also observed in *Costesia glomerata* Linnaeus (Hymenoptera: Braconidae) when it attacks different instars of *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae) (Brodeur et al., 1996).

In addition, all the host species exihibited dropping behaviour when being attacked by *D. tasmanica*. Dropping is a common way to avoid parasitism among lepidopterant larvae (Godfray, 1994) and this behaviour was also found in a previous study investigating the behaviour of larvae of these species feeding on capeweed (*Arctotheca calendula* L.) (Arctotheca) (Cordingley & Danthanarayana, 1976). In this study, the observed frequency of dropping was low and not significant different among host species. Overall, observations indicated that defensive behaviours resulted in a small probability of escape by all host larvae.

Our results indicate that populations of *D. tasmanica* should be conserved if alternative hosts *M. divulsana* and *A. rudisana* are present. Both species are susceptible to parasitism by *D. tasmanica,* and small differences in its behaviour in response to these alternative hosts are not substantial. Conservation of populations of *D. tasmanica* should promote biological control of LBAM. However, further investigation should be done on host preferences and the consequences of these preferences on the life-history variables of the wasp, such as body size, fecundity, sex ratio and developmental rates. Further study to select host plants to support these larval species that can be incorporated into landscapes where vineyards are planted should be another avenue for investigation in developing conservation biological control to suppress light brown apple moth.

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Author contribution

HTB conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript. MY and MAK supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.

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Tables

Table 1. Behavioural catalogue for *Dolichogenidea tasmanica,* females, foraging for leaf-rolller hosts

Behaviour type	**Description**
Antennating (An)	Walking while drumming the substrate with antennal tips
Flying (Fl)	Flying
Grooming (Gr)	Preening any part of the body (e.g. antennae, legs and wings)
Probing (Pr)	Walking while drumming the substrate with anttenae and jabbing with ovipositor
Stationary (Sta)	Standing with still moving antennae
Stinging (Sti)	Insert its ovipositor into the host by curving its abdoment under its body
Walking (Wa)	Moving without using antennae to touch the substrate

Table 2. Characterization of host selection behaviour of female *D. tasmanica* in response to different larval host species after first landing on infested leaves.

	Mean duration ± SE		
Behavioural types	***A. rudisana***	***M. divulsana***	***E. postvittana***
Antennating	90.34 ± 14.66	106.25 ± 13.72	102.15 ± 14.40
Flying	3.60 ± 1.37	2.03 ± 0.76	2.11 ± 1.05
Grooming	31.55 ± 8.04	23.49 ± 5.91	30.37 ± 7.32
Pointing	1.61 ± 0.87^{a}	2.02 ± 0.50^{b}	1.53 ± 1.17^{a}
Probing	29.72 ± 6.63^{a}	95.26 ± 19.64^{b}	31.39 ± 9.11^{a}
Resting	17.12 ± 9.29	22.35 ± 11.77	17.53 ± 6.05
Stationary	3.61 ± 1.33	1.85 ± 0.75	2.29 ± 1.00
All Stinging	17.10 ± 2.97	17.50 ± 3.09	11.01 ± 1.60
Walking	3.35 ± 0.72	6.63 ± 1.31	4.51 ± 0.92

	Mean frequency/min ± SE		
Behavioural types	***A. rudisana***	***M. divulsana***	***E. postvittana***
Antennating	3.14 ± 0.27	2.85 ± 0.22	2.87 ± 0.22
Flying	0.32 ± 0.10	0.20 ± 0.12	0.23 ± 0.14
Grooming	0.63 ± 0.10	0.49 ± 0.08	0.58 ± 0.08
Pointing	0.15 ± 0.06^{a}	0.31 ± 0.08^{b}	0.10 ± 0.04^{a}
Probing	1.54 ± 0.16	1.65 ± 0.11	1.52 ± 0.19
Resting	0.22 ± 0.06	0.26 ± 0.06	0.19 ± 0.05
Stationary	0.25 ± 0.10	0.12 ± 0.05	0.08 ± 0.03
All Stinging	0.59 ± 0.11	0.46 ± 0.10	0.49 ± 0.08
Walking	0.39 ± 0.09	0.56 ± 0.10	0.50 ± 0.08
	n = 35	**n = 36**	**n = 39**

Different letters within rows indicate significant differences among species ($P < 0.05$)

Table 3. Mean (± SE) leaf damage areas (mm^2) and perimeter lengths (mm) caused by feeding of *A. rudisana*, *M. divulsana* and *E. postvittana* on test leaf of *P. lanceolata*.

	Mean leaf damage areas ± SE	**Mean perimeter lengths ± SE**
A. rudisana	7.23 ± 0.85	22.40 ± 1.78
M. divulsana	5.48 ± 0.56	17.78 ± 5.94
E. postvittana	7.08 ± 0.96	23.24 ± 2.52
	n = 25	**n = 25**

Figure legends

Figure 1. Cumulative survival curves for time to initiate first fly. Different letters indicate significant differences among host species (*P* < 0.05).

Figure 2. Proportion of time for each type of behaviour responded to different host species after landing on an infested leaf. See Table 1 for definition of behaviours and associated abbreviations. Bold text and different letters indicate which associated behaviours are significantly different among species (*P* < 0.05).

Figure 3. Cumulative survival functions for first sting duration of three host species. Different letters indicate significant differences among the species (*P* < 0.05).

Figure 4. Cumulative survival curves for interval between first landing on a leaf until first sting. Different letters indicate significant differences among the species (*P* < 0.05).

Figure 5. Proportions of wasps in each species that made no sting, one and more than one stings among hosts.

Figure 1

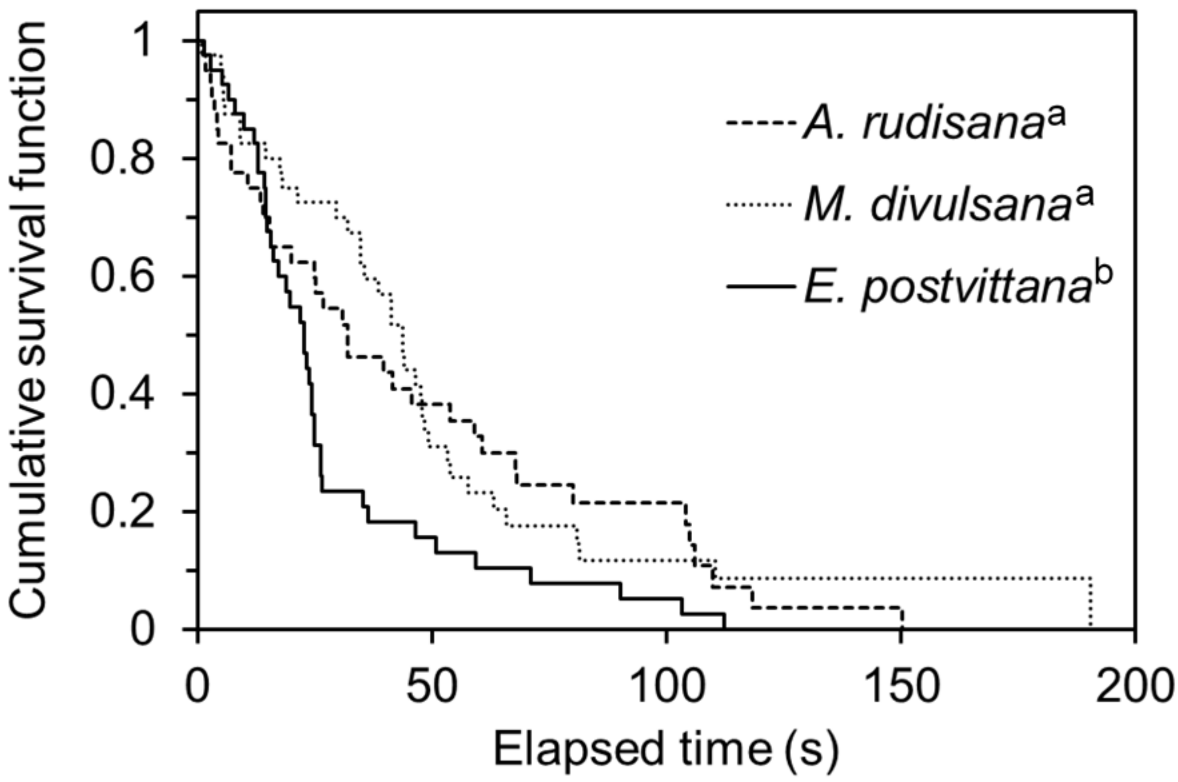
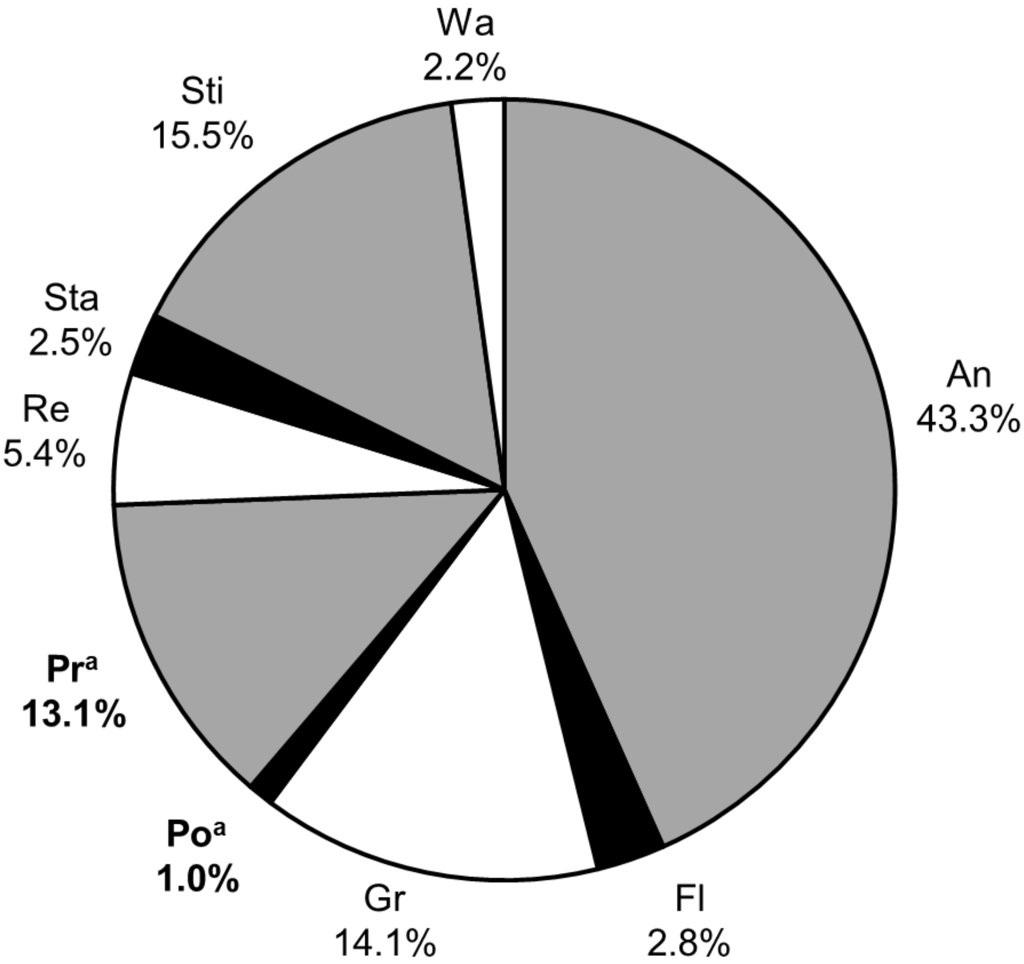
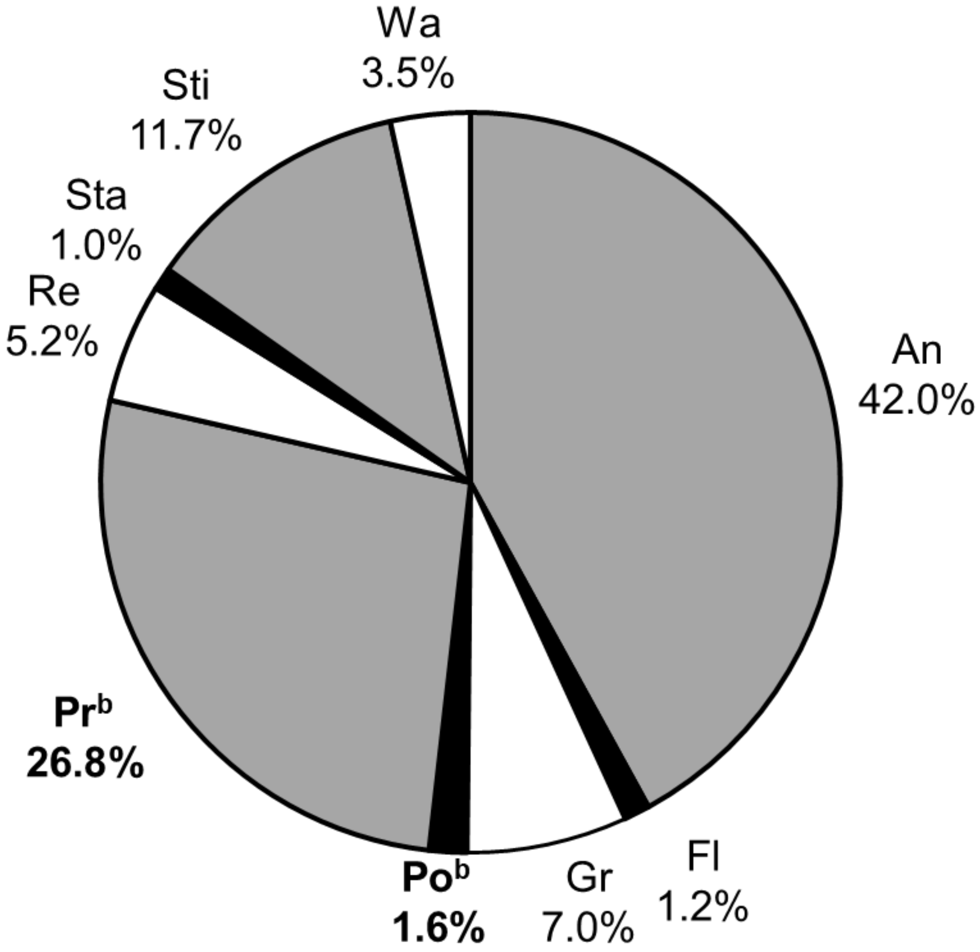

Cumulative survival function
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
Elapsed time (s)
A. rudisana[a]
M. divulsana[a]
E. postvittana[b]

Figure 2

a) *A. rudisana*



b) *M. divulsana*



c) *E. postvittana*

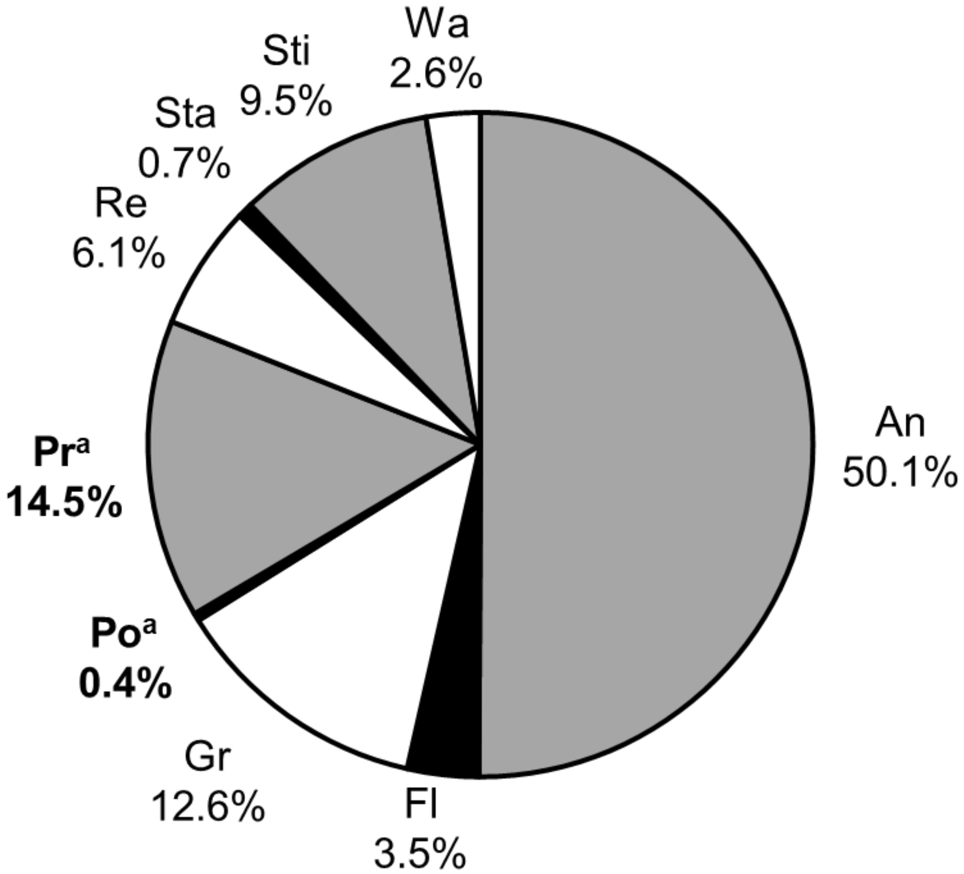
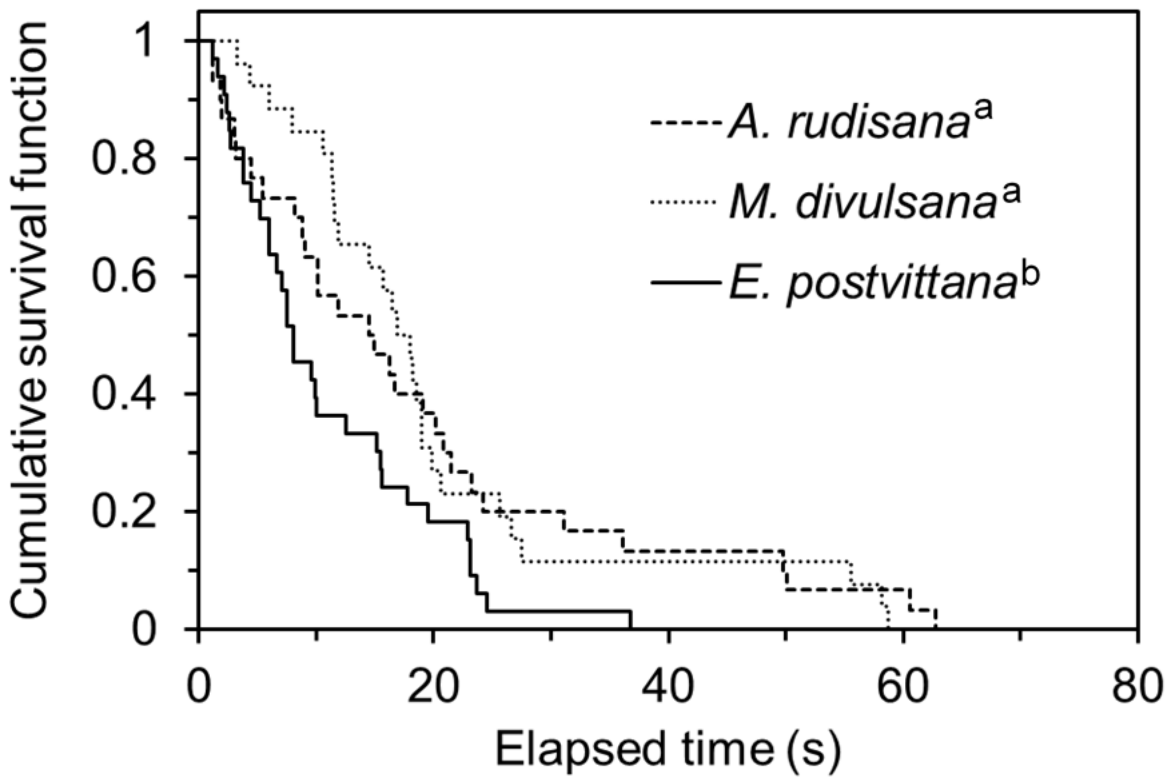


Figure 3



Cumulative survival function
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
Elapsed time (s)
A. rudisana a
M. divulsana a
E. postvittana b

Figure 4

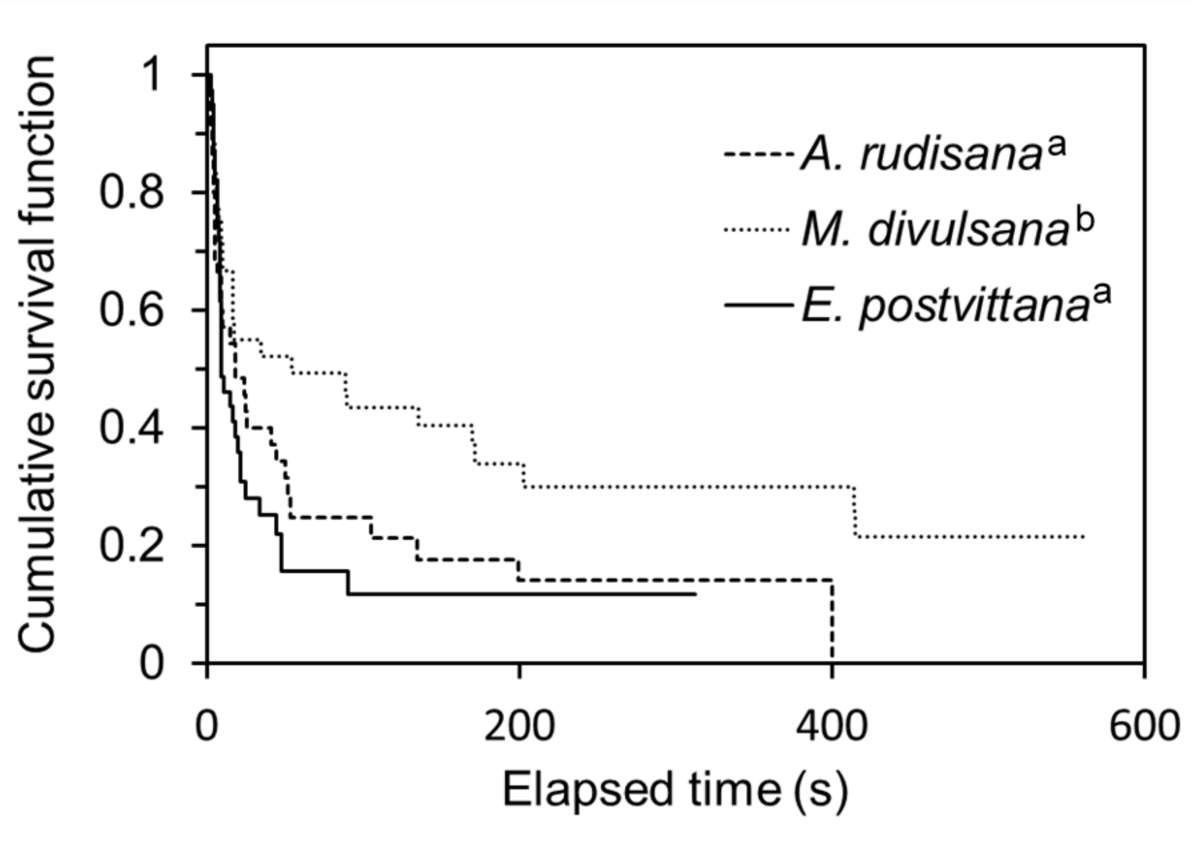
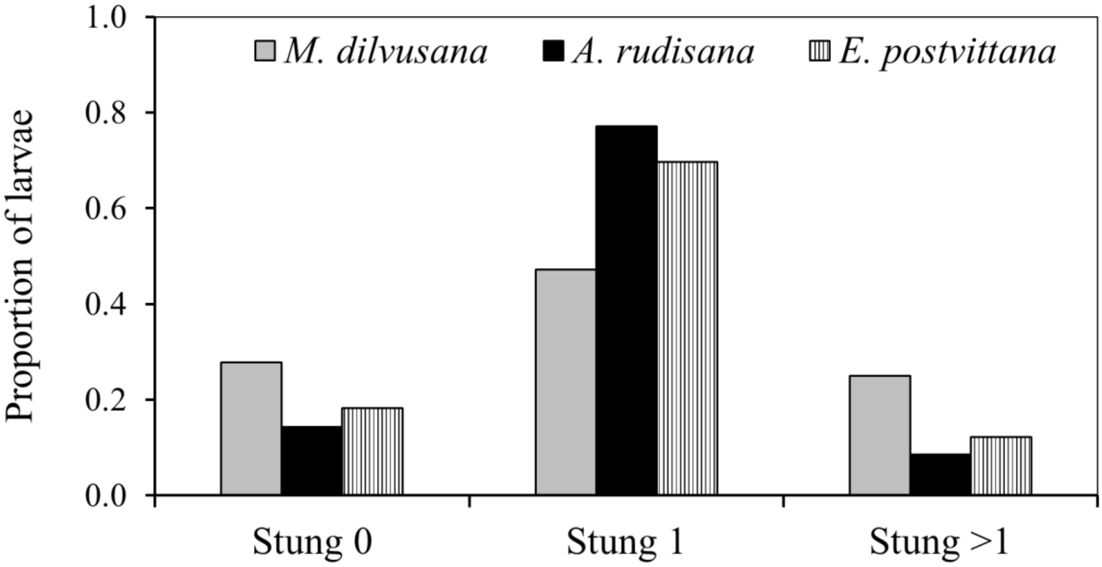

Cumulative survival function
1
0.8
0.6
0.4
0.2
0
0
200
400
600
Elapsed time (s)
A. rudisana^a
M. divulsana^b
E. postvittana^a

Figure 5



M. dilvusana
A. rudisana
E. postvittana
Proportion of larvae
1.0
0.8
0.6
0.4
0.2
0.0
Stung 0
Stung 1
Stung >1

CHAPTER THREE

Host selections of *Dolichogenidea tasmanica* and the implications for the conservation biological control of light brown apple moth, *Epiphyas postvittana*

Statement of Authorship

Title of Paper	Host selection of *Dolichogenidea tasmanica*: implications for conservation biological control of light brown apple moth
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Hieu Trung Bui		
Contribution to the Paper	Conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript.		
Overall percentage (%)	80%		
Signature		Date	01 June 2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Maryam Yazdani		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	1 June 2018

Statement of Authorship

Title of Paper	Host selection of *Dolichogenidea tasmanica*: implications for conservation biological control of light brown apple moth
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	This chapter is prepared in publication style for Biocontrol Science and Technology

Principal Author

Name of Principal Author (Candidate)	Hieu Trung Bui		
Contribution to the Paper	Conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript.		
Overall percentage (%)	80%		
Signature		Date	

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Maryam Yazdani		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	4/06/2018

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	

Host selection of *Dolichogenidea tasmanica*: implications for conservation biological control of light brown apple moth

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Abstract

An understanding of host selection by parasitoids can help in evaluating alternative host candidates to make conservation biological control of a target pest more reliable. This study investigated the host selection behaviour of *Dolichogenidae tasmanica* (Hymenoptera: Braconidae) in response to two related tortricid host species (Lepidoptera). *Epiphyas postvittana* is the biological control target in Australian vineyards, while *Merophyas divulsana* was selected to evaluate its potential as an alternative host. We quantified the effects of host species, ages and experiences on the landing preferences of the parasitoid in dual choice tests. The effects of these host species on developmental time, sex ratio and body size of adult parasitoids were also examined. During all observations, *D. tasmanica* exhibited no statistically significant preferences in response to these hosts, possibly due to low statistical power and what seems to be a very small difference. Host species did not affect the body size of the wasp, but did influence its developmental time, possibly due to a trade-off between body size and developmental time of the wasp, which took longer in the smaller host, *M. divulsana*. The sex ratio was similar within host instars and between host species. These results suggest that *M. divulsana* should be a promising alternative host to support the local parasitoid population in vineyards as part of a conservation biological control program that targets *E. postvittana*.

KEYWORDS:

alternative hosts, *Epiphyas postvittana, Merophyas divulsana*, preference, vineyards

1 | INTRODUCTION

The impact of parasitoids on a target pest may be enhanced by the availability of alternative hosts (Murdoch, Chesson, & Chesson, 1985; Powell & Wright, 1988; Jervis, Lee, & Heimpel, 2004). Holt and Lawton (1994) used the term “apparent competition” effects since the presence of one herbivore increases the risk of parasitism for another one via shared natural enemies. Through such indirect interactions, biological control of insect pests could be enhanced through the provision of alternative non-pest hosts to support local parasitoid populations (Holt & Lawton, 1994; Langer & Hance, 2004; Gillespie, Gurr, & Wratten, 2016). Although the importance of alternative hosts for the control of insect pests has been recognised, it is still less reliable in practice due to the lack of knowledge of the alternative host needs of many parasitoid species (Gillespie et al., 2016). Moreover, the presence of alternative hosts in turn may affect the control of a target pest if they are more significantly preferred by their shared parasitoids. To select appropriate alternative hosts, it is also important to know if switching from an alternative to the target host may have negative influences on the development and fitness of parasitoids or not. Thus, understanding of the host selection and the consequences of the selection in parasitoids is the first step to the successful implementation of any conservation biological control program that involves alternative hosts.

Host selection by a parasitoid is mediated by natural selection and may not be constant across time. The selection of a host is critically based on its suitability in size and nutrition for the offspring’s development, as determined by chemical, visual, tactile and/or physical contact with the host and its associated products (Vinson 1976; Vet & Dicke 1992). In addition, when hosts of different species are present in a particular habitat, the differences in availability, detectability and defensive behaviour of hosts are also significantly affected the host selection of parasitoids (Chow & Mackauer, 1991; Godfray, 1994; Harvey & Thompson, 1995; Hopkinson, Zalucki, & Murray, 2013). Also, host selection can be influenced by learning cues during natal and/or adult parasitoid experience (Vet & Dicke, 1992; Turling, Wäckers, Vet, & Tumlinson, 1993). Many parasitoids under choice tests prefer to attack hosts of species in or on which they previously developed (Morris & Fellowes, 2002; Giunti et al., 2015). If the host preferences of a parasitoid are weakly fixed genetically, contact with natal hosts or products associated with their hosts during pre-adult stages can reinforce its preference for that host species (Hérar, Keller, Lewis, & Tumlinson, 1988). Consequently, if a parasitoid developed on an alternative host, it may be less responsive to the target pest (Van Driesche, Hoddle, & Center, 2008). During searching for hosts, natal experiences of parasitoids may be altered through adaptive learning. Positive impacts of learning for parasitoids may include optimization of foraging efficiency (Vet & Groenewold, 1990; Vinson, 1998) and increase in the likelihood of encountering more suitable hosts (Papaj & Vet, 1990; Dutton, Mattiacci, & Dorn, 2000). In addition, the effects of learning on host selection behaviour are strongly correlated with rewarding experiences, i.e. oviposition (Vet & Dicke 1992; Costa, Ricard, Davison, & Turlings, 2010; Giunti et al., 2015, Yazdani & Keller, 2016).

Dolichogenidea tasmanica (Cameron) (Hymenoptera: Braconidae) is native to Australia and is a solitary, koinobiont, and generalist endoparasitoid (Dumbleton, 1935). Host stages (Yazdani, Glatz, & Keller, 2015) and adaptive learning (Feng, Wratten, Sandhu, & Keller, 2015; Yazdani & Keller, 2016) are reported to have impacts on the host selections of *D. tasmanica*. An empirical study found that parasitism rates by *D. tasmanica* varied significantly among host species, which suggests the host selection behaviour of the wasp may depend on insect host species or host plants, or that some host species are more suitable for this parasitoid's offspring (Suckling, Burnip, Gibb, Daly, & Amstrong 2001). Subsequent experiments revealed that host plants affect the foraging success of *D. tasmanica* (Feng et al 2015). Therefore, it is of theoretical and practical interst to understand the host selections of the parasitoid and the consequences of the selections on its fitness.

The light brown apple moth (LBAM), *Epiphyas postvittana* (Walker), and lucerne moth, *Merophyas divulsana* (Walker), are two tortricid (Lepidoptera) species associated with vineyards. They are active throughout year and share some natural enemies, including *D. tasmanica* (Feng, Kravchuk, Sandhu, Wratten, & Keller, 2017). The LBAM is the most damaging insect pest of grapevines in Australia (Scholefield & Morison, 2010). The lucerne moth is one of several species that have the potential to be promoted as alternative hosts for *D. tasmanica* and other beneficial insects associated with vineyards. LBAM is larger than the lucerne moth.

The aim of this study is to investigate the host selection behaviour of *D. tasmanica* in response to *E. postvittana* and *M. divulsana.* The effects of host species and stages, natal host experience, and adaptive learning on host selection were investigated. The effects of host species on the development and adult parasitoid size were also studied. The results will inform the potential role of alternative hosts in conservation biological control of LBAM.

2 | MATERIALS AND METHODS

2.1 | Insect cultures and plants

A laboratory culture of *E. postvittana* has been maintained for more than 200 generations (Yazdani, Glatz, et al., 2015). Cultures of *M. divulsana* and *D. tasmanica* were established from specimens collected at the Waite Conservation

Reserve, Urrbrae, South Australia in September 2014. To maintain genetic diversity of these laboratory cultures, field-collected individuals from the Waite Conservation Reserve and McLaren Vale, South Australia were added to the respective colonies at least every two months.

Tortricid cultures were maintained at 22 ± 2 º C and a 12 L: 12 D photoperiod on an artificial diet developed by Cunningham (2007). Colonies of *D. tasmanica* were reared on larvae of either *E. postvittana* or *M. divulsana* that fed on plantain (*Plantago lanceolata* L., Plantaginaceae) in screened insect cages (600 × 600 × 600 mm) provided with honey and water at 23 ± 2 ˚C under a 14 L: 10 D photoperiod.

Plantago lanceolata was selected as a standard host plant because both tortricid species feed on it. The plant was grown from seed in plastic trays filled with University of California (UC) soil mix (SARDI Plant Growth Services, Plant Research Centre, 2b Hartley Grove, Adelaide, South Australia). Seedlings were individually transferred to forestry tubes (50 × 50 × 120 mm) filled with UC soil mix and kept in a glasshouse under a natural photoperiod for a month prior to experiments.

2.2 | Sizes of tortricid host instars

Head capsule was used as the index of larval size for *E. postvittana* and *M. divulsana*. Five egg masses which contained 40 to 50 eggs each were separately placed in a 100 ml plastic cup in an incubator at 22 ± 2 ˚C under a 14 L: 10 D photoperiod. When 80% of eggs had hatched, six larvae were randomly selected from each cup to rear individually in CSIRO 32-well plastic trays (10 ml/well) on an artificial diet at the same conditions. Head capsule width was measured under a dissecting microscope at a magnification of 40X, using a calibrated ocular micrometer (precision = ± 0.0125 mm). Head capsule width of first instar larvae was measured after few hours of transferring new emerged larva from cups to wells. Head capsule width of latter instars was measured just after moulting when the remains of old head was observed. Measurements concluded when larvae pupated. Only healthy larvae that developed to the adult stage were included in the statistical analysis. The differences between head capsule widths of each instar of *E. postvittana* (n = 26) and *M. divulsana* (n = 23) were analysed with Mann-Whitney U tests with IBM SPSS 22 (IBM-SPSS Inc., Chicago, IL) because the data were not normally distributed.

2.3 | Host choice tests

2.3.1 | Material preparation

Two different colonies of *D. tasmanica* that were reared on either larval *E. postvittana* or *M. divulsana* were used in the experiments (hereinafter referred as Ep and Md culture respectively). When cocoons formed, they were transferred to insect rearing cages (245 × 245 × 245 mm) and kept in another room at 23 ± 2 ˚C under the natural photoperiod. This helped to keep them isolated from cues associated with host species. Prior to an experiment, each individual wasp was separately kept in a glass vial (18 mm diam. x 50 mm) with a drop of honey. All wasps used in the experiment were two- or three-day old mated females.

For each host species, three first-instar host larvae of either *E. postvittana* or *M. divulsana* were placed on a potted *P. lanceolata* sixty hours before the experiment in a glasshouse. Each inoculated plant was covered by a polythene bread bag with 30µm micro-perforations (185mm × 540mm) to prevent the neonate larvae from escaping.

2.3.2 | Experimental procedure

Host preferences of *D. tasmanica* were investigated in a wind tunnel (Keller, 1990). Wasps from both colonies were given a dual-choice situation in which two volatile sources associated with each host species were placed in pairs at a wind speed of 20 cm/s at 23 ± 2 ˚C. During a test, a pair of host-infested plants were placed in the up-wind of the wind tunnel. The distance between the infested plants was 20 cm and from the plant to release point was 40 cm. The position of the plant and order of wasp culture were randomised across the replicates. To stimulate the wasp, each was exposed to a leaf of *P. lanceolata* that had physically been damaged by pressing a glass vial onto surface prior to observations.

A single parasitoid was released from a glass vial (18 mm diam. x 50 mm). A “choice’ was recorded when a female wasp landed on either infested plant. After the wasp landed on the plant, it was captured without allowing it to oviposit. The wasp was then kept in a glass vial (same above) and was released second time to the same pair of infested plants after the first release of a following wasp. The second flight was done to detect changes, if any, of host plant choices between the first and second release time that would be associated with a non-rewarding experience. Wasps that did not respond within ten minutes or land elsewhere were recorded as “no response”. The first choice of wasp to host were recorded. The experiment for each wasp species ended when forty wasps made a choice. The plants were kept for 3-4 days until the larvae developed into the second instar. The tests were repeated using the same procedure with naïve wasps.

The data were analysed using a binominal test, with 0.5 as the null hypothesis in IBM SPSS 22. Sequential Bonferroni correction were also applied to adjust *P* value. The experimental design has a statistical power of 0.92 to detect a 3:1 preference for one species over the other at $P = 0.05$. In addition, chi-square tests for association (IBM SPSS 22) were conducted for each colony of *D. tasmanica* to determine if experience (first *vs* second flight) and host instar can alter the landing preferences of the parasitoids. Differences were considered significant at $P< 0.05$.

2.4 | Effects of host species on development and adult sizes of *D. tasmanica*

An independent experiment was conducted to evaluate if switching from the alternative host, *M. divulsana*, to the pest host, *E. postvittana*, could affect on the development and adult size of *D. tasmanica*. Female *D. tasmanica* were reared on *M. divulsana*. Five second instar larvae of either host species were infested on a leaf of *P. lanceolata* in a 100 ml round plastic container one day before the experiment. A two- or three-day old mated female was then released to parasitise these larvae for two hours. Thirty wasps were tested for each host species. Each larva was then individually reared in the 100 ml container provided with fresh leaves of *P. lanceolate* in an incubator at 20 ± 2 °C under a 14 L: 10 D photoperiod. Larvae were checked daily until they pupated, died, or parasitoid cocoons and later adult wasps emerged. The developmental time from egg to spinning cocoon and from cocoon to adult wasp emergence, as well as sex ratio were recorded. The head capsule, thorax and hind tibia length were measured as indicators of adult wasp size. The measurements were made with a Leica M80 stereomicroscope (Leica Microsystems, Australia) under a magnification of 20X. Leica Las X imaging analysis software (Leica Microsystems, Australia) was used to measure adult wasp sizes.

The effects of host species and sex on the developmental time, head capsule size, thorax and hind tibia length of adult *D. tasmanica* were statically analysed using two-way MANOVA in R version 3.4.3 (R Core Team, 2017). All data were subjected to logarithmic transformation to stabilise variances.

3 | Results

3.1 | Tortricid head capsule measurement

The head capsule widths of *E. postivittana* were significantly larger than those of *M. divulsana* in all instars, indicating that *E. postivittana* is a larger species than *M. divulsana* (Table 1).

3.2 | Host selections of *D. tasmanica* in choice tests

For Ep cultures, the number of *D. tasmanica* that were not responded when given a choice of first and second instar larvae was eight (16.67%) and nine (18.37%) respectively. While these figures presented in Md cultures were nine (18.37%) and five (11.11%). Chi-square tested showed no difference between number of no-responding wasps from these cultures in the choices of first instars (χ^2 (*df*=1, n = 97) = 0.05, $P = 0.826$) and second instars (χ^2 (*df*=1, n = 94) = 0.974, $P = 0.324$).

For both Ep and Md cultures of *D. tasmanica*, when given a choice between first and second instar larvae of *E. postivittana* and *M. divulsana*, no evidence revealed that *D. tasmanica* preferred one host over another in both release times (Figure 1) (n = 40). In addition, natal host and host species showed no effect on the host choice of the wasp. However, in the most cases more *E. postivittana* was selected by the wasps although statistical analysis revealed no evidence (Figure 1).

3.3 | Effect of experience on host selections

No relationship was found between release times and host choices made by *D. tasmanica* (Figure 1). In other words, experience with cues associated with host species without rewarding an oviposition had no effects on the subsequent selection of host species by the wasp.

3.4 | Effects of host ages on host choices

Due to insignificant differences found between release times, data of first and second release times were pooled for first and second instar larvae. For Ep culture, binomial tests with Sequential Bonferroni correction indicated that no statistically significant differences were detected between the frequency of choices between *E. postivittana* and *M. divulsana* in either first instar ($P = 0.219$ > Critical Bonferroni $P = 0.0167$) or second instar ($P = 0.738$ > critical $P = 0.05$). Similar results were also found in Md culture, with $P = 0.018$ > Critical $P = 0.0125$ and $P = 0.219$ > Critical $P = 0.025$ in first and second instar of these host species respectively.

In addition, Chi-square tests indicated no relationship detected between host instars and host choices made by females of *D. tasmanica* which reared on either *E. postivittana* or *M. divulsana* (χ^2(df = 1, n = 80) = 0.655, P=0.418 and χ^2 (df = 1, n = 80) = 0.404, $P = 0.525$ respectively).

3.5 | Developmental time and adult sizes of *D. tasmanica* reared on different host species

Developmental time of *D. tasmanica* was significantly different between species, except for time duration from cocoon to adult emergence (Figure 2). Particularly, mean development time of eggs, larvae and total time of *D. tasmanica* were significantly longer when they developed in *M. divulsana* than in *E. postivittana*. In addition, parasitoid adult sizes were only affected by sex as females were larger than males (Figure 3).

Number of females and males presented in *E. postivittana* and *M. divulsana* were 46 and 54 and 42 and 31 respectively. The proportion of male and female was not different either in *E. postvittana* (χ^2 = 0.64, df = 1, $P = 0.424$) or *M. divulsana*

($\chi^2 = 0.66$, $df = 1$, $P = 0.198$). Similar results were also found between these two species ($\chi^2 = 2.246$, $df = 1$, $P = 0.134$).

4 | Discussion

During all choice tests, there was no evidence that the parasitoid showed a preference for either host species. It is well documented that the selection of a host by parasitoids relies on the cues associated with the host (Vet & Dicke, 1992; Vinson, 1976; Vinson, Bin & Vet, 1998). For braconid parasitoids, the cues are chemical, visual and tactile (Wäckers, 1994; Segura, et al., 2007). The most common source of attractant and arrestant semiochemical cues used by searching parasitoids are produced when their host feeds or defecates (Godfray, 1994). Many parasitoid wasps are more frequently attracted to larger host larvae that produce greater quantities of attractive semiochemicals (Agelopoulos et al., 1995; Rukmowati Brotodjojo and Walter, 2006; Hopkinson et al., 2013), which is known to be the case for *D. tasmanica* (Yazdani, Glatz, et al. 2015). Hence, even if *E. postvittana* produces equally attractive semiochemicals to *D. tasmanica* compared to *M. divulsana*, it should be more attractive than because the larvae of *E. postvittana* are significantly larger than those of *M. divulsana* in every instar (Table 1). As a result, *E. postvittana* is likely to be more quickly detected from a longer distance by *D. tasmanica.* Moreover, the first and second instar larvae of *M. divulsana* often feed inside the leaf surface of *P. lanceolata*, while the larvae of *E. postvittana* feed on the leaf surface. Feeding in leaf mines may produce fewer external cues, which minimises the chances of discovery by parasitoids (Godfray, 1994). If plant damage, and consequently overall production of attractive semiochemicals, is proportional to body size, and semiochemicals are equally attractive between species, then the expected probability of *D. tasmanica* choosing *E. postvittana* is 0.57 or less based on the differences in sizes of head capsule widths (Table 1). In order to have a statistical power of 0.8 for detecting a difference between species this small at $P = 0.05$, the number of samples must be 399. Therefore, the experimental design was not powerful enough to detect such a small difference based on the difference in the expected quantity of semiochemicals produced.

Host selection also depends on the quality of cues. The absence of a preference for one of the host species suggests that *D. tasmanica* does not perceive differences between them or, if it does, then it does not have an innate preference based on the cues it perceives.

In this study, the natal host had no effect on host choices by *D. tasmanica*. The learning of cues from the natal host may occur at two stages of a parasitoid's life-time, during the pre-adult stages (pre-imaginal experience) and/or during adult emergence or as a newly-emerged adult (Turlings et al., 1993). Learning in insects may be influenced by numerous factors such as genetics (Powell & Wright, 1988; Poppy, Powell, Pennacchio, 1997), the chemical legacy from larval environments retained in adult parasitoids (Corbet, 1985; Hérard et al., 1988), or memory under neurological impacts (reviewed in Giunti et al. 2015). Giunti et al. (2015) found that learning of volatiles emitted from the natal host-plant complex occurs only during, or immediately after, adult emergence in many parasitoids. Leaning in pre-imaginal stages was postulated in Hopkins' host-selection principle (Hopkins, 1916), but conclusive evidence of such learning ability in parasitoids is not substantial (Gandolfi, Mattiacci, & Dorn, 2003). Our results are consistent with the body of work that does not support Hopkin's host-selection principle (Barron, 2001). In our experiment, the cocoons of *D. tasmanica* were removed from the host-plant complex and adults emerged in a clean environment. Early adult learning apparently did not happen, as there was no difference in the observed host preferences of the wasp.

The study also revealed that brief experience with cues from the host insect and host plant have no impact on host selection by *D. tasmanica.* However, associative learning of host-related cues in this species has been observed (Yazdani & Keller, 2016; Yi et al., 2016). This inconsistency in observations is likely to be due to differences in the opportunity to oviposit in a host. In our experiments, females did not allow to sting host larvae after making a choice. Rewarding experiences with hosts, i.e. contact with a host or successful oviposition, significantly increase the preferences of many parasitoids (for examples see Costa et al. (2010)). But neither *D. tasmanica* (Yazdani & Keller, 2016) nor *Cotesia marginiventris* (Hymenoptera: Braconidae) (Costa et al., 2010) displayed changes in preferences to odours associated with hosts after unrewarding experiences.

When *D. tasmanica* developed in the two different host species, it displayed developmental biology that is consistent with being a koinobiont. Adult sizes were only different between the sexes, not the host species (Fig 3), whereas its overall developmental time was influenced by host species and not sex (Fig 2). It seems that developmental plasticity has evolved to allow *D. tasmanica* to vary its developmental time in hosts of different sizes in order to reach the same adult size. In koinobiont-host interactions, the host is only killed when it provides sufficient nutrition for a parasitoid to achieve a minimum viable body size for survival (Harvey & Strand, 2002). When developing in a small host where the nutritional resource is limited, parasitoids have to trade-off between adult body size and development time (Harvey & Strand, 2002; Harvey & Malcicka, 2016). To achieve such body sizes, *D. tasmanica* had to prolong its development in the smaller host, *M. divulsana* to obtain enough nutrition. No differences in development time of pupae of *D. tasmanica* were detected in either host species or sex. No nutrition from the host is required in this stage for completing development. In a previous study the development of *D. tasmanica* in younger, smaller instars of *E. postvittana* took longer than in older, larger instars, but the size of adults was unaffected by host age (Yazdani, Feng, Glatz, & Keller, 2015). There is a positive relationship between parasitoid body size and fitness in many hymenopteran parasitoids, as indicated by variation in searching efficacy, lifetime fecundity and longevity (Visser, 1994; West, Flanagan, & Godfray, 1996; Bezemer, Harvey, & Mills, 2005). It is critical that parasitoids do not have reduced fitness when they attack alternative hosts that are part of a conservation in the biological control program directed against LBAM.

Because of haplodiploid sex determination, females of hymenopteran parasitoids can decide which sex to allocate when parasitising a host. In solitary parasitoids, it is expected that more female than male offspring will be allocated to high quality hosts (Louise, Datema, Janssen, & Snellen, 1994), which is often indicated by host size. Our data indicate that host size does not affect sex allocation in *D. tasmanica*. This is consistent with the biology of koinobionts *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) (Sequeira & Mackauer 1992) and *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae) (Rivero, 2000). Host quality may vary independently of host size or total biomass. In koinobiont parasitoids the relationship between the size of a host at a given time and its quality for parasitoid growth and development may be nonlinear.

Our result indicated that *M. divulsana* should be a promising alternative host candidate to promote and enhance populations of *D. tasmanica*. It is a smaller host but is large enough to maximize the size of female progeny. Our results indicate that *D. tasmanica* should ready to attack either of these hosts when they are encountered. As no *M. divulsana* were found feeding on grapevines in our field sampling (unpublished data), it is unlikely to be pest if it is promoted as alternative host in vineyards.

Further development is required in the field to finds ways to promote the presence of *M. divulsana* in and around vineyards. It is important to select host plants that are not a refuge for pests. Ideally host plants for *M. divulsana* should be perennial native species that are well adapted to local conditions and can provide both food for alternative hosts, and nectar, pollen and shelter for beneficial insects.

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AUTHOR CONTRIBUTION

HTB conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript. MY and MAK supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.

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TABLES

TABLE 1 Mean (± SD) head capsule widths (mm) of larval *E. postvittana* and *M. dilvusana* reared in an artifical diet in an incubator at 22 ± 2 ^{0}C under a 14 L: 10 D photoperiod.

	First instar	Second instar	Third instar	Fourth instar	Fifth instar
E. postvittana (n= 26)	0.21 ± 0.01^a	0.31 ± 0.01^a	0.55 ± 0.02^a	0.88 ± 0.03^a	1.25 ± 0.05^a
*M. divulsana (*n = 23)	0.20 ± 0.01^b	0.27 ± 0.02^b	0.42 ± 0.02^b	0.66 ± 0.02^b	0.99 ± 0.04^b

Different letters within rows indicate significant differences between species (Mann-Whitney test, $P < 0.01$)

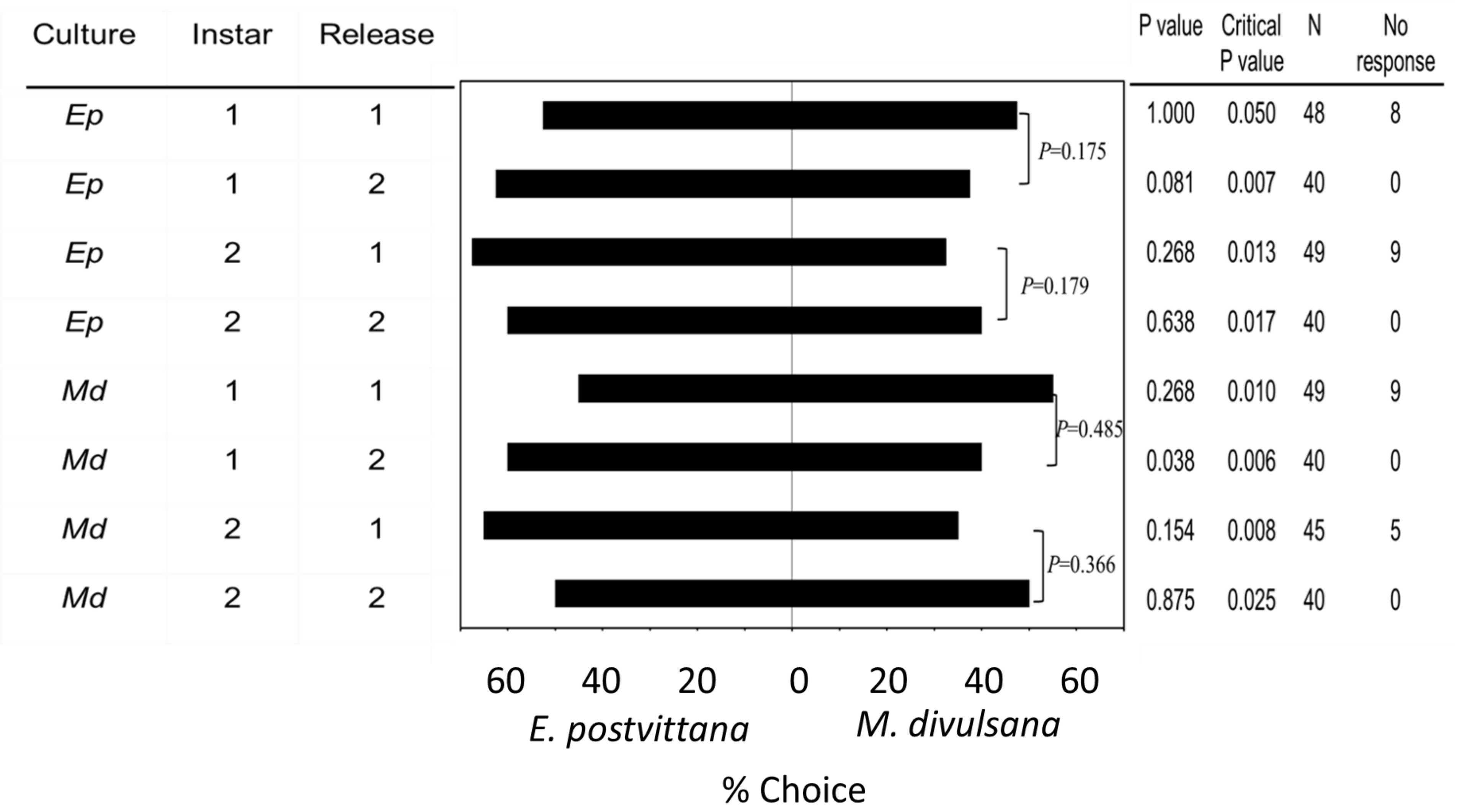
FIGURE LEGENDS

FIGURE 1 Distribution of choices made by *Dolichogenidea tasmanica* in response to plants infested with either first or second instar of either *Epiphyas postvittana* or *Merophyas divulsana* after two releases. Ep and Md refer to culture of *D. tasmanica* reared in *E. postvittana* and *M. divulsana* respectively. No evidence revealed that *D. tasmanica* preferred one host over another in both release times (Binominal tests with Sequential Bonferroni correction). Also, no relationship was found between release times and host choices made by the wasp (Chi-square tests).

FIGURE 2 Developmental time of *Dolichogenidea tasmanica* reared on second instar larvae of either *Epiphyas postvittana* or *Merophyas divulsana.* E-C: duration time from egg to spinning cocoon; C-A: developmental time from cocoon to adult wasp emergence; Total: Total developmental time from egg to adult emergence. Asterisks indicate significant differences in developmental time of parasitoids reared in different host species (MANOVA test, $P < 0.001$).

FIGURE 3 Adult sizes (mm) of *Dolichogenidea tasmanica* reared on second instar larvae of either *Epiphyas postvittana* or *Merophyas divulsana*. H-C: Head capsule width, T-L: Thorax Length and H-T: Hind Tibia Length. Asterisks indicate significant differences between female and male (MANOVA test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$).

FIGURE 1



Culture	Instar	Release	P value	Critical P value	N	No response
Ep	1	1	1.000	0.050	48	8
Ep	1	2	0.081	0.007	40	0
Ep	2	1	0.268	0.013	49	9
Ep	2	2	0.638	0.017	40	0
Md	1	1	0.268	0.010	49	9
Md	1	2	0.038	0.006	40	0
Md	2	1	0.154	0.008	45	5
Md	2	2	0.875	0.025	40	0

FIGURE 2

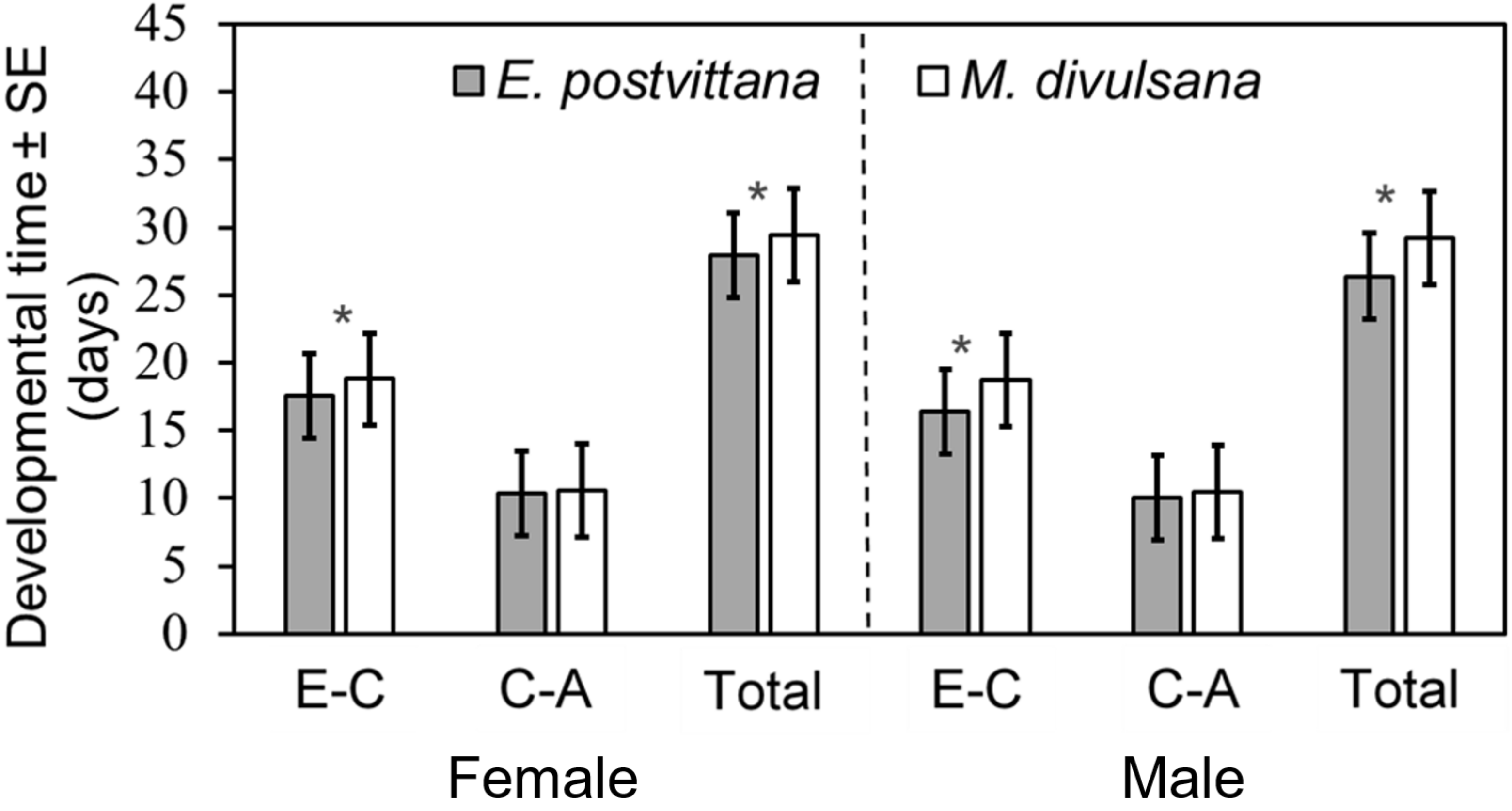
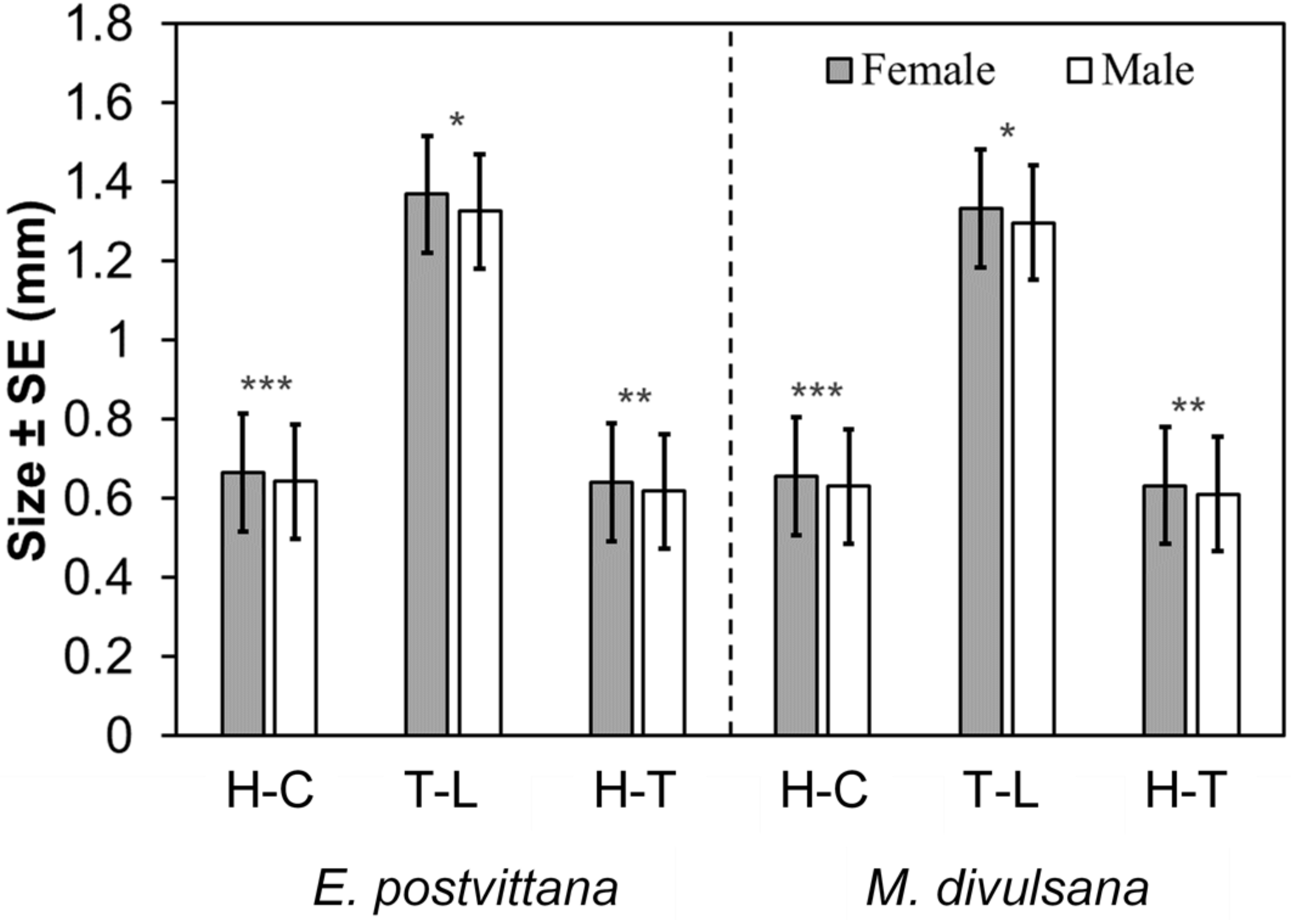

E. postvittana
M. divulsana
Developmental time ± SE (days)
45
40
35
30
25
20
15
10
5
0
*
*
*
*
E-C
C-A
Total
E-C
C-A
Total
Female
Male

FIGURE 3



Size ± SE (mm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Female
Male
***
*
**
***
*
**
H-C
T-L
H-T
H-C
T-L
H-T
E. postvittana
M. divulsana

CHAPTER FOUR

Towards conservation biological control of *Epiphyas postvittana* (Lepidoptera:Tortricidae) in vineyards: potential value of native plants

Statement of Authorship

Title of Paper	Towards conservation biological control of *Epiphyas postvittana* (Lepidoptera: Tortricidae) in vineyards: potential value of native plants
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	This chapter is prepared in publication style forJournal of Pest Science

Principal Author

Name of Principal Author (Candidate)	Hieu Trung Bui		
Contribution to the Paper	Conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript.		
Overall percentage (%)	80%		
Signature		Date	01 June 2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Michael Nash		
Contribution to the Paper	Provided suggestions and feedback on experimental designs and analysis; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	

Name of Co-Author	Maryam Yazdani		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	1 June 2018

Statement of Authorship

Title of Paper	Towards conservation biological control of *Epiphyas postvittana* (Lepidoptera: Tortricidae) in vineyards: potential value of native plants
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Principal Author

Name of Principal Author (Candidate)	Hieu Trung Bui		
Contribution to the Paper	Conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript.		
Overall percentage (%)	80%		
Signature		Date	

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Michael Nash		
Contribution to the Paper	Provided suggestions and feedback on experimental designs and analysis; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	4 June 2018

Name of Co-Author	Maryam Yazdani		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	Supervised the project; provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.		
Signature		Date	

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Towards conservation biological control of *Epiphyas postvittana* (Lepidoptera: Tortricidae) in vineyards: potential value of native plants

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Abstract

Biological control of insect pests often relies on the seasonal colonisation by natural enemies from surrounding non-crop habitats. Providing complementary non-crop plants within an agroecosystem may aid biological control in the primary crop by providing natural enemies with plant foods, refuges, and alternative hosts or prey. Such resources may support natural enemies when the crop is dormant and also sustain their population through a crop's growth and production cycle, which may in turn result in a greater or more reliable pest control. In South Australia, this approach was tested by growing five native species adjacent to vineyards to enhance biological control of light brown apple moth. Similar leafroller abundance and parasitism rates were found between blocks (with and without complementary plants). Absence of a difference was possibly due to the proximity of the experimental treatment blocks and movement of parasitoids. The diversity of parasitoids increased over the three years of the study. The results imply potential benefits of stabilising leafroller management in Australia.

Keywords Biological control · Light brown apple moth · native floral plant · Natural enemy

Key message

- Conservation biological control of insect pests requires plants that provide limiting resources needed by natural enemies.
- Native flowering plant species were evaluated for their suitability for conservation biological control of light brown apple moths and other leafrollers in vineyards in South Australia.
- The results imply potential benefits of stabilising leafroller management in Australia.

Author Contribution Statement

HTB conceived the project, designed experiments, conducted experiments, analysed data and wrote the manuscript. MY and MAK supervised the project. MY, MN and MAK provided feedback on experimental designs and analyses; reviewed the manuscript and provided suggestions to improve it.

Introduction

Enhancement of biological control of insect pests in many crops, such as vineyards, often relies on the seasonal colonisation by natural enemies from overwintering habitats (Wissinger, 1997; Skirvin et al., 2011). The success of this approach often requires (1) attraction of natural enemies early in the cropping season and (2) enhancement of the natural enemy populations throughout a crop's growth period (Simpson et al., 2011). However, the lack of food and other resources needed by natural enemies within some modern agroecosystems (monocultures) has long been suspected to be an impediment to the viability of such an approach (Masetti et al., 2010; Wäckers & van Rijn, 2012). Within-crop habitat manipulation, particularly the use of flowering field margins, may fulfil these requirements by providing natural enemies with more suitable microclimate, plant food resources (e.g. nectar and pollen), refuges and alternative hosts or prey (Altieri & Letourneau, 1982; Thomas et al., 1992; Gurr & Wratten, 1999; Landis et al., 2000). The provision of extra resources has potential to increase natural enemy population size, and thus provide sufficient numbers for colonisation into crops earlier, which may in turn result in a greater or more reliable pest suppression.

A recent review (Garcia et al., 2018) indicates inconsistent or limited effects from supplementary plant resources in vineyards on enhancing pest controls. Confounding factors include some plant species that can shelter pests. For example, *Myrtus communis* L.; *Leptospermum laevigatum* (Sol. ex Gaertn.) F. Muell. and *Pittosporum tobira* (Thunb.) W.T. Aiton are often heavily infested with the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) in California (Wang et al., 2012), and hence in some instances increase this pest's abundance. In addition, Berndt et al. (2006) found that the effects of floral resources do not always result in an increase of parasitism rates by natural enemies. These findings demonstrate the importance of selecting plants that provide limiting resources for use in habitat manipulation, and further highlight the importance of assessing of the risks and benefits of these plants in conservation biological control in vineyards. Ideally, the plants should be a mixture of “selective” species that benefit the natural enemy community more than the pests and must be appropriate for on-farm conditions (Lavandero et al., 2006; Wäckers et al., 2007; Wäckers & van Rijn, 2012).

In Australia, *E. postvittana* (Walker) (Lepidoptera: Tortricidae), is an important pest of wine grapes. Parasitism of LBAM by indigenous parasitoids is an important mortality factor (Paull & Austin, 2006). In Australia, 25 species of parasitoids and hyperparasitoids are associated with LBAM (Paull and Austin 2006). Few Hymenopteran parasitoid species were reared from larval leafrollers that were collected in and adjacent to vineyards in Adelaide Hills, South Australia found, notably *Dolichogenidea tasmanica* (Cameron), *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae), and *Phytodietus celsissimus* (Turner) (Hymenoptera: Ichneumonidae) (Feng et al., 2017). Among these parasitoids, *D. tasmanica* is the most abundant species, followed by *T. unimaculatus*. However, the parasitism rates of leafrollers by parasitoids were often less than 20% (Paull, 2007; Feng et al., 2017).

Selecting plant resources for biological control of leafrollers in South Australian vineyards may be difficult due to the biology of pests and conditions of vineyards. LBAM adults may gain benefits from nectar provided by flowering plants, which increases longevity (Begum et al., 2006). In addition, LBAM larvae are highly polyphagous (Suckling & Brockerhoff, 2010), so this may render a plant species inappropriate for enhancing biological control. Until recently, the use of non-indigenous cover crops such as buckwheat *Fagopyrum esculentum* (Moench) (Ploygonceae) has been the most common practice in vineyards to enhance natural enemies of insect pests (Orre-Gordon et al., 2013). However, under Mediterranean conditions in South Australia, plant species that are not adapted do not establish successfully (Danne et al., 2010). In addition, cover crops are typically removed annually to prevent competition for moisture when vines are growing over the summer months. Hence, indigenous plant species must be more sustainable alternatives to the currently recommended species based on overseas studies. Numerous studies suggest the use of perennial (Landis et al., 2000) and indigenous species (Fiedler et al., 2008; Isaacs et al., 2009) in conservation biological control. These plants are often well adapted, cover a large flowering period, can provide year-round resources such as shelter, and especially serve as overwintering sites to support natural enemies. They can provide greater multiple ecosystem services than non-indigenous and annual species in addition to improving biological control (Isaacs et al., 2009; Danne et al., 2010). In Australia, indigenous plants often support low densities of pests and high densities of their natural enemies, while weeds harbour more pests (Gagic et al., 2018).

The aim of this three-year field study was to evaluate the potential impacts of candidate native floral plants on biological control of insect pests in vineyards. To test the effects of the supplementary resources on leafroller control we measured three expected responses: the change in parasitism rates, the change in abundance of leafrollers and the change of beneficial insect diversity in vineyards adjacent to indigenous plants.

Methods

Sites descriptions

Field experiments were conducted at five vineyards in the Southern Vales Wine Region of South Australia (Table 1; Fig. 1) from 2015 to 2018. The sites were similar in respect to variety (Shiraz) and pest management. No insecticide had been applied to the vineyards for at least five years due to the low density of insect pests. Winter weeds were left to encourage undervine weed growth and used as mulch during winter months. Herbicides were only applied to control the weeds in spring. Fungicides were applied to control powdery mildew. There were some differences among sites such as vine age, midrow management, watering program and surrounding landscape (Table 1).

Selection of native plant species

Five criteria were used to select plants: (1) native to South Australia; (2) flowering in spring or early summer; (3) annual rainfall requirement ≤ 350 mm; (4) adaptable to a wide range of soil types and (5) easy to manage (pruning possible). Five species were selected: *Bursaria spinosa* Cavanilles subsp. *spinosa* (Pittosporaceae) (Christmas bush); *Hakea mitchellii* Meissner (Proteaceae) (Desert Hakea); *Leptospermum lanigerum* (Sol. ex Aiton) Smith (Myrtaceae) (Woolly Tea-tree); *Melaleuca lanceolata* Otto (Myrtaceae) (Dryland Tea-tree) and *Myoporum petiolatum* Chinnock (Myoporaceae) (Sticky Boobialla) The plants were supplied in forestry tubes (50mm square by 120 mm deep) by the State Flora Nursery, Belair National Park, South Australia.

In the 2016-2017 season, only *M. petiolatum* produced flowers, while all these plant species bloomed in 2017-2018 (Table 2).

Treatment design

At each site, two rows of native floral plants were planted parallel to the vine rows at a distance of 3-4 m from the vines, thereby minimising disruption to farm activities. A staggered spacing was used to allow for plant growth over time. The distance between rows and between each plant was 1m. Eight (8) plants per species were planted along each row. Therefore, 80 plants were planted at each site (2 rows by 5 spp. by 8 plants). These plants were planted in August 2015. Re-planting was carried out in October 2015 to replace any dead plants. Tree guards were installed to protect the plants from animals, wind and conserve moisture in the first year of planting. The plants were watered as needed after planting, and then as per the watering program for vines. Weeds growing within experimental blocks were removed by a petrol line trimmer.

In a vineyard, the treatment block (T) was 40 m long by 10 vine rows adjacent to supplementary floral plants. At the opposite corner of the vineyard, the same area without proximity to supplementary floral plants was used as a control block (C). The closest distance between these blocks within a vineyard was 80 m, while the farthest was 190 m.

Sampling of insects

The 2015/16 sampling was conducted at five sites to provide baseline information about parasitism rates and diversity of natural enemies, in order to refine experiment design in subsequent years. In the establishment year, it was assumed no effects could be detected. Sampling was carried out on 10-20th November,15-25th December 2015 and 20-27th January 2016. Leafrollers were collected from vines in both treatment and control blocks at the five sites. Sampling was terminated when 30 individual specimens were collected.

Due to destruction of the plants by animals at site 4 in 2016, the field experiments in 2016/17 and 2017/18 were conducted at the four remaining sites. The 2016/17 samples were collected on 20-29th November, 22-30th December 2016 and 20-30th January 2017. The number of leafrollers collected increased to 50 per block per site, to increase precision. The 2017/18 field samples were collected on 15-22h November, 20-28st December 2016 and 20-30th January 2018. The sampling plan was changed from the previous year, to enable comparison of leafroller abundance between treatment and control blocks. All leafrollers in the first 40 m of the first four rows were collected. If the number of specimens collected in samples was under 50, then supplementary sampling was carried out until 50 larvae were found to determine parasitism rates and parasitoid diversity more precisely.

The leafroller larvae collected in field samples were reared in a 100 ml plastic cups at 23 ± 2 ^{0}C under the natural photoperiod in the laboratory. Larvae were checked frequently until they pupated, died or parasitoid cocoons and adults emerged. The numbers of larvae that were parasitised by each parasitoid species, un-parasitised and dead was recorded.

Insects were sampled on native plants to determine if these plants harbour any leafrollers and parasitoids. Four native plants of each species were randomly selected in each site and thoroughly inspected. Leafrollers were reared and levels of parasitism were assed using the same method as was used for the vineyard samples.

Voucher specimens have been deposited in the Waite Insect Collection at the University of Adelaide.

Data analysis

The proportion of each parasitoid species collected was calculated as an indicator of the diversity of natural enemies. The proportion of leafroller species was calculated for only identified individuals collected. The unidentified individuals included those that died of unknown causes before reaching adulthood and those that died due to parasitism. The data were pooled for all treatments and sites, and calculated for each sampling period within a year.

Each year, the proportion of parasitised leafrollers was analysed using a generalised linear mixed model (GLMM) with binominal error distribution and logit link function using Genstat (18th ed., VSN International Ltd). Leafrollers that died of unknown causes before reaching adulthood rather than by parasitism were excluded from the analysis.

The numbers of leafrollers collected in the first four rows of vines between treatment and control blocks in the 2017-2018 field season were analysed using GLMM with a Poisson error distribution and logarithmic link function. This statistical analysis only included data from November and December 2017, due to very low leafroller abundance in January 2018.

In GLMM models, site was used as random effect while treatment and sampling period were used as fixed effects. Wald tests were used to examine significance of the fixed effects.

The Jaccard Similarity Indices (Jaccard, 1908) was calculated to determine the degree of overlap between total parasitoid species collected within and among sampling periods over the term of the study. These indices give an indication of the level of changes that occurred in parasitoid diversity.

Results

Leafroller complex and effects of floral plants on pest abundance in vineyards

The pest leafroller complex in the experimental vineyards consist of two species, *E. postvittana* and *Acropolitis rudisana* (Walker) (Lepidoptera: Tortricidae). Among them, *E. postvittana* was the most common species, constituting more than 85% of identified leafrollers collected from vine canopy (Table 3).

In the 2017/18 season, the number of leafrollers collected in first four vine rows within 40 m of treatment blocks varied significantly according to sampling period (Wald χ^2 = 14.16, *df* = 1, *P* = 0.004), but not between treatment blocks (Wald χ^2 = 1.12, *df* = 1, *P* = 0.317) (Fig. 2). The was no evidence of an interaction between treatments and sampling periods that affected leafroller abundance (Wald χ^2 = 0.18, *df* = 1, *P* = 0.678).

Effects of indigenous plants on parasitism rates of leafrollers and natural enemy diversity

The effects of flowering native plants on parasitism of leafrollers (Table 4) were not statistically significant in both two sampling years, the 2016/17 (Wald χ^2 = 3.58, *df* = 1, *P* = 0.078) and 2017/18 (Wald χ^2 = 0.01, *df* = 1, *P* = 0.939). Sampling periods had a significant effect on the parasitism rates in the 2016/17 (Wald χ^2 = 38.45, *df*=2, *P* <0.001) while no effect was detected in the 2017/18 (Wald χ^2 = 0.02, *df*=1, *P* = 0.891). No interaction between fixed factors was observed for both the 2016/17 (Wald χ^2 = 2.37, *df* = 2, *P* = 0.332) and the 2017/18 (Wald χ^2 = 0.65, *df* = 1, *P* = 0.441) sampling seasons. In the first two years of field sampling, the parasitism rates increased during the season (Fig. 4). In the 2017/18 season, parasitism rates dropped between November and January.

Overall, *D. tasmanica* was the most abundant parasitoid collected in vineyards throughout the study, followed by *Bracon sp.* and *T. unimaculatus*. Only *D. tasmanica* and *T. unimaculatus* were found in November of each year. *Goniozus jacintae* (Farrugia) (Hymenoptera: Bethylidae) was not collected in the 2015/16 sampling season, but it was common in both 2016/17 and 2017/18 (Fig. 3). Five other Hymenopteran parasitoids were found in vineyards: *Elasmus sp.* (Eulophidae), *Perilampus sp.* (Perilampidae), *Phytodietus celsissimus* (Turner) (Ichneumonidae), *Plectochorus sp.* (Ichneumonidae) and *Temelucha minuta* (Morley) (Ichneumonidae). In addition to parasitic wasps, parasitic Tachinidae (Diptera) were found in the vineyards during the 2017/18 season.

A review of Jaccard Similarity Indices indicated that the diversity of parasitoid species changed during the growing season, with more species observed as the growing season progressed (Table 5a). Between years, the largest change in parasitoid species diversity was observed in early season colonisation, with 78% dissimilarity in species collected in November 2015/16 vs. November 2017/18 (Table 5b). Overall, there was increasing parasitoid diversity over the three years of the study (Table 5b).

Insect species found on native flowering plants

All native floral species were a host for *E. postvittana*. However, *H. mitchellii* is unlikely a preferred host for leafrollers as only one larva of E*. postvittana* was found on it. *Acropolitis rudisana* was found on all plants species, except for *H. mitchellii.* Another leafroller species, *Holocola spodostola* (Turner) was collected only on *M. lanceolata* while *Strepsicrates ejectana* (Walker) was found on both *M. lanceolata* and *L. lanigerum*. Several parasitoid species were found attacking leafrollers on these native plants. However, the host species they attacked were not identified (Fig. 4).

Discussion

Although significant variation was observed in leafroller seasonal abundance and rates of parasitism, no differences were found in these measurements between the blocks that were adjacent to native plants and those that were not in this study. Several factors may be responsible for these outcomes. The treatment blocks may have been too close together (Russell, 1989), allowing the movement of parasitoids between the two blocks. *Dolichogenidea tasmanica*, the most abundant parasitoid observed in this study, is reported to be able to disperse at least 30 m in one week (Scarratt et al., 2008). In addition, in the present study, the closest distance between test blocks was 80 m while the farthest was 190 m, so the treatment effects may have been masked by movement of parasitoids. In addition, it is also possible that floral resource plantings may not large enough to have an impact on parasitoid populations (Bell et al., 2006; Thomson & Hoffmann, 2010; Tscharntke et al., 2016) or that natural enemies had sufficient other resources in the vicinity of the experimental vineyards (Keller & Baker, 2002). Observations in vineyards during winter and spring months indicated that flowering weeds can be important alternative nectar resources for parasitoids. Moreover, leafroller species including *E. postvittana*, *A. rudisana* and *Merophyas divulsana*, which are hosts of *D. tasmanica* and other parasitoids (Feng et al., 2017), were also found on weeds that are commonly present in vineyards such as *Plantago lanceolata* L. (Plantaginaceae). This would have diluted the treatment effects in our study. The effects of native flowering plants on an insect community may also require a longer time before any detectable effect occurs (Gurr et al. 2017). Finally, the influences of surrounding landscapes would affect populations of leafrollers and their parasitoids at a local and regional scale (Thomson & Hoffmann, 2010; Veres et al., 2013). For example, there was a greater abundance of leafrollers at site 5, which is adjacent to the Onkaparinga River National Park, compared to the other sites, which are surrounded by other vineyards and other managed landscapes. Adding floral plants may be ineffective in highly complex landscapes where the addition of supplementary plants is small compared to what is already present (Gurr et al., 2017). This is especially relevant for highly mobile natural enemies. Native flowering plants provide complementary resources for natural enemies over the entire year (shelter and alternative hosts), or during the crucial spring and early summer period (nectar and pollen). Where these resources are limiting, adding them to a landscape, particularly planting them adjacent to productive crops like grapevines, has the potential to make biocontrol more reliable. It is well known that nectar increases the longevity (Dyer & Landis, 1996; Johanowicz & Mitchell, 2000) and fecundity (Tylianakis et al., 2004; Winkler et al., 2006) of many parasitoids. *Dolichogenidea tasmanica* is no exception (Berndt & Wratten, 2005). Clearly, the provision of limiting nectar sources within or beside vineyards should increase the host searching efficiency of parasitoids (Jervis et al., 2004). In addition, native plants that support low densities of leafrollers can serve as a refuge for parasitoids during winter and when vineyards are sprayed with pesticides. This will enable natural enemies to “lay in wait” (Murdoch et al., 1985), and be ready to suppress LBAM when they recolonise vineyards. The relative abundance of both *E. postvittana* and *A. rudisana* on the native plants was low during our study (Figure 3), which suggests that they are unlikely to be a serious pest concern in this situation. In contrast, the LBAM and other leafroller species that feed on the native plants can serve as a source of alternative hosts for parasitoids and prey for predators, and thereby make them a reliable source of natural enemies for vineyards.

Two trends in changing parasitoid diversity warrant further investigation (Table 5a & b). On the one hand the diversity of parasitoids that colonised vineyards early in the growing season (November) increased each year, as did their overall observed diversity. These suggest that native plants may have played role in promoting parasitoid diversity. Greater parasitoid diversity that is more consistently present in vineyards should contribute to a more stable biocontrol system. Both greater replication and a longer study period are needed to demonstrate conclusively that these trends were caused by adding plants to the system.

Adding native plants did not increase pest pressure. No insecticide had been applied by grape growers in the vineyards for five years before the study commenced or during the three years of study, suggesting that the populations of leafrollers were low and certainly did not exceed the action thresholds used by the collaborating grape growers. Also, pest abundances did not increase in vineyard blocks adjacent to native plants. Thus, the low pest densities were maintained in the presence of the additional native plants.

In this study, the beneficial effects of the native flowering plants on biological control of leafrollers were not clearly demonstrated. However, our study does not eliminate these species from being selected to enhance biological control. Rather, it appears that better experimental designs and longer-term assessment are required to clearly determine if these or other plant species provide benefits that would justify their cultivation. Future experiments should be spatially designed on a larger scale with a knowledge of natural enemy dispersion. Moreover, it is important to determine if the resources that the plants provide are limiting to populations of parasitoids. The benefits of adding native plants to a crop landscape are likely to be worthwhile only where they provide limiting resource (Schellhorn et al. 2015). Finally, it is also important to evaluate the effects of the native plants on predators that also contribute to biological pest control.

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Compliance with ethical standards

Conflict of interest All authors have declared that no conflict of interest exists.

Ethic statement No human participants and/or animals were involved in this study. We confirm that this work meets the international ethical guidelines and journal' policy on these matters, including adherence to the legal requirements of Australia where the work was conducted.

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Table 1 Description of field sites.

Site	Location	Organic	Year of planting	Mid-row cover crops	Watering
1	35°12'28.31"S 138°31'47.11"E	No	1998	Permanent volunteer mid-row sward	Late December
2	35°13'12.23"S 138°33'26.58"E	Yes	2003	Annual cereal crop	Late December
3	35°12'27.12"S 138°33'11.05"E	No	1992	Permanent volunteer mid-row sward	Late November
4	35°10'19.00"S 138°33'3.79"E	No	2007	Generally, an annual cereal crop	Late December
5	35°10'7.26"S 138°34'2.91"E	No	2007	Permanent Fescue sward	November

Table 2 Plant species used, their floral colour and period

Plant Species (family)	**Common names**	**Flowering period**	**Flowering colour**
Bursaria spinosa Cavanilles subsp. *spinosa* (Pittosporaceae)	Christmas bush	Early December to late January	White
Hakea mitchellii Meissner (Proteaceae)	Desert hakea	Mid-October to mid-December	White
Leptospermum lanigerum (Sol. ex Aiton) Smith (Myrtaceae)	Green tea tree	Mid-November to late January	White
Melalueca lanceolate Otto (Myrtaceae)	Dryland tea tree	Early January to mid-February	White
Myoporum petiolatum Chinnock (Myoporaceae)	Sticky boobialla	Mid of July to early November	White

Table 3 Outcome of rearing leafrollers collected in three seasons at experimental vineyards, with treatments and sites combined. Dead leafrollers are those died of unknown reasons before reaching adulthood. The parasitised ones are recorded as the emerging parasitoid species

	2015-2016			**2016-2017**			**2017-2018**		
	Nov	Dec	Jan	Nov	Dec	Jan	Nov	Dec	Jan
Leafroller species									
Epiphyas postvittana	201	81	48	251	220	164	330	207	30
Acropolitis rudisana	22	5	0	39	36	2	36	25	4
Dead leafrollers	15	4	4	57	47	57	104	75	9
Parasitoid species									
Dolichogenidea tasmanica	58	30	32	43	70	117	146	117	8
Bracon sp.	0	7	11	0	13	51	17	5	4
Therophilus unimaculatus	4	1	2	8	4	3	29	24	0
Goniozus jacintae	0	0	0	0	5	4	30	16	2
Other parasitoids	0	0	1	2	5	2	33	1	1
Total	300	128	98	400	400	400	725	470	58

Table 4 Mean parasitism rates (±SE) (%) of leafrollers by parasitoids between Treatment (proximity to supplementary floral plants) and Control blocks (without proximity to these plants). Data were pooled from all sites (n = 4).

Mean parasitism rates (±SE) (%)	**2016-17**			**2017-18**	
	Nov	**Dec**	**Jan**	**Nov**	**Dec**
Treatment blocks (T)	16.43 ± 0.02	30.12 ± 0.12	63.24 ± 0.05	43.56 ± 0.05	39.19 ± 0.04
Control blocks (C)	14.76 ± 0.04	25.66 ± 0.06	41.24 ± 0.05	39.49 ± 0.02	42.55 ± 0.07

Table 5 The Jaccard Similarity Indices of total parasitoid species numbers within years **(a)** and between years **(b)**

(a)

Sampling time comparison	**Within year Jaccard Indices**		
	2015/16	**2016/17**	**2017/18**
November vs December	0.67	0.43	0.56
December vs January	0.75	0.71	0.50
November vs January	0.50	0.60	0.30

(b)

Sampling time comparison	**Between year Jaccard Indices**		
	2015/16 vs 2016/17	**2016/17 vs 2017/18**	**2016/16 vs 2017/18**
November	0.67	0.33	0.22
December	0.43	0.71	0.60
January	0.80	0.50	0.33
Overall	0.57	0.70	0.40

Figure Legends

Fig. 1 Locations of study sites

Fig. 2 Number of leafrollers collected in the first four vine rows within treatment and control blocks (40 m by four rows) in the 2017/18 field experiment.

Fig. 3 Proportion of leafrollers parasitised by different parasitoid species from November, December and January, using pooled data for both treatment blocks from five sites for the 2015/16 sampling season and four sites for the 2016/17 and 2017/18 seasons.

Fig. 4 Leafrollers and parasitoids collected on four native flowering plants of each species, with 2016 and 2017 indicated the 2016/17 and 2017/18 field experiments, respectively.

Fig. 1

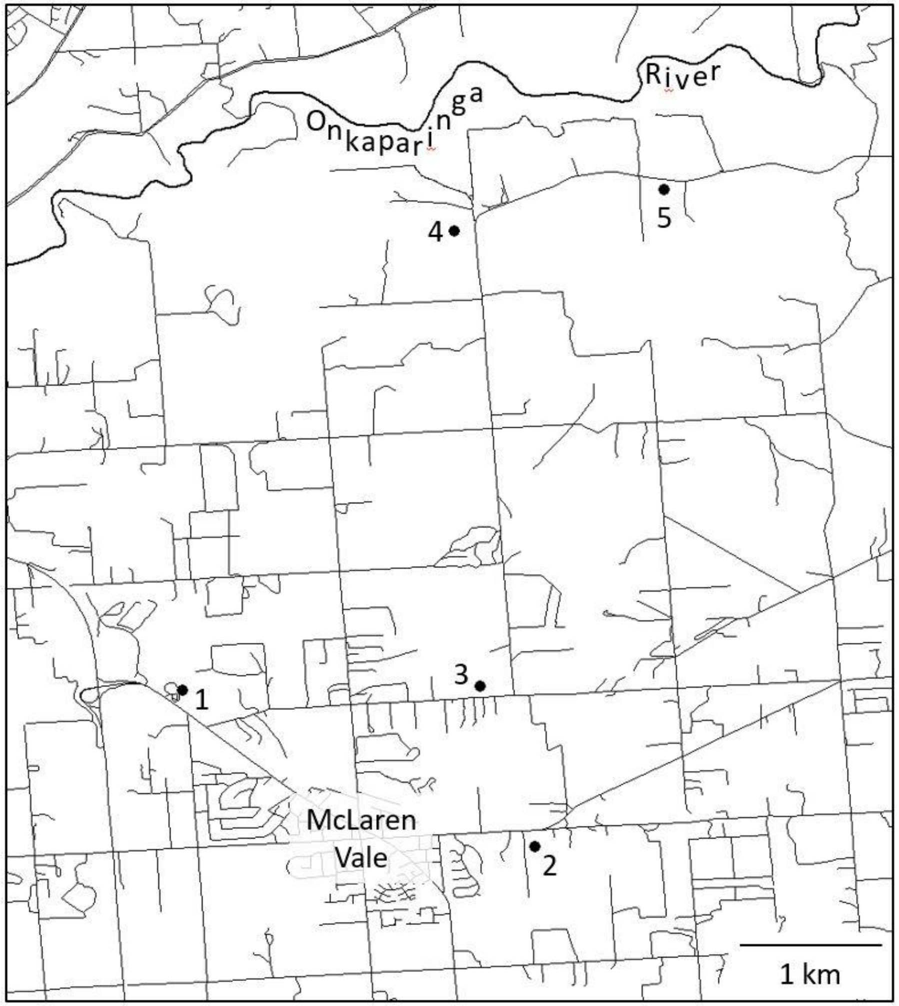
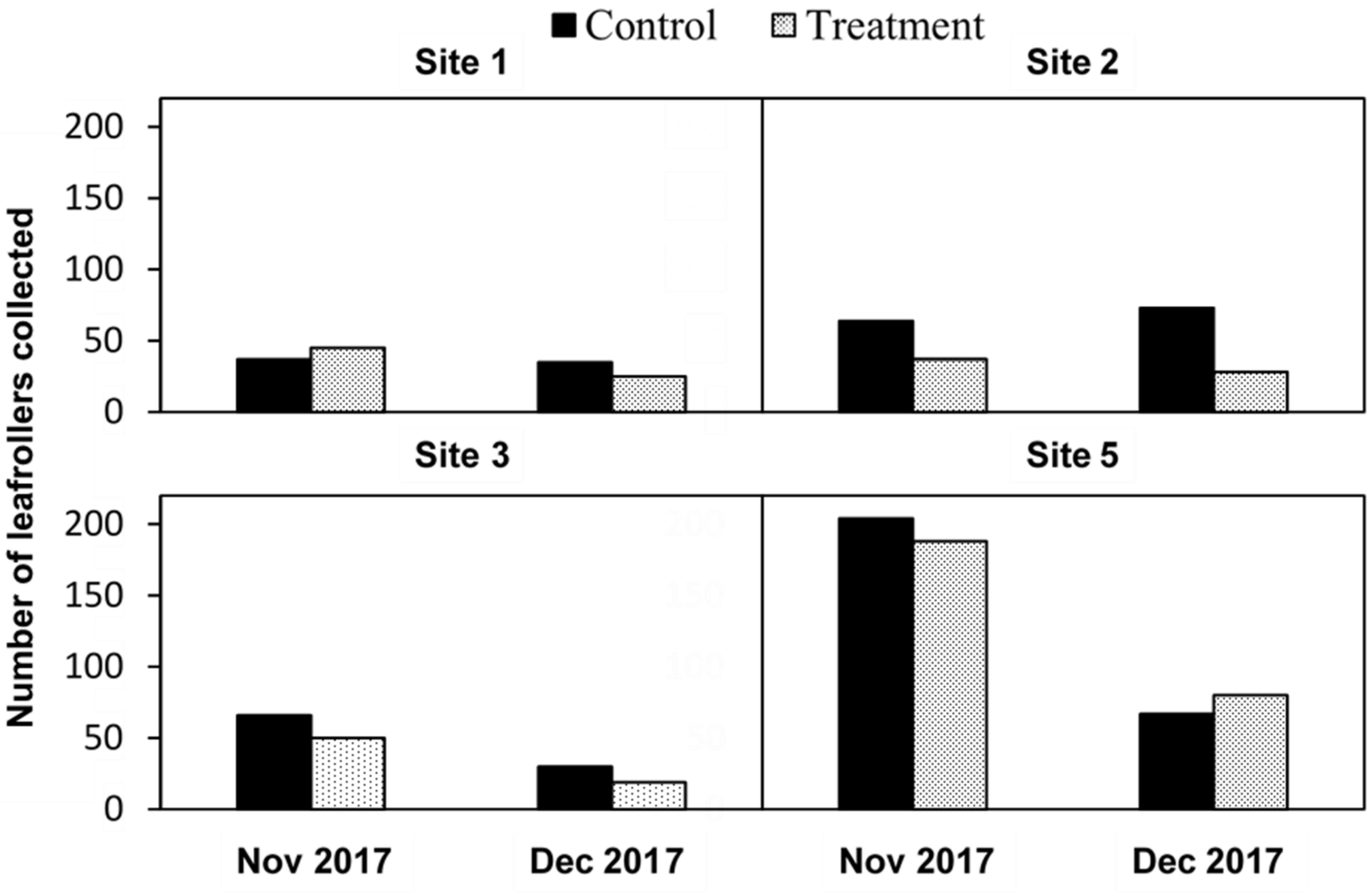


Fig. 2



■ Control
▨ Treatment
Site 1
Site 2
Site 3
Site 5
Number of leafrollers collected
0
50
100
150
200
Nov 2017
Dec 2017

Fig. 3

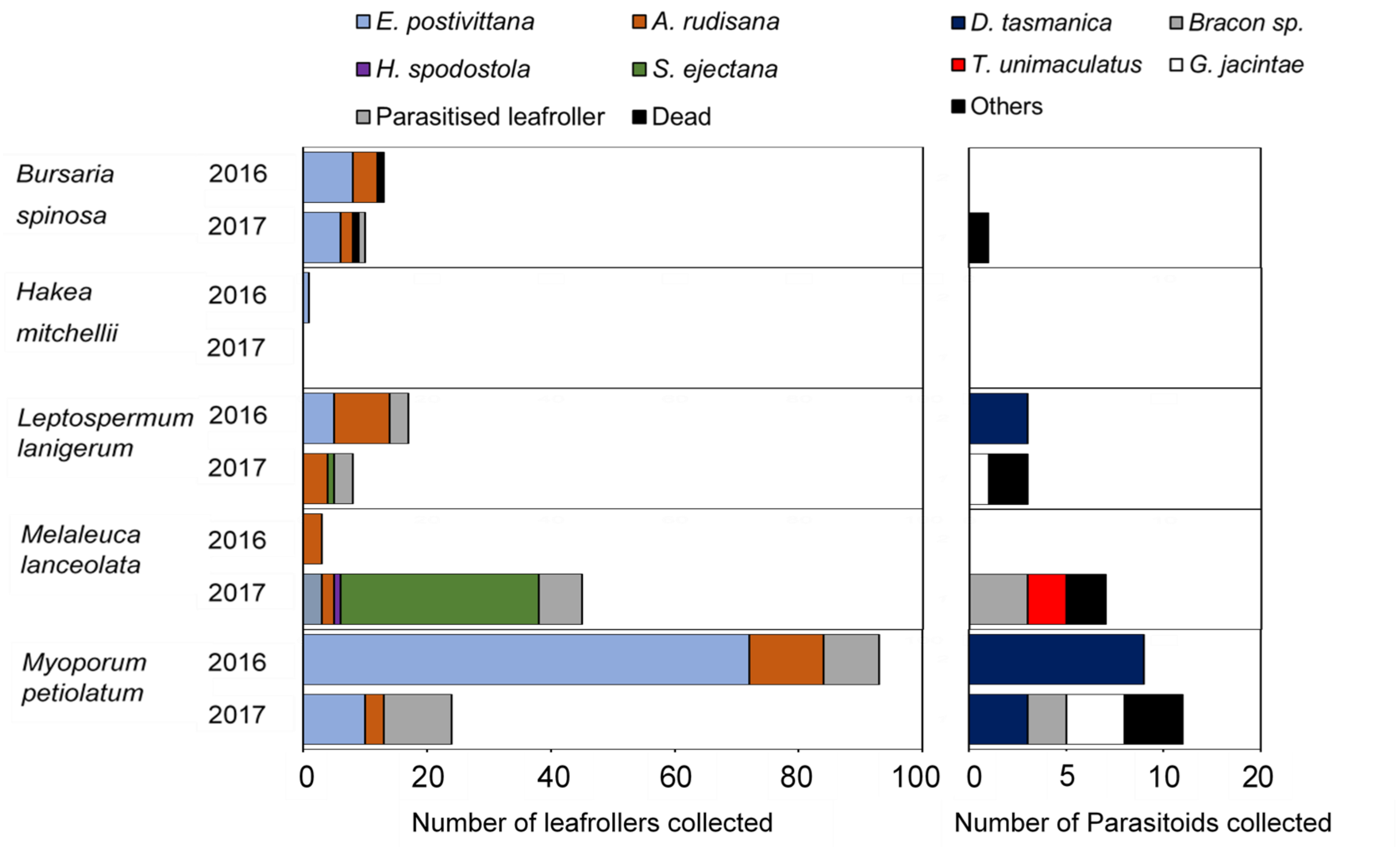
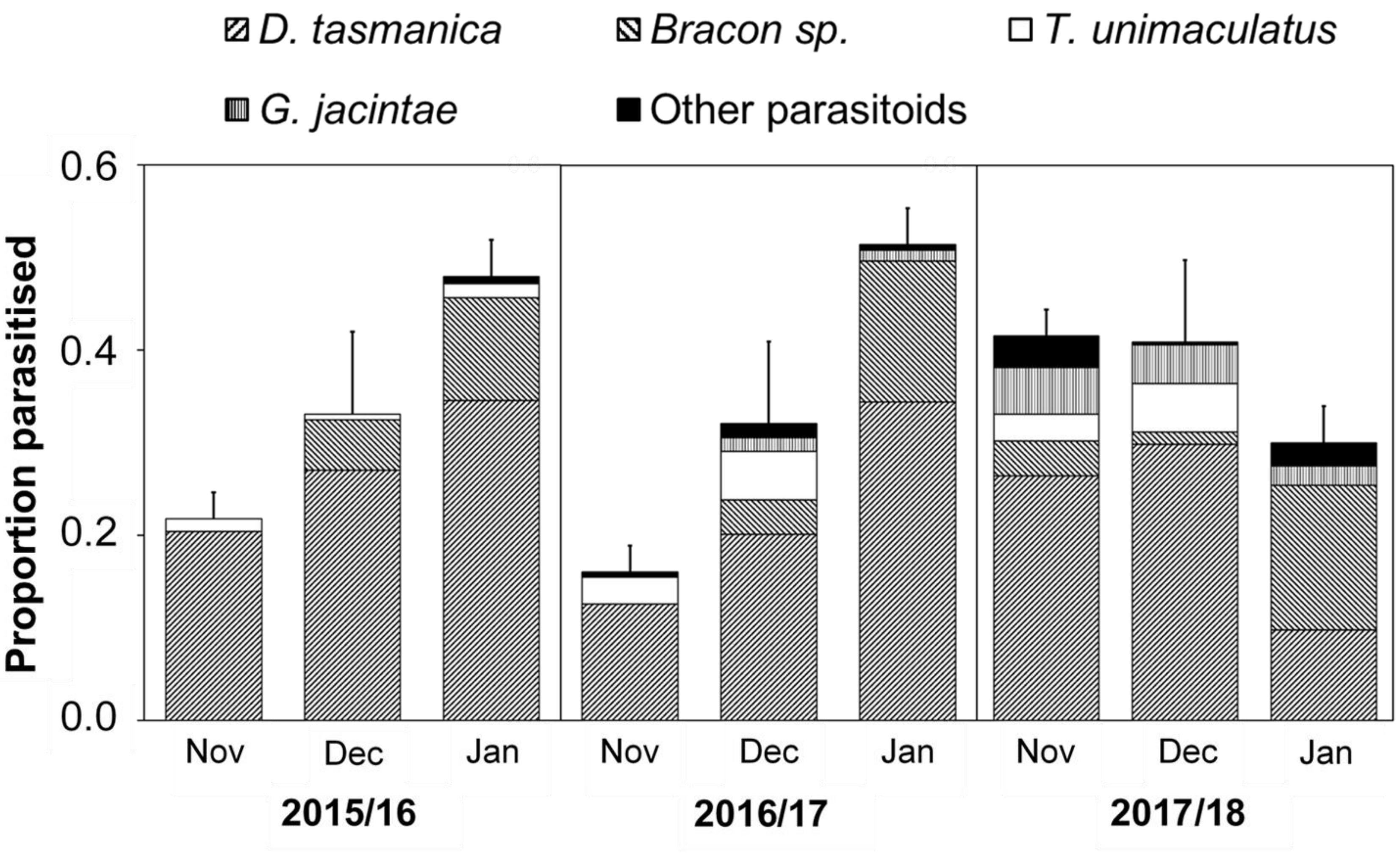

E. postivittana
A. rudisana
H. spodostola
S. ejectana
Parasitised leafroller
Dead
D. tasmanica
Bracon sp.
T. unimaculatus
G. jacintae
Others
Bursaria spinosa
Hakea mitchellii
Leptospermum lanigerum
Melaleuca lanceolata
Myoporum petiolatum
2016
2017
0
20
40
60
80
100
Number of leafrollers collected
0
5
10
20
Number of Parasitoids collected

Fig. 4


D. tasmanica
Bracon sp.
T. unimaculatus
G. jacintae
Other parasitoids
Proportion parasitised
0.6
0.4
0.2
0.0
Nov
Dec
Jan
Nov
Dec
Jan
Nov
Dec
Jan
2015/16
2016/17
2017/18

CHAPTER FIVE

General discussion and synthesis

5.1. General discussion

The present study aimed to enhance the conservation biological control of LBAM by parasitic wasps by examining how the provision of alternative hosts and native flowering plants can sustain parasitoids like *Dolichogenidea tasmanica*. Though the research's results, suitable native plants and alternative host insects that could support conservation biological control of LBAM in South Australian vineyards were evaluated. The findings point to potential opportunities to expand the range of supplementary species and contribute to making conservation biological control more reliable.

One of key findings of the study is to demonstrate that the population of *D. tasmanica* should be conserved, and LBAM more reliably suppressed, if the alternative hosts, *M. divulsana* and *A. rudisana,* are present (Chapter 2 and 3). This study and previous studies (Danthanarayana, 1980; Danthanarayana, 1983; Suckling et al., 1998; Paull & Austin, 2006; Paul, 2007; Feng, Kravchuk, Sandhu, Wratten, & Keller, 2017) have demonstrated, a wide range of parasitoids and predators attack LBAM and other leafrollers in vineyards. What was observed in *D. tasmanica* in the present study implies that the presence of alternative hosts should also enhance populations of other natural enemies, thus contributing to increasing or maintaining their diversity in vineyard ecosystems. Studies on a wide range of agroecosystems have shown that a higher diversity of natural enemies can increase overall rates of mortality and stabilise pest control through niche partitioning, facilitation, and a higher probability of having efficient natural enemies (reviewed in Rusch et al. 2016). However, some negative effects on biological control such as intraguild predator and behavioural interference have been postulated (reviewed in Rusch et al. 2016). Thus, there is a need to study the effects of alternative hosts on a natural enemy community rather than on a single species, or at least groups of species should be identified as key biological control agents. In addition to *D. tasmanica*, the present study has has indicated that *Bracon sp.*, *G. jacintae* and *T. unimaculatus*, and perhaps others natural enemy species reported in the litterature (Danthanarayana, 1980; Paull & Austin, 2006), should be studied further.

A field study was conducted to evaluate the potential impacts of candidate native plants on biological control of leafrollers, especially LBAM (Chapter 4). The beneficial effects of the five native plants on biological control of leafrollers were not clearly demonstrated. The absence of differences of parasitism rates and pest abundance was possibly due to the proximity of the experimental treatment areas and the mobility of parasitoids. However, the results imply potential benefits of the plants for better leafroller management. Field records demonstrated that the native plants can provide complementary resources for natural enemies over the entire year (shelter and alternative hosts), or during the crucial spring and early summer period (nectar and pollen), which can enhance their activities in vineyards. In addition, the native plants may have played a role in promoting increased parasitoid diversity as was shown by increasing parasitoid species numbers that appeared earlier each year and increased in total over the three-year period of study (Table 5 a & b). Moreover, provision of native plants did not increase pest pressure in vineyards. Hence, it is important to note that incorporating native plants beside vineyards should contribute to stabilising leafroller biological control, if not improving it. Both better experimental designs and longer-term assessment are required to clearly determine if these or other plant species provide benefits that would justify their cultivation.

5.2. From theory to practice of LBAM management in vineyards

The incorporation of supplementary resources should be applied to cropping systems where limiting resources for natural enemies are identified (Landis, Wratten, & Gurr, 2000; Schellhorn, Gagic, & Bommarco, 2015). Providing these limiting resources to enhance viable beneficial arthropod populations should take a landscape perspective. The continuity and linkages of all resources over the entire year, rather than only during the crop growing season, are crucial (Schellhorn et al., 2015). The benefits of biological control should be assessed not only on an increased parasitism rates, but also stabilising them and making pest suppression more reliable.

It would be expected that biological control practices, such as the use of alternative hosts and native flowering plants, should be adopted by grape growers as a part of a leafroller management strategy in Australia. To do this, it is crucial to give them evidence that the practices could provide greater or more stable productivity. In addition, there is a need to demonstrate to them the costs and benefits of the implementation of conservation biological control practices in comparison to the costs of crop losses caused by pests and/or economic costs associated with the use of pesticides or other pest management practices. To do this, economic thresholds for LBAM should be revised and updated with the current market value of grapevines (Scarratt, 2005). Methods to estimate overall costs of pests have been developed (Scholefield, & Morison, 2010), and updated crop loss estimates are now needed.

The adoption of conservation biological control practices is not yet common in Australia vineyards. However, the propagation of native plants in and around some vineyards to provide multiple ecosystem services, in addition to biological control, is occurring in such places as Taltarni Vineyards, Victoria, Australia

(Bailey, 2012) and in the Canterbury Region of New Zealand (Meurk, Wratten, & Sam, 2006). This indicates that there is some interest in the wine industry to adopt conservation biological control within viticultural systems.

5.3. Future research

The results of research presented in this thesis pointed out that there is a need to undertake further studies that could inform conservation biological control of LBAM. These should include (1) studies on the impact of natural enemy communities on leafroller populations; (2) Movement of natural enemies, particularly during vineyard colonisation in spring; (3) Density dependent responses of natural enemies in the field; (4) Long-term studies of landscape effects of supplementary vegetation and the multiple ecosystem services that they provide.

Impacts of natural enemy community on leafroller population

Conservation biological control depends on a community of natural enemies rather than a single natural enemy species. Predators, prasitoids and other beneficial organisms, together, contribute to the natural mortarlity of pests. Many predators and parasitoids are omnivorous so they need non-host food for their survival and performance, along with shelter sites and alternative hosts or prey. Recent reviews (Letourneau et al., 2011; Begg et al., 2017) indicate that increasing plant diversity in agroecosystems can result in greater natural enemy enhancement and herbivore suppression. Particularly, in Australian vineyards, the provision of perennial native grasses has been linked to a higher abundance of a range of predators and parasitoids (Danne et al., 2010). Therefore, in order to enhance sustainable pest management through habitat management, it is worthwhile to investigate the ecology of the natural enemy communities.

Movement of natural enemies

Understanding the dispersal of natural enemies from floral supplementary resources is crucial for designing conservation biological control programs as it determines the spatial distribution of floral resource subsidies used to enhance natural enemies. The results of this study (chapter 4) and other research (Keller & Baker, 2002; Bell et al., 2006) have shown that there is a need to understand the dispersal of natural enemies when deploying floral subsidies in an agroecosystem. In addition, this knowledge will help in elucidating the ability of natural enemies to supress pests at both local and landscape scales and their temporal and spatial population dynamics. Numerous techniques have been developed to quantify the movement or dispersal of insects in the field, such as the use of rubidium (Corbett et al., 1996; Pickett et al., 2004; Scarratt et al., 2008) or dyes (Verhulst et al., 2013) to mark natura enemies. However, it is difficult to follow movement of insects in the field due to their relatively small size. Thus, laboratory observations with the use of dispersal models have been applied to quantify important insect dispersal parameters (Zhou et al., 2003). However, there is little work investigating on movement of natural enemies in vineyards, except for study on *Dolichogenidae spp.* (Bell et al., 2006) and *D. tasmanica* (Scarratt et al., 2008). While the dispersal of other natural enmies is still unknown, future work should investigate this, so as to make biological control more reliable.

Density dependent responses of natural enemies

The ability of natural enemies to exhibit a rapid numerical response to increasing pest density is critical in biological control. The provision of floral plant resources may alter the nature of the functional response curve through effects on survival, searching efficiency an eff production of natural enemies (Hassell & Comins, 1978; Jervis et al., 2004). In large-scale field experiments, (Paull et al.,

2013) found that the response of *D. tasmanica* to the density of LBAM was inversely density-dependent. They speculated that this is possibly due to inadequate resources such as nectar, alternative hosts and shelter which were not available or were in short supply in vineyards. In contrast, Yazdani et al. (2016) conducted experiments in wind tunnels and enclosed cages, and found that in both cases the wasp parasitised LBAM in a density-dependent manner at low host densities. Hence the responses of *D. tasmanica* to different host densities under field conditions are not well understood. It is necessary to further investigate its functional responses in conjunction with other aspects of biology. The numerous and functional response of other parasitoid species that occur commonly vineyards such as *Bracon sp.* and *T. unimaculatus* should also be investigated. Finally, there is a need of studying the effects of alternative hosts on the suppression of LBAM, particularly switching behaviours, because they can result in a type III functional response to each of the two host species (Hassell et al., 1977).

Landscape effects

Landscape structure can influence pest and natural enemy communities through the provision of limiting resources (Tscharntke et al 2005; Bianchi et al., 2006; Thomson & Hoffmann, 2010; Veres et al., 2013; Schellhorn et al., 2015). For example, many pests and beneficial species need to move over the landscape to search for alternative hosts or overwintering sites. A meta-analysis of 46 landscape-level studies (Rebecca et al., 2011) found a strong positive relationship between landscape complexity and natural enemy abundance and diversity, predation and parasitism. More recently, Veres et al. (2013) found 45 out 72 independent case studies reported that landscapes with higher proportion of semi-natural habitats can have positive effects on a reduction of pest abundance and an increase of natural pest control in fields. However, this also indicates that habitat complexity does not always result in a greater pest control. The provision of floral resources may not be effective in highly complex landscapes where adding resources through supplement plants provides benefits that are very minor compared to what is already present (Gurr et al., 2017) or in landscapes where the supplemental vegetation is not sufficient in amount to support natural enemies effectively (Tscharntke et al., 2016; Gurr et al., 2017). To support viable populations of beneficial insects, landscape must provide resources that can be accessed when needed, not only in the crop-growing season, but also throughout the entire year (Schellhorn et al., 2015).

In Australia, natural and semi-natural habitats adjacent to vineyards can affect the abundance and diversity of natural enemies (Thomson & Hoffmann, 2010; Thomson & Hoffmann, 2013). However, the effects are varied across species and the size of adjacent woody vegetation. Thus, it is necessary to place conservation biological control under landscape perspective. In this regard, it will be important to determine the scale and spatial arrangement of plantings of native species that can deliver the most cost-effective benefits for biological control and other ecosystem services.

Long-term studies of habitat management

To elucidate the effects of perennial flowering plants in vineyards, long-term studies of habitat manipulation are needed. When increasing plant biodiversity to enhance pest suppression services, Gurr et al. (2017) argued that complementarity - where natural enemies attack pests in different ways, at different times, and/or different places - can be greater in mature compared to immature plant communities. Thus, if habitat manipulation using perennial supplementary plants is deployed in perennial crops like vineyards, longer term studies are required (5-

10 years) to accurately assess the shifting effects of habitat manipulation on biological pest control (Gurr et al., 2017). In addition, monitoring pest and beneficial insect populations over different seasons, particularly during the mild Australian winter, is crucial to evaluate the impacts of floral resource subsidies on enhancing biological control.

Multiple ecosystem services

The use of native plants can provide multiple ecosystem services, in addition to enhanced biological control. For example, biodiversity conservation of native species and ecosystem restoration can be achieved by using plant species that are indigenous to an area. Flowering plants can provide recreational values for visitors and improve landscape aesthetics (Orre-Gordon et al., 2013). Finally, if the presence of native plants reduces the need for pesticides, then Australian producers could receive financial benefits for their “clean and green” wines. Thus, it is worthwhile to investigate how selected native plant species can not only increase sustainable pest control but also increase other ecosystem services.

Conclusion

Overall, the present study contributes to the development of sustainable biological control of LBAM and other pests in vineyards. The findings of the study could also be transferable to other cropping systems where tortricids are present. This study and previous studies (Scarratt, 2005; Berndt et al., 2006; Danne et al., 2010;) have demonstrated that the provision of floral resources in or adjacent to vineyards can contribute to sustainable management of leafrollers and that conservation biological control practices can be included as a component of an integrated pest management strategy in Australian vineyards.

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