The potential functional diversity offered by native insectary plants to support populations of predatory arthropods in Australian vineyards

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A thesis submitted for the Degree of Doctor of Philosophy

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28 February 2019

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Abstract

This study investigated the role that native insectary plants can play in promoting predatory arthropods, and thereby to enhance biological control of vineyard pests in Australia. I also set out to clarify if light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) is the main lepidopteran pest of grapevines in vineyards.

Economic damage is caused to grapevines each season by pest species and *E. postvittana* is considered the dominant insect pest in Australian vineyards. However, recent observations suggested that species of tortricids other than *E. postvittana* may also act as pests. I investigated which tortricids are present in local vineyards, and whether the diversity of tortricids varied significantly among vineyards. I used molecular methods to determine the species of tortricids present in the canopies of grapevines over two growing seasons. This study confirms that *E. postvittana* is the most common tortricid pest in South Australian vineyards. *Acropolitis rudisana* (Walker) (Lepidoptera: Tortricidae), lucerne leafroller, *Merophyas divulsana* (Walker) (Lepidoptera: Tortricidae), and cotton tipworm, *Crocidosema plebejana* (Zeller) (Lepidoptera: Tortricidae) are also present in grapevine canopies but have not been reported previously.

I also sought to determine if three native candidate native insectary plants, Christmas bush, *Bursaria spinosa* (Cav.) (Apiales: Pittosporaceae), prickly tea-tree, *Leptospermum continentale* (Forst. and G.Forst) (Myrtales: Myrtaceae), and wallaby grasses, *Rytidosperma* ssp. (DC) (Poales: Poaceae) have the capacity to support populations of predatory arthropods throughout the year, and if they may also provide habitat for economically damaging vineyards pests. Surveys were conducted in Adelaide Hills, Barossa Valley and Eden Valley vineyards over a 12-month period. The data were analysed to answer the following questions. What is the biological and functional diversity associated with each plant species? What are the features of an effective, functional native insectary plant assemblage for use in and around vineyards? What is the level of similarity and dissimilarity between the arthropod faunas of each plant species?

Each plant species was found to support diverse predator species, which should attack a range of other arthropod pests across their life stages. It may also be possible to increase the functional diversity of predatory arthropods by more than three times when *B. spinosa* or *L. continentale* is incorporated into a landscape containing vineyards. *Rytidosperma* ssp. provides valuable complementary habitat for predatory species other than those commonly found in association with the woody perennials. When *Rytidosperma* ssp. are included in a plant assemblage with each woody plant species, this could result in an average net increase in predator morphospecies richness of at least 27%.

Species distribution modelling was used to examine the potential range of each plant species under different climatic conditions. The insectary plants are naturally adapted to all of the major wine growing regions within Australia. Vineyard managers are encouraged to explore the use of *B. spinosa, L. continentale* and *Rytidosperma* ssp. as insectary plants in their vineyards. This information could help wine grape growers to manage pests like LBAM, save time and money by producing grapes with lower pest incidence, while enhancing the biodiversity of their vineyards.

Declaration

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

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Mary Retallack
28 February 2019

Statement of Authorship

Title of Paper	Which species of Tortricidae leafrollers are key insect pests in South Australian vineyards?
Publication Status	☑ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Published in Transactions of the Royal Society of South Australia

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Contribution to the Paper	MR collected samples, conducted molecular laboratory analysis, interpreted data and wrote the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

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

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Contribution to the Paper	MK contributed to the manuscript production, helped in developing the idea, provided guidance throughout and assisted with statistical analysis.		
Signature	Michael A. Kell	Date	3 December 2018

Statement of Authorship

Title of Paper	Predatory arthropods associated with potential insectary plants for Australian vineyards
Publication Status	☑ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Accepted for publication in the Australian Journal of Grape and Wine Research

Principal Author

Name of Principal Author (Candidate)	Mary Joy Retallack		
Contribution to the Paper	MR collected samples, identified arthropods, interpreted data and wrote the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Contribution to the Paper	LT contributed to the manuscript production.		
Signature		Date	28 November 2018

Name of Co-Author	Emeritus Prof. Michael A Keller		
Contribution to the Paper	MK contributed to the manuscript production, helped in developing the idea, provided guidance throughout and assisted with statistical analysis.		
Signature	Michael A. Kell	Date	3 December 2018

Statement of Authorship

Title of Paper	The potential bio- and functional diversity offered by native insectary plants when planted in and around Australian vineyards
Publication Status	☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Submitted to the Australian Journal of Grape and Wine Research

Principal Author

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Contribution to the Paper	DM contributed to the manuscript production, helped in developing the idea, and assisted with statistical analysis.		
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Name of Co-Author	Dr. Linda J Thomson		
Contribution to the Paper	LT contributed to the manuscript production.		
Signature		Date	28 November 2018

Name of Co-Author	Emeritus Prof. Michael A Keller		
Contribution to the Paper	MK contributed to the manuscript production, helped in developing the idea, provided guidance throughout and assisted with statistical analysis.		
Signature	Michael A. Keller	Date	3 December 2018

Statement of Authorship

Title of Paper	Plant species distribution modelling of three native insectary plants
Publication Status	☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Submitted to the Australian Journal of Grape and Wine Research

Principal Author

Name of Principal Author (Candidate)	Mary Joy Retallack		
Contribution to the Paper	MR collected samples, identified arthropods, interpreted data and wrote the manuscript.		
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Signature		Date	28 November 2018

Name of Co-Author	Emeritus Prof. Michael A Keller		
Contribution to the Paper	MK contributed to the manuscript production, helped in developing the idea, provided guidance throughout and assisted with modelling analysis.		
Signature	Michael A. Keller	Date	3 December 2018

Acknowledgements

This research was made possible with scholarships awarded by the Australian Government Research Training Program (Australian Postgraduate Award), The University of Adelaide C. J. Everard Supplementary Scholarship, SARDI Women's Suffrage Centenary Bursary, Grape and Wine Research and Development Corporation PhD Supplementary Scholarship [GWR Ph1209]. Project operating support was provided via the Grape and Wine Research and Development Corporation and via the Adelaide Hills Wine Region Post Graduate Biodiversity Study Grant.

I gratefully acknowledge the viticulturists who allowed me to collect data in their vineyards located in the Adelaide Hills, Barossa and McLaren Vale wine regions; Darren Arney, John Good, Dan Falkenberg, Anton Groffen, Matt Hatwell, Prue Henschke, Janet and Erinn Klein, Craig Markby, David Paxton, Edd Perkins, and Fiona Wood.

Grateful thanks to my supervisors Emeritus Professor Michael Keller, Associate Professor Duncan Mackay and Dr. Linda Thomson. Your insights and guidance have been invaluable.

Thank you to my colleagues Dr. Kelly Hill, Dr. Fung Yi, Dr. Kay Anantanawat, and Dr. Amanda Choo who assisted me with molecular methods in the laboratory, Glenys M Wood who assisted greatly with arthropod identification, Dr. Maryam Yazdani, Dr. Kym Perry, Dr. Hieu Trung Bui, Kala Bhandari, Dr. Katja Hogendoorn, Dr. Michael A. Nash, Dr. Maarten van Helden and all those who have contributed their time and guidance along the way.

With much gratitude to my Aunty Beth who always provided unwavering support and encouragement. Thank you to my family and friends who shared many of glasses of wine and provided support over the course of my studies; Olive, Jim Retallack, Rachael von Einem, Kylie Clark, Jodi Heath, John Waller, Pat Hamilton, Jodie Sard, Fran Parker, Nick Spencer, Bella, Nicole McPheeters, Stuart Pettigrew, Josie Gordon, Mary Raynes, Megan Marshall and Judith Bradsen.

To the next generation, my nieces Evelyn Retallack, Claire Retallack and Bianca Marshall. Stay inquisitive, be kind and generous.

"When we kill off the natural enemies of a pest, we inherit their work."

Dr. Carl Huffaker, The University of California

In fond memory of

My Dad, M.J. Retallack 07/03/1939 - 21/09/1987

My Aunty, M.B. Spackman 24/07/1929 - 29/11/2017

Preface

The research discussed in this thesis has led to the generation of three journal papers that have either been accepted for publication, or have been submitted to scientific journals, seven journal articles, two conference posters, six conference or symposium presentations, a book contribution and numerous workshop presentations.

Journal papers (peer reviewed)

Retallack, M.J., D. Mackay, L.J. Thomson, and M.A. Keller (unpublished). The potential biological and functional diversity offered by native insectary plants when planted in and around Australian vineyards. *in preparation*.

Retallack, M.J., Thomson, L.J, and Keller, M.A. (2019). Predatory arthropods associated with potential native insectary plants for Australian vineyards. *Australian Journal of Grape and Wine Research,* doi: 10.1111/ajgw.12383.

Retallack, M.J., D. Mackay, L.J. Thomson, and M.A. Keller (2018). Which species of Tortricidae leafrollers are key insect pests in South Australian vineyards? *Transactions of the Royal Society of South Australia* 142, 130-142. doi: 10.1080/03721426.2018.1510656.

Journal articles (non-peer reviewed)

Retallack, M.J. (2019). Millipedes! How to manage populations so they do not become damaging at vintage. *The Australian and New Zealand Grapegrower and Winemaker, in press*.

Retallack, M.J. (2019). Ways to monitor arthropod activity on native insectary plants. *The Australian and New Zealand Grapegrower and Winemaker,* February, 661, 40-43. https://winetitles.com.au/gwm/articles/february-661/ways-to-monitor-arthropod-activity-on-native-insectary-plants/

Retallack, M.J. (2019). The functional diversity of predator arthropods in vineyards. *The Australian and New Zealand Grapegrower and Winemaker,* January, 660, 23-26. https://winetitles.com.au/gwm/articles/january-660/the-functional-diversity-of-predator-arthropods-in-vineyards/

Retallack, M.J. (2018). Practical examples of ways to establish native insectary plants in and around vineyards. *The Australian and New Zealand Grapegrower and Winemaker,* December, 659, 38-41. https://winetitles.com.au/gwm/articles/december-659/practical-examples-of-ways-to-establish-native-insectary-plants-in-and-around-vineyards/

Retallack, M.J. (2018). The role of native insectary plants and their contribution to conservation biological control in vineyards. *The Australian and New Zealand Grapegrower and Winemaker*, November, 658, 30-35. https://winetitles.com.au/gwm/articles/november-658/the-role-of-native-insectary-plants-and-their-contribution-to-conservation-biological-control-in-vineyards/

Retallack, M.J. (2018). The importance of biodiversity and ecosystem services in production landscapes. *The Australian and New Zealand Grapegrower and Winemaker,* October, 657, 36-43. https://winetitles.com.au/gwm/articles/october-657/the-importance-of-biodiversity-and-ecosystem-services-in-production-landscapes/

Retallack, M.J., and Keller, M.A. (2018). Which species of Tortricid leafroller do I have in my vineyard? *The Australian and New Zealand Grapegrower and Winemaker*, September, 656, 36-42. https://winetitles.com.au/gwm/articles/september-656/which-species-of-tortricid-leafroller-do-i-have-in-my-vineyard/

Conference posters

Retallack, M.J. (2016). LBAM is the key insect pest in Adelaide Hills and McLaren Vale vineyards. *16th Australian Wine Industry Technical Conference*, 24 - 28 July 2016, Adelaide, SA.

Retallack, M.J. (2013). Evaluating native insectary plant species to boost beneficial arthropod populations. *15th Australian Wine Industry Technical Conference*, 13 - 18 July 2013, Sydney, NSW.

Oral conference and symposium presentations

Retallack, M.J. (2018). Bio-diverse ecosystems in vineyards and applications for production landscapes. Australian Biological Farming Conference 2018, 10 - 11 November 2018, Southern Cross University, Gold Coast Campus, Bilinga, Australia.

Retallack, M.J. (2018). Native insectary plants have the capacity to boost predatory arthropod populations in Australian vineyards. Crush 2018, 26 September 2018, Adelaide, Australia.

Retallack, M.J. (2018). Free biological control of insect pests? Yes please! Adelaide Australian Society of Viticulture and Oenology Seminar 'Frontline pest and disease management for healthy vineyards'. 25 July 2018, Mildura, Australia.

Retallack, M.J. (2017). Which species of leafrollers (Lepidoptera: Tortricidae) are key insect pests in South Australian vineyards? Crush 2017, 14 November 2017, Adelaide, Australia.

Retallack, M.J. (2016). Evaluating native insectary plants to boost beneficial arthropod populations in vineyards. 9th International Cool Climate Wine Symposium, 26 - 28 May 2016 Brighton, UK.

Retallack, M.J. (2015). Evaluating native insectary plant species to boost beneficial arthropod populations in vineyards. Postgraduate Symposium. 22 July 2015. School of Agriculture Food and Wine, Adelaide, Australia.

Oral workshop presentations

Retallack, M.J. (2018). 'Hero' native insectary plants suited to use in Adelaide Hills vineyards. Adelaide Hills Wine Region planting and regeneration field day, 22 November 2018, Lenswood and Mount Barker Summit, Australia.

Retallack, M.J. (2017). Native insectary plants have the capacity to boost predatory arthropod populations in Adelaide Hills and Barossa vineyards. Adelaide Hills Wine Region Innovation Day workshop. 30 November 2017, Lenswood, Australia.

Retallack, M.J. (2017). Which species of leafrollers (Lepidoptera: Tortricidae) are key insect pests in South Australian vineyards? Adelaide Hills Wine Region Innovation Day workshop. 30 November 2017, Lenswood, Australia.

Retallack, M.J. (2016). Evaluating native insectary plants to boost beneficial arthropod populations in vineyards (initial PhD findings). Enhancing biodiversity and insectary workshops. Port Phillip and Westernport CMA. 15 September 2016, Bulla, Victoria.

Retallack, M.J. (2016). Evaluating native insectary plants to boost beneficial arthropod populations in vineyards (initial PhD findings). Enhancing biodiversity and insectary workshops. Port Phillip and Westernport CMA. 14 September 2016, Cockatoo, Victoria.

Retallack, M.J. (2016). Evaluating native insectary plants to boost beneficial arthropod populations in vineyards (initial PhD findings). Enhancing biodiversity and insectary workshops. Port Phillip and Westernport CMA. 13 September 2016, Main Ridge, Victoria.

Retallack, M.J. (2014). Which native plants can help to boost predators in vineyards? Creating resilient landscapes on your property - showcasing work in progress on Barossa vineyards field day and workshop, Barossa Grape Wine and Tourism Association, 19 November 2014. Nuriootpa, Australia.

Book contribution

Hall, C. (2016) Wild Barossa - How plants, people and wildlife connect. Tanunda, SA. http://www.wildbarossa.com.au M. Retallack contribution, pp18 and 58. Photos pp 18b, c, 25a, c, 26c, d and 58b.

CHAPTER ONE

General introduction

Introduction

The Australian wine sector has had a long and proud history of exporting table wine since the 1890s (Anderson, 2018). The pursuit of growing quality wine grapes in Australia has led to a range of production challenges in the vineyard. The management of pests is required to minimise the economic damage caused by fruit quality and yield losses (Scholefield and Morison, 2010). Over the last two decades there has been ongoing work on the role of native insectary plants and their contribution towards 'conservation biological control' (CBC) (Gurr et al. 1998; Landis et al. 2000b; Begum et al. 2006; Tsitsilas et al. 2006; Thomson et al. 2007; Scarratt et al. 2008; Thomson and Hoffmann, 2009b; Thomson and Hoffmann, 2010; Thomson et al. 2010b; Bui, 2018).

An insectary plant is a plant that provides food, shelter and alternative prey or hosts (Barnes et al. 2010; Gurr et al. 2017), which nourish and support the presence of predatory arthropods in association with a focal crop plant, such as grapevines. Predatory arthropods contribute towards the biological control of economically damaging pests. However, this knowledge has not resulted in widespread uptake amongst growers. This may be due to a lack of confidence, and understanding of where and how to incorporate native insectary plantings.

The wine sector is currently in the process of rebounding after a decade of downturn due to the widespread effects of the millennium drought (Goodwin and O'Connell, 2017). External economic forces have also negatively impacted on export growth (Anderson, 2018) and this phenomenon has been largely outside the control of a grower. Annual wine exports are now growing steadily again. Export sales generate \$2.7 billion (Wine Australia, 2018a) and domestic sales account for an additional \$3.3 billion per annum (Wine Australia, 2018d). This provides a greater financial capability for growers to trial new management approaches in the vineyard. There is also a growing awareness by grape growers of ways to build competitive advantage along the value chain and in key export markets. Australian producers are well placed to demonstrate their environmental stewardship credentials and promote their 'clean and green' image (Bernard et al. 2007; Thomson et al. 2007; Thomson and Hoffmann, 2009a; Hoffmann and Thomson, 2011; Penfold, 2018; Ratten, 2018).

In response to the potential challenges posed by insect damage, vineyard managers employ a range of control options to combat economically damaging pests. An integrated pest management (IPM) framework comprises the use of biological, cultural, and chemical control options (Wilson and Daane, 2017). However, over the past 60 years, growers have become increasingly reliant on chemical pest control options as a first response (Parry and Schellhorn, 2013).

The use of chemicals will invariably result in collateral damage to non-target species (Thomson and Hoffmann, 2006a, 2007a; Thomson and Nash, 2009; Bernard et al. 2010; Pennington et al. 2018). This has led to a review of plant protection practices, and a greater emphasis is now placed on biological control to regulate plant pests both in Australia (Bernard et al. 2007) and overseas (Pertot et al. 2017).

There has been growing interest in the importance of ecology within vineyards overseas (Viers and Kelsey, 2007; Viers et al. 2013; Rabolin et al. 2017; Winter et al. 2018), and also in the field of environmental stewardship in Australia. A number of evaluation and continual improvement programs, which include rudimentary measures of areas setting aside for biodiversity, and pest and disease management practices, have been tailored for use by Australian vineyard owners including Entwine Australia and Sustainable Australia Winegrowing (SAW) (Hoffmann and Thomson, 2011; Santiago-Brown et al. 2015; AWRI, 2018b; Flores, 2018). There has also been growing awareness amongst grape growers about enhancing biodiversity (Retallack, 2010) and the establishment of insectary plants in association with vineyards (Retallack, 2011; Thomas, 2018). In keeping with the focus on biological control measures, there has also been growing interest in the application of CBC within Australian vineyards (Gurr et al. 1998; Begum et al. 2006; Tsitsilas et al. 2006; Thomson et al. 2007; Scarratt et al. 2008; Thomson and Hoffmann, 2009b; Thomson and Hoffmann, 2010; Thomson et al. 2010b). Here I focus on expanding the available knowledge of pests found in South Australian vineyards, predator richness and abundance, and the capacity of native insectary plants to support populations of predatory arthropods that contribute to biological control of pests.

Economic damage is caused in Australian vineyards each season by pest species (Scholefield and Morison, 2010). Light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) is the principal insect pest that causes economic damage in Australian vineyards. *Epiphyas postvittana* causes damage to flower clusters resulting in yield losses and damage to berry skins. Damaged skins provide infection sites for *Botrytis cinerea* and other bunch moulds, which may result in a reduction in fruit quality and yield losses (Ferguson, 1995). However, recent observations suggested that species of tortricids other than *E. postvittana* may also be present. Other common vineyard pests include the Australian grapevine moth, weevils, mealybugs, scales and mites (Thomson et al. 2007).

In this thesis, the associations between larval tortricids and grapevines were investigated via a field study using molecular methods to addressing the following questions.

1. Which tortricids are present in South Australian vineyards, and
2. Does the diversity of tortricids vary significantly among vineyards?

The answer to these questions may change the management approaches adopted for leafroller management. This study focuses on wine grapes as a ‘model’ horticultural crop, given they can tolerate cosmetic damage, unlike crops such as apples or cherries, where aesthetics determine market acceptance.

Biological control is a key component of arthropod-mediated ecosystem services (AMES), which are used to manage pests in production systems (Isaacs et al. 2009). Predatory arthropods found in association with insectary plants have the capacity to provide biological pest control in vineyards (Paull, 2007; Nicholls et al. 2008; Thomson and Hoffmann, 2009b; Thomson et al. 2010b). CBC involves the implementation of practices that protect and enhance the reproduction, survival, and efficacy of natural enemies of pests (DeBach, 1974; Barbosa, 1998; van Emden, 2003; Fiedler et al. 2008; Begg et al. 2017). Insectary plants need to be attractive to predators and parasitic species (‘predators’) but not to pests, and be easy to establish and maintain, without actively competing with grapevines. A lack of biodiversity can lead to instability within a production system (Altieri, 1999; Gurr et al. 2004) such as a vineyard. Loss of species may also threaten ecosystem functioning (Tilman et al. 1996). Landscape simplification often results in an increase in pest pressure (Altieri and Letourneau, 1982; Altieri, 1994; Altieri and Nicholls, 2002; Gurr et al. 2004), and insecticide use to combat outbreaks of pests (Meehan et al. 2011). A reduction in semi-natural habitat has also been linked with a reduction of biological pest control in cultivated land by up to 46%, when compared with more complex landscapes (Rusch et al. 2016). Enhanced biodiversity is often promoted as an important indicator of vineyard health (Altieri, 1999; Gurr et al. 2003; Barnes et al. 2010; Winter et al. 2018) and non-crop plants may have the capacity to maintain and enhance biodiversity (van Emden, 1965, 2003). There is current interest in biodiversity loss due to crop production and the consequent alteration in ecosystem services provision. The presence of non-crop vegetation including native insectary plants (Schellhorn et al. 2015), may be an important contributor of functional diversity and ecosystem services (Close et al. 2009; Mace et al. 2012).

Native plants are preferred as supplementary flora, as they are locally-adapted to Australia’s dry and hot climatic conditions (Danne et al. 2010; Pandey et al. 2018). They are consistently reported as having a low occurrence of pests (Parry et al. 2015), a high occurrence of natural enemies (Gurr et al. 2017; Gagic et al. 2018) and they have the capacity to contribute towards CBC (Danne et al. 2010; Thomson and Hoffmann, 2010; Wood et al. 2011; Pandey et al. 2018). Exotic insectary plants are recognised for their provisioning services overseas (Wratten et al. 2003a; Ambrosino et al. 2006; Fiedler and Landis, 2007a), but they are not easy to establish and maintain in the dry Australian environment (Bone et al. 2009; Thomson et al. 2010a).

Grapevines are deciduous and the reduction in the resources that they provide for insects during dormancy may result in a ‘resource bottleneck’. For example, the lack of provisioning resources during plant dormancy, or throughout the overwintering period may result in an interruption in the presence of predators that may otherwise breed continuously (Schellhorn et al. 2015). However, it is possible that ecosystem services could be extended by planting a range of suitable native, woody, evergreen perennial plants to ensure habitat permanency, and synchrony of provisioning services is continual. Thus, the incorporation of native insectary plants in association with vineyards may also provide biodiversity enhancement and ecosystem service benefits at the landscape and regional scale. Relatively little work has been done on the use of specific native plant species in the field of CBC within Australia. I chose to address this gap in knowledge.

I also sought to determine if candidate insectary plants:

1. Have the capacity to support populations of predatory arthropods throughout the year, and
2. If they may also provide habitat for economically damaging vineyards pests.

The data were analysed to answer these additional questions:

1. What is the biological and functional diversity associated with each plant species? This will highlight the potential contribution of each plant.
2. What are the features of an effective, functional native insectary plant assemblage for use in and around vineyards? This will highlight the number of plants needed for effective function.
3. What is the level of similarity and dissimilarity between the arthropod faunas of each plant species? This will provide an indication of the versatility of each plant.

To address these aims, I used arthropod data collected in association with three selected native insectary plants, *B. spinosa, L. continentale* and *Rytidosperma* ssp. Adjacent plantings of grapevines, *Vitis vinifera* were also sampled. Grapevines were included as the focal horticultural crop for comparison with the locally-adapted native insectary plants. Vineyards are a managed habitat where pest insects may cause substantial damage (Scholefield and Morison, 2010). The areas in and around vineyards are also where insectary plants could be incorporated to support predators and contribute towards the biocontrol of economically damaging pests. Relationships between arthropods and plants were explored using common diversity indices to determine which plant assemblages have the capacity to provide enhanced biodiversity and effective functional diversity, when incorporated in and around Australian vineyards. The existing and potential geographic distribution of each plant species was also assessed in order to determine suitability for planting each in association with grapevines in regions around Australia.

Summary of the project

I set out to investigate the role that native insectary plants can play in promoting predatory arthropods that provide biological control of vineyard pests in Australia, and to clarify if *E. postvittana* is the main lepidopteran pest of grapevines in vineyards.

The first aim of the study was to determine which tortricids are present in South Australian vineyards. This is important, as little was known about the complex of leafroller larvae present on grapevine canopies in Australia. It was not clear if species other than *E. postvittana* were present, and if so, do they contribute to economic damage to grapevines and need to be managed separately? Therefore, an investigation of tortricid larvae present in grapevine canopies was undertaken using molecular methods, to determine the species of larvae with confidence over two seasons in Adelaide Hills and McLaren Vale vineyards. The results are presented in Chapter 3.

In addition to LBAM, there are a number of other economically damaging pests of grapevines including other moths, weevils, grape phylloxera, mealybugs, scales and mites. A truly integrated approach to pest management is needed to ensure pest damage can be minimised. CBC using native insectary plants is one way to help extend the presence and activity of predatory arthropods. The study focused on wine grapes as a 'model' horticultural crop, given grapevines can tolerate more aesthetic damage than crops such as apples or cherries, where appearance is a major driver of consumer acceptance. The results of this study may be applicable to a wide range of production systems.

The aim of the study reported in Chapter 4 was to evaluate the performance of candidate native insectary plants, which have the potential to be used in association with Australian vineyards, and identify the predatory arthropods associated with each. This is important to determine, if selected candidate insectary plants have the capacity to support populations of predators throughout the year, or if they may provide habitat for economically damaging vineyards pests. Surveys were conducted at four sites in the Adelaide Hills and two in the Barossa wine region. Three sampling methods were employed, tailored to each plant species. These results could help wine grape growers save time and resources in producing fruit with lower pest incidence by enhancing the biodiversity of their vineyards.

Similarly, I wanted to determine if the candidate insectary plants were able to simply enhance general biodiversity of all arthropod species found in association with each plant, or if they had the capacity to extend the effective functional diversity. In this study (Chapter 5), I focus on the contribution made by a group of predatory arthropods. This is important as they have functional traits that could contribute to pest regulation within the production ecosystem.

I assessed the potential biological and functional diversity offered by different native plant assemblages in association with Australian vineyards using common diversity indices. This will allow vineyard managers to select native insectary plants that have the capacity to provide targeted benefits, which are tailored to attract different functional groups of predators.

The final study outlined in Chapter 6, explores the existing and potential geographic distribution of each native insectary plant. This is important, as the plants assessed in this study are located within South Australia. For the outcomes of these studies to have broader appeal, it is important to determine the potential suitability of planting each species in association with grapevines around Australia. Climatic data for Australia were obtained for a historical thirty-year time period. The existing geographic distributions of each plant species were determined by using currently available geographical distribution records. Ecological niche models were produced for each species using a maximum entropy species distribution modelling algorithm to predict the potential niche of each species under different climatic conditions.

A summary of all these observations are synthesised in Chapter 7. A general discussion is presented bringing together each of these topic areas under the banner of 'from theory to practice'. Future research suggestions are incorporated throughout the manuscript.

Four appendices are provided for completeness. Appendix 1 covers the desktop review of candidate insectary plants. Appendix 2 provides a summary of predatory arthropods. Appendix 3 expands on the sampling methods used. Appendix 4 presents photos taken at each survey site during the sampling period to provide a clear impression of what was done and where.

Scope and structure of thesis

This thesis comprises a series of manuscripts each prepared for publication (Chapters 3, 4 and 5), as well as an additional paper (Chapter 6). Each are presented in the journal's required format. As each manuscript is intended to be read independently, so there is some repetition that flows over into the introduction, literature review and discussion chapters (Chapters 1, 2 and 7). References for the general introduction, literature review and discussion are presented at the end of Chapter 7. References for Chapters three, four, five and six are included within each of those chapters.

CHAPTER TWO

Literature review

Background

This review of the literature provides valuable background information and context for each of the themes and topics addressed in this thesis. Here I review the literature on conservation biological control in vineyards, with a particular focus on native insectary plants and predatory arthropods. I start by introducing the concept of biodiversity and explore the negative impacts that may occur as a result of landscape simplification and the loss of biodiversity it causes. I go on to highlight the important role of ecosystem services, including conservation biological control. I discuss different functional groups of arthropods including pests and the associated predators, which contribute to biocontrol of economically damaging pest species. Finally, I highlight the preferred attributes and potential contribution of locally-adapted native insectary plants for use in production systems. They have the potential to support populations of predators via the provision of food, shelter and alternative prey and enhance the overall biodiversity in vineyards.

Biological and functional diversity

Biological diversity refers to the variety of plants, animals and micro-organisms that live and interact within an ecosystem (Wilson and Peter, 1988; Cardinale et al. 2012). They provide valuable ecological services to humans (Pimentel et al. 1992). Biodiversity is typically measured as ‘richness’ (the number of unique life forms), ‘evenness’ (the consistency among life forms) and ‘heterogeneity’ (the dissimilarity among life forms) (Cardinale et al. 2012). A measure of functional diversity is often used to refer to the variety and number of species that fulfil different functional roles (Colwell, 2009) including the biological control of pests by predators. I have adopted the measure of the richness and abundance of predators to represent an objective measure of functionality.

Landscape simplification

When diverse natural systems are replaced with monocultures, this often has a negative impact on biodiversity and species richness (Hooper et al. 2005; Meehan et al. 2011). A simplistic ecological network with fewer connections could lead to instability within a production system (Altieri, 1999; Gurr et al. 2004). Where there is fragmentation of the landscape, there is often an increase in pest pressure on crops and a greater reliance on chemical control options (Meehan et al. 2011; Orre-Gordon et al. 2013). Fragmented landscapes can also have a negative effect on the abundance and diversity of predators (Steffan-Dewenter, 2003) and reduce their capacity to provide biological pest control (Kruess and Tscharntke, 1994). The effects of habitat loss and fragmentation on herbivores and predators are contingent on species and landscape (Tscharntke and Brandl, 2004).

Biodiversity loss

Loss of habitat is regarded as the greatest threat to biodiversity (Brooks et al. 2002). It is generally regarded that as the proportion of suitable habitat in the landscape is reduced to less than 30% of original vegetation cover, that this will cause a loss of biodiversity, that is, a reduction in species numbers and population densities for all fauna (Andren, 1994; Hanski, 2011). Conversely, in structurally complex landscapes predation and parasitism tends to be higher and crop damage lower than in simple landscapes (Marino and Landis, 1996; Thies and Tscharntke, 1999; Tscharntke et al. 2002b).

A number of consensus statements are proposed in the literature, which help to sum up the significance of biodiversity loss and its potential impact on humanity (Cardinale et al. 2012):

- There is indisputable evidence that the efficiency of multiple ecosystem functions is reduced as biodiversity is lost. These functions include biocontrol (Kruess and Tscharntke, 1994), and the production, decomposition and nutrient cycling of biological biomass (Balvanera et al. 2006; Cardinale et al. 2011).
- Initial losses of biodiversity in complex ecosystems have relatively low impacts on the functioning of ecosystems but both the rate of change within an ecosystem and its capacity to function accelerate as biodiversity loss increases (Cardinale et al. 2006).
- Loss of diversity across trophic levels has the potential to influence ecosystem processes more strongly than diversity loss within trophic levels (Duffy et al. 2007; Estes et al. 2011).
- A reduction in the diversity of functional characteristics of organisms will have large impacts on the extent of ecosystem functions (Petchey and Gaston, 2006; Laureto et al. 2015).
- Conversely, there is growing evidence that as biodiversity increases, so does the stability of ecosystem functions through time (Cottingham et al. 2001; Jiang and Pu, 2009).
- Diverse communities tend to be more productive, as they contain a variety of species with different functional traits that can increase productivity via the greater biomass produced (Cardinale et al. 2012).

Habitat management involving the manipulation of vegetation in production systems can exert direct suppressive effects on pests and promote predatory arthropods (Gurr et al. 2017). It is not considered that corridors, or revegetation can compensate for the overall loss of habitat provided by original plant cover (Harrison and Bruna, 1999). However, it is possible to implement restorative ecological practices that contribute to bridging this gap, by restoring indigenous plant communities (Altieri, 1999; Montoya et al. 2012). Stands of native vegetation adjacent to perennial production areas including vineyards, have been associated with increased biodiversity benefits (Thomson and Hoffmann, 2010; Smith et al. 2015).

For example, within a vineyard existing vegetation structures such as windbreaks, vegetation corridors, mid-row, under-vine and headland plantings can be enhanced to provide resources for predators that contribute to pest control throughout the year. More work is needed to assess the value of biodiversity corridors and 'stepping stones' between regions (Duelli and Obrist, 2003) for those species who are not affected by local vegetation but may respond to landscape changes at the regional scale.

The role of ecosystem services

Ecosystem services are the benefits that humans derive from ecosystems (Mace et al. 2012). They are often classified into categories of provisioning, regulating, cultural and supporting services (Close et al. 2009). This study focuses on the capacity of native insectary plants to provide 'provisioning' resources, such as food (pollen and nectar), shelter, and alternative prey (Barnes et al. 2010; Gurr et al. 2017), that nourish predators and extend their presence in a vineyard (Gurr et al. 1998). In turn, predators provide 'regulating' ecosystem services which contributes to biological control of insect pests.

Biocontrol

Biocontrol is a key component of AMES (Isaacs et al. 2009), which rely on the colonisation of vineyards by predatory arthropods each season due to the resource 'bottleneck' which may occur over the winter period when resources are limited (Schellhorn et al. 2015). My project aims to broaden our understanding of ecosystem services through ecological engineering with native species (Gurr et al. 2004). The benefits of preserving native vegetation near horticultural production areas include CBC and biodiversity enhancement (Gurr et al. 2003; Bianchi et al. 2006; Fiedler et al. 2008; Frank et al. 2008). Perennial cover crops function as a 'ecological turn-table', which have the capacity to activate and influence key processes and components of the agroecosystem (Altieri, 1999). The broad range of additional benefits include weed suppression, erosion control, aesthetics, nutrient cycling, soil water retention, soil organic carbon and soil biological activity (Gurr et al. 2003; Nicholls and Altieri, 2003; Pfiffner and Wyss, 2004; Fiedler et al. 2008).

Conservation biological control

Conservation biological control (CBC) is defined as the conservation and augmentation of predatory arthropods that are already in place or are readily available (Barbosa, 1998). This may include environmental modification to protect and enhance populations of natural enemies (DeBach, 1964). CBC is one of four strategies of 'biological control' described by Eilenberg et al. (2001), which also include 'classical', 'inoculation', and 'inundation' biocontrol of arthropods. Relatively little work has been done on the use of specific native plant species in the field to enhance CBC within Australia. However, this approach could provide innovative, practical and sustainable solutions for wine grape growers.

Enhancing biodiversity

Enhanced biodiversity can lead to greater natural biological control, resilience within the system and improved ecosystem services (Altieri, 1991; Andow, 1991; Stamps and Linit, 1997). The resilience of a system describes its capacity to reorganise after local disturbance (Tscharntke et al. 2005), or in response to environmental changes (Oliver et al. 2015). It is generally regarded that if a greater diversity and species richness are present, then it is less likely that individual weeds or arthropod pest species will dominate (Bianchi et al. 2006). The system may also be better able to recover from disruptions including extreme weather events (Yachi and Loreau, 1999). By adopting optimised management practices and promoting the richness of the natural enemies present, they could reduce the density of a widespread group of herbivorous pests and this may lead to increased yield (Cardinale et al. 2003).

Minimising negative effects

An understanding of the specific attributes insectary plants provide is important. Increasing biodiversity in general is no guarantee of pest suppression (Gurr et al. 2003; Begg et al. 2017), and may have unintended consequences, such as the enhancement of pest populations (Baggen and Gurr, 1998; Winkler, 2005; Ambrosino et al. 2006; Fiedler and Landis, 2007a).

Measuring benefits

Biodiversity enhancement is often promoted as an important indicator of vineyard health. However, the measurement of biodiversity is difficult. Thomson et al. (2007) suggest that a surrogate indicator such as predatory invertebrates, which have a direct impact on pest abundance, can be used as one way to assess the benefits of enhancing biodiversity. Therefore, I have focussed my efforts on identifying which predators are present on native vegetation and are likely to contribute to the control of key vineyard pests.

Arthropods

Functional groups: pests

Morphology of Tortricidae

Tortricidae is a diverse family of moths which have a wide range of host plants (Brown et al. 2010). In the larval stage (Figure 1a and b) they are called leafrollers because the caterpillars build protective feeding shelters, by folding leaves over their bodies and using silk webbing to secure these structures (Figure 1c). However, the distinguishing morphology of species of

Tortricidae has not been thoroughly elucidated. There are several morphological characteristics that can be used to identify larvae to the sub-family Tortricinae, including the presence of an anal comb that is used to flick away fecal pellets from their shelters (Figure 1d), and is almost always present (Brown, 2011; Gilligan, 2014a; Gilligan, 2014b). However, there are no definitive morphological characters that can be used to identify Tortricidae at the larval stage to species (Whittle et al. 1991a). Therefore, molecular methods such as DNA barcoding with short DNA sequences in a standardised region of the genome are required to determine larval stages of species of Tortricidae with confidence (Hajibabaei et al. 2006; Barr et al. 2009; Barr et al. 2011).

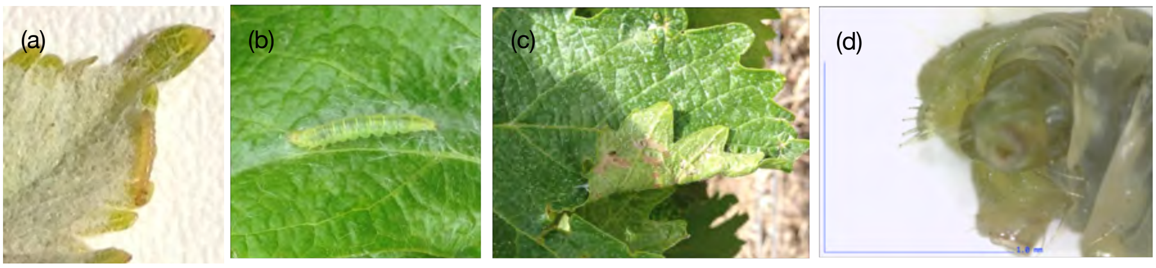

Figure 1. 1st or 2nd instar tortricid larva (a), 5th or 6th instar inside a silk refuge (b), folded grapevine leaf providing shelter (c), the presence of an anal comb is used to identify tortricid larvae to family (d). Photos: Mary Retallack

Epiphyas postvittana

LBAM, *Epiphyas postvittana* is an Australian native leafroller which was first described in 1863 (Geier and Briese, 1981). It is a damaging pest of grapevines in Australia and it has been introduced into England, Ireland, Japan, Sweden and the USA (Suckling and Brockerhoff, 2010). Typically, there are three (spring, summer and autumn-winter), and occasionally four LBAM generations (Magarey et al. 1994). The economic cost of pests and diseases was calculated in Australian vineyards in 2010 by Scholefield and Morison (2010). They found LBAM is a number four priority pest (behind powdery mildew, downy mildew and *Botrytis*), and the number one priority insect pest identified for future research. Damage to grape skins caused by LBAM provides infection sites and may predispose bunches to bunch moulds. Annual losses from *Botrytis* and other bunch rots and LBAM were estimated at $52 million and $18 million respectively, with a combined national economic impact of $70 million p.a.

Acropolitis rudisana

Acropolitis rudisana (Walker) (Lepidoptera: Tortricidae) was first described in 1863 and is widespread in eastern Australia. There is a scarcity of published information about *A. rudisana*. However, Feng et al. (2016) recently found *A. rudisana* present in woody habitats adjacent to vineyards.

Merophyas divulsana

The lucerne leafroller, *M. divulsana* (Walker) (Lepidoptera: Tortricidae) is a significant pest of cultivated lucerne, *Medicago sativa* (L.) (Allsopp et al. 1983a; Whittle et al. 1991a) and is a native to Australia. *Vitis vinifera* is not regarded as a key host species and little is known about the presence of *A. rudisana* in perennial horticultural crops. Feng et al. (2016) also found *M. divulsana* present on cover plants in vineyards.

Other pests

There are a many other pests that also contribute to economic damage of grapevines. For example it was estimated in 2010 that losses of $0.5 million per year can be attributed to garden weevils, grape phylloxera, mealybugs, scales and trunk boring insects (Scholefield and Morison, 2010). Other vineyard pests include Australian grapevine moth, elephant weevil and mites (Bernard et al. 2007; Thomson et al. 2007).

Functional groups: predators

Generalist predators feed on a range of host species and are often voracious feeders on eggs, larvae and adult stages. Many predators, like spiders, brown and green lacewings, ladybird beetles and predatory bugs are commonly found in vineyards (Thomson et al. 2007; Thomson and Hoffmann, 2008). A range of generalist predators contribute to the control of LBAM (Bernard et al. 2006b). The main predators and parasitoids of *E. postvittana* include neuropteran larvae (lacewings), spiders, earwigs, ladybird, carabid and rove beetles, predatory Hemiptera (shield and damsel bugs), predatory Diptera (hover flies and robber flies) and parasitic wasps (Bernard et al. 2006b; Frank et al. 2007; Paull, 2007; Thomson and Hoffmann, 2009a, 2010; Hogg et al. 2014; Yazdani et al. 2015; Yazdani and Keller, 2017). Some predators feed on LBAM eggs (MacLellan, 1973; Danthanarayana, 1980; Paull and Austin, 2006). It is reported that up to 90% of newly hatched leafroller larvae may be killed by predators in the absence of toxic chemicals (Helson, 1939; Waterhouse and Sands, 2001).

There are at least 25 known parasitoids of eggs, caterpillars and pupae of LBAM (Paull and Austin, 2006; Paull, 2007). *Trichogramma* ssp. (Hymenoptera: Trichogrammatidae) wasps are only able to parasitise LBAM eggs (Glenn et al. 1997; Glenn and Hoffmann, 1997) but no other life stage. This along with low levels of parasitism and late season activity, may naturally limit their ability to control LBAM in isolation (Bernard et al. 2006a). However, young LBAM instars can be parasitised by *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae), but parasitism is only possible up to and including the third instar (Yazdani et al. 2015). Whereas, *Goniozus s*sp. (Hymenoptera: Bethylidae), can parasitise third and fourth stage instars (Danthanarayana, 1980).

Intraguild predation

Diverse assemblages of predatory arthropods are frequently more effective in reducing the density of herbivore pests than simplistic communities (Denoth et al. 2002; Cardinale et al. 2003). However, it has been postulated that diverse predatory arthropod communities may sometimes inhibit control because of the tendency of predatory arthropods to attack each other through intraguild predation (Cardinale, et al. 2012). However, there have been no empirical research findings to unanimously support this claim. For example, Polis et al. (1989) found that the combined effects of competition and predation often produce concurrent differences within interacting populations. Rosenheim (1998) found that both natural enemies and resources both may play important roles in the regulation of herbivorous arthropods, not just higher order predators in isolation. Wise (2006) concluded that the extent to which cannibalism limits the strength of trophic cascades initiated by spiders remains largely unknown. Lucas and Rosenheim (2011) reported on the impact of the density of extraguild prey and how this may lead to an increase in the density of an intraguild heteropteran predator. These observations underscore the point that the intraguild interactions in natural communities are complex (Karp et al. 2018) and may impact on pest populations in a multitude of ways. It follows that greater measurement of the impact of predator density on herbivores, and their foraging preferences in vineyard systems are needed also.

Insectary plants

Native insectary habitat

The use of non-crop plants as insectary was reported as early as the mid 1960s (van Emden, 1965). It is well documented that predatory arthropods have the capacity to provide ‘regulatory’ services such as biological pest control in vineyards (Nicholls et al. 2000; Williams and Martinson, 2000; Viggiani, 2003; Altieri et al. 2005; Paull, 2007; Thomson and Hoffmann, 2009a; Simpson et al. 2011). Many natural enemies that attack crop pests are native (Gagic et al. 2018), and an increase in predator richness and abundance is reported where there are stands of native vegetation adjacent to cropping areas (Landis et al. 2000b; Landis et al. 2005; Parry et al. 2015). Native perennial ground covers may provide food and habitat, and be more compatible with crop management than exotic annuals (Daane et al. 2018). For example, the longevity of parasitoid wasps increases up to 3.4x when they are exposed to flowering shoots of *Leptospermum* ssp. when compared to buckwheat (Pandey et al. 2018). The presence of insectary resources to nourish predatory arthropods in vineyards may be a promising way to attract and maintain populations of predators. However, there are still many unknowns and of these, the relationships linking non-crop pants, predators, and levels of pest suppression are also particularly complex (Karp et al. 2018).

SNAP

SNAP is an acronym used to describe arthropod 'provisioning' services - shelter, nectar, alternative prey and pollen (Barnes et al. 2010; Gurr et al. 2017). These are essential resources required by predators to survive and thrive (Coombes and Sotherton, 1986; Corbett and Plant, 1993; Eubanks and Denno, 1999; Landis et al. 2000b; van Emden, 2003; Altieri and Nicholls, 2004). Shelter (Gurr et al. 1998; Nentwig et al. 1998), non-host food (Baggen et al. 1999; Wilkinson and Landis, 2005), including nectar (Siekmann et al. 2004; Lavandero et al. 2005; Heil, 2015; Gillespie et al. 2016; Zemenick et al. 2018), pollen (Hickman et al. 1995; Patt et al. 2003; Villenave et al. 2006; Andrade et al. 2018) and alternative prey/hosts (Menalled et al. 1999; Agusti et al. 2003; van Emden, 2003), all to contribute to sustaining populations of predators. While floral resource availability is important, the provision of structural refuges, alternative prey and other attractive qualities may be critical to support particular predatory functional groups (Hogg and Daane, 2015). By focusing on select perennial insectary plants it may be possible to configure plantings to support particular beneficial taxa (Gareau et al. 2013). In addition to the insectary plants included in this study, is estimated that there are more than 21,000 flowering plants that are native to Australia (ANBC, 2015), which may provide additional insectary options in each geographic range. Future research is required to elucidate the potential of a broader suite of native insectary plants to extend the richness and abundance of predatory arthropods in vineyards.

'Shotgun' versus directed approaches

There are two main ways of incorporating insectary plantings in and around production areas.

1. The first is a 'shotgun' approach which involves the planting of a diversity of plant types, or seeds mixes with the assumption that 'diversity is better', and will be beneficial to pest control (Gurr et al. 2005).
2. The second, targeted and more directed approach is preferred, which takes into account optimal forms of diversity (Jervis et al. 2004), floral resources (Berndt et al. 2002; Berndt and Wratten, 2005), and community dynamics within food webs (Polis and Strong, 1996)

By employing a rigorous screening and ranking process to identify which species will best attract predators, it is less likely that populations of key insect pests will be increased (Fiedler and Landis, 2007a, b), and provisioning benefits that are favourable to predators can be achieved.

Introduced insectary species

A small suite of plants has come to dominate the habitat management literature and they are frequently used in areas outside of their native ranges (Fiedler and Landis, 2007b; Fiedler et al. 2008; Shields et al. 2016). For example, New Zealand researchers have focussed on the role of exotic species such as buckwheat, *Fagopyrum esculentum* (Moench) (Caryophyllales: Polygonaceae) (Stephens et al. 1998; Berndt et al. 2000; Berndt et al. 2002), alyssum, *Lobularia maritima* (L.) Desv. (Brassicales: Brassicaceae) (Berndt and Wratten, 2005) and phacelia, *Phacelia tanacetifolia* (Benth.) (Boraginales: Boraginaceae) (Wratten et al. 2003b). These plants have been trialled in Australia with varying degrees of success (Bone et al. 2009; Thomson et al. 2010a; Thomson and Penfold, 2012) even though they are recognised for their provisioning services overseas (Wratten et al. 2003a; Ambrosino et al. 2006; Fiedler and Landis, 2007a). In Australia they are not always easy to establish and maintain. Hence, locally-adapted native plants are preferred as supplementary flora, as they are naturally adapted to Australia’s various climatic conditions (Danne et al. 2010; Pandey et al. 2018).

It has also been reported that the presence and longevity of LBAM may be extended in the presence of buckwheat (Begum et al. 2006) and its fecundity could be enhanced by the availability of nectar plants such as borage, *Borago officinalis* (L.) (Boraginales: Boraginaceae), white clover, *Trifolium repens* (L.) (Fabales: Fabaceae), and brown mustard, *Brassica juncea* (L.) Czern. (Brassicales: Brassicaceae) (Begum, et al. 2006). Therefore, it appears that the use of introduced plant species may be counterproductive in some situations.

Screening and ranking candidate insectary species

Careful screening of candidate insectary plants is required to ensure success. They need to be attractive to predators and be easy to establish and maintain, without actively competing with the crop, or providing habitat for pests. A range of functional attributes is deemed important As a general rule, it is suggested that growers focus their efforts on selecting insectary plants that provide multiple benefits (Fiedler et al. 2008). While it is not the focus of this study, there may be merit in considering Australian native food crops as dual use insectary plants with the potential to provide value added benefits as a cash crop.

Criteria that were used to guide the process of screening and ranking candidate plants (Landis et al. 2000b; Fiedler and Landis, 2007b; Fiedler et al. 2008; Isaacs et al. 2009) are presented below:

1) Plant species that are native to the local area, naturally adapted and suitable for use in and around vineyards, with little or no inputs (irrigation, fertiliser) required post establishment.

    a. Use plants that are commercially available, or seed that is easy to source, collect and/or strike.
    b. Plants that can fill flowering gaps to collectively provide floral services throughout the entire year.

2) A diversity of different locally-adapted native plants, representing different morphologies and height strata.
    a. Flower size - an abundance of smaller flowers is preferred, otherwise bees may deplete the available resources, if only larger flowers are present (Conner and Rush, 1996; Hegland and Totland, 2005).
    b. Flower morphology and accessibility of floral resources - depth and width; some flowers are 'buzz pollinated' and their resources can only be accessed by native bees, or the nectar from long, narrow flowers may only be accessed via species with long mouthparts i.e. butterflies (Orr and Pleasants, 1996; Wackers et al. 1996; Jervis, 1998; Baggen et al. 1999; Houston and Ladd, 2002; Patt et al. 2003; Fenster et al. 2004).
    c. Flower colour may impact on attractiveness to different predators and parasitoids. For example, the parasitoid wasp, *Trichogramma carverae* (Oatman and Pinto) (Hymenoptera: Trichogrammatidae) is reported to associate with white flowers of alyssum to a greater extent than to other colours of the same cultivar (Begum et al. 2004).
    d. Flower phenology and synchronicity throughout the year (Long et al. 1998; Stephens et al. 1998; Rebek et al. 2005; Winkler, 2005).
    e. Plants that prolifically produce pollen and/or nectar (Zhao et al. 1992).

3) Attractiveness to predators (Bugg and Wilson, 1989; Maingay et al. 1991; Patt et al. 1997).
    a. The timing of pollen and nectar production coincides with the needs of predators and parasitoids, especially during spring/summer when biocontrol is critical (Jervis et al. 1993; Colley and Luna, 2000; Siekmann et al. 2001).

4) Plants that do not provide resources for herbivorous pests (Baggen and Gurr, 1998; Ambrosino et al. 2006; Fiedler and Landis, 2007a).

Candidate native insectary plants

Three candidate insectary plants, Christmas bush, *Bursaria spinosa* (Cav.) (Apiales: Pittosporaceae) (Figure 2), prickly tea-tree, *Leptospermum continentale* (Forst. and G.Forst) (Myrtales: Myrtaceae) (Figure 3), and wallaby grasses, *Rytidosperma* ssp. (DC) (Poales: Poaceae) (Figure 4) were selected for assessment based on their attributes and availability (Appendix 1).

Figure 2. Christmas bush, *B. spinosa* exhibits a natural sprawling habit (a), flowers (b), and distinctive seed pods (c). Photos: Mary Retallack

Figure 3. Prickly tea-tree, *L. continentale* during establishment (a), shrubs established as a shelterbelt (b), and the pollen and nectar producing flowers (c). Photos: Mary Retallack

Figure 4. Wallaby grass, *Rytidosperma* ssp. seed head (a), planted as a mix of species in the vineyard mid-row (b), and the underground biomass produced by the roots (c). Photos: Mary Retallack

Location of insectary plantings

The structure and composition of the adjacent landscape will have an influence on the capacity of the habitat to encourage predatory arthropods into production areas (Colunga-Garcia et al. 1997; Thies et al. 2003). Predatory arthropods will respond differently to the size, location and diversity of insectary plantings (Banks, 2000; Tscharntke et al. 2007; Fraser et al. 2008; Werling and Gratton, 2008). The spatial capacity of predators to prey on pest species will be determined by the distance they disperse into the vineyard from insectary plantings and their movement capabilities (Lewis, 1965; Pollard, 1968; Bugg, 1993; Landis, 1994; Roland and Taylor, 1997). Their migration may also depend on visual preferences and plant volatile cues (Suckling et al. 2012c). Local research indicates it may be challenging to encourage certain parasitoid species into the vineyard. Feng et al. (2015b) found *D. tasmanica* parasitised the most *E. postvittana* larvae in vineyards, while *Therophilus unimaculatus* (Turner) (Hymenoptera: Braconidae) was most active in adjacent native vegetation.

The spatial area explored by predators during their lifetime may not be sufficient to ensure their movement into the vineyard. 'Islands' of insectary vegetation may be required within production landscapes (Thomas et al. 1991) to facilitate the movement of individuals among the vines. The vineyard floor provides an example of this utility. The mid-row area covers about two thirds of the vineyard area and provides a suitable area to plant native cover crops and facilitate connectivity between patches (Thomson et al. 2009; Danne et al. 2010; Penfold, 2010; Penfold and McCarthy, 2010). It may also be possible to plant low growing insectary plants species that are suited to the undervine area (Penfold, 2018). These plants must be naturally adapted to a site and have a low requirement for water and ongoing maintenance. This is important so they do not overly compete with grapevines or have a detrimental effect on their vigour.

Spatial movement

Movement between plants enables natural enemies to find floral resources, alternative prey/hosts, and seek refuge from adverse conditions and resource bottlenecks (Schellhorn et al. 2015), which occur when SNAP is less available. Native perennial plants may provide valuable habitat for mobile predators (Letourneau et al. 2012). Some predators are more mobile than others and have the capacity to colonise areas more quickly (Hogg and Daane, 2018). It is reported that ground beetles move up to 200 m from boundary plantings into adjacent crops, minute pirate bugs and predatory thrips can disperse up to 36 m (Nicholls et al. 2001; Irvin et al. 2018), and parasitoids up to 80 m from buckwheat refuges (Lavandero et al. 2005). Spiderlings are well known for their capacity to passively colonise new areas via aerial dispersal techniques including 'ballooning' which involves moving through the air on silken threads over large distances (Greenstone, 1990; Kevan and Greco, 2001; Venturino et al. 2006; Simonneau et al. 2016). The direction of travel either along or across rows will also be of interest, as this will provide insights to the best location of an insectary. An outstanding issue is the uncertainty that all predators will readily move between native vegetation and vineyards. To build on my research, further work is required to quantify the movement of predators from insectary plants into the vineyard.

Associations between predators and insectary plants

One of the key areas of focus must be to determine when predators are present in the vineyard in relation to abundance of pest species. However there is little information available describing the key relationships between predators in the vineyard and native insectary resources in Australia. Wood et al. (2011) found that brown lacewings most likely breed on native wallaby grass, *Rytidosperma bipartitum* (Link) A.M. Humphreys and H.P.Linder (Poales: Poaceae) (previously *Austrodanthonia linkii*), and perhaps other grasses. This association may be important for manipulating populations of brown lacewing, *Micromus tasmaniae* (Rambur) (Neuroptera: Hemerobiidae) at the property scale. The benefits of planting wallaby grasses are also supported by Thomson and Hoffmann (2009a) who found direct evidence of the effects of the native cover crops in enhancing predators, as predation of LBAM eggs increased when *Rytidosperma* ssp. and windmill grass, *Chloris truncata* (R.Br.) (Poales: Poaceae) were present. Danne et al. (2010) found predation levels of sentinel eggs of *E. postvittana*, were increased in native cover crops, which included species of *Rytidosperma* ssp., *C. truncata* and two species of saltbush, berry saltbush, *Atriplex semibaccata* (R.Br.) (Caryophyllales: Amaranthaceae) and sprawling saltbush, *A. suberecta* (I.Verd.) compared with introduced oats, *Avena sativa* (L.) (Poales: Poaceae). Similarly, wolf spiders are nocturnal, ground dwelling hunters whose presence is enhanced by grassy understorey, adjacent pasture and woody vegetation (Tsitsilas et al. 2006; Thomson and Hoffmann, 2009b; D'Alberto et al. 2012). Coccinellid ladybird beetles, which are predators of mealybugs, whiteflies, psyllids, scale, aphids (Hodek and Honek, 2009), lepidopteran (moth) and coleopteran (weevil) immatures (Weber and Lundgren, 2009) and possibly grape phylloxera (Kogel et al. 2013), benefit from nectar and pollen resources (Landis et al. 2000b). Thomson and Hoffmann (2006b) found the distribution of spiders, predatory mites, predatory and parasitic flies and parasitoids within a vineyard were positively influenced by native vegetation at the margins. Paull (2007) also discovered a novel interaction between the predatory mite, *Anystis baccarum* (L.) (Acari: Anystidae), an abundant predator in the vine canopy, the parasitoid *D. tasmanica*, and larval *E. postvittana*. The activity of *D. tasmanica* rendered larval LBAM more susceptible to attack by *A. baccarum*.

Multi-species interactions

Plant diversification promotes diverse arthropod communities that may provide greater stability of ecosystem provisioning (Lichtenberg et al. 2017). A integrated approach to pest control is needed that embraces a range of predatory arthropods that are either present at the same time, and/or succeed one another (Waterhouse and Sands, 2001). The capacity of multiple species to provide pest control is enhanced by their capacity to attack different life stages of the pest (Holt and Lawton, 1994; Losey and Denno, 1999; Cardinale et al. 2003). These predators may be supported by multiple insectary resources of different strata, located throughout the production landscape. It is also reported that the populations of predators may be more abundant in six year old than one year old insectary plantings (Denys and Tscharntke, 2002). This emphasizes the importance of habitat age for natural enemies and possible biological control. Multi-species interactions will occur between predator and prey. Further work is required to explore the relationships of different functional arthropod groups in production landscapes, as well as the dynamics of host and prey densities at different times of the year, and the dynamics of multi-herbivore species presence.

Seasonal synchrony and overwintering

The seasonality of ecosystem services can be extended by planting a range of suitable native perennial plants that provide floral resources at key times. This helps to ensure habitat permanency and synchrony of provisioning services is continual throughout the year (Losey and Denno, 1999). An understanding of the overwintering requirements of predators may be critical to ensuring that their population base is sufficiently large at the start of the following season (Sotherton, 1984; Thomas et al. 1991; Nicholls et al. 2001; Horton and Lewis, 2003; Schmidt et al. 2005; Stephens et al. 2006; Lorenzon et al. 2015). Similarly, access to suitable floral resources and alternative prey may help to sustain predatory populations throughout the period of grape vine dormancy (Schellhorn et al. 2015).

Manipulating the structure and habit of insectary plantings

It may be possible to manipulate the flowering time, structure and habit of insectary plants. For example, mowing of grass swards can be used to manipulate the timing of flowering and the provision of pollen for predators such as predatory mites (Smith and Papacek, 1991). Mowing of alternative rows can be used to provide habitat and shelter for predators, including spiders that live and reproduce in long grass (Bernard et al. 2006a; Wood et al. 2011). Alternatively, grasses can be slashed to a minimum height of 10 cm to preserve habitat. It may be possible to prune or hedge woody plant species to induce a density of flower clusters or encourage a compact habit. Some species may also provide concurrent flowering over several months. More work is needed on the capacity to manipulate insectary plants to engineer structure and inflorescence production at times that are of benefit to production landscapes.

Drawbacks of using native plants as insectary

There are a number of potential drawbacks of using native perennial plants. For example, the time taken to establish woody plants may be several years. Floral provisions and shelter may be low compared to annuals until perennial plants are well established (Isaacs et al. 2009). It may be difficult to source seeds locally, or native seed of local provenance in commercial quantities, and the seed may be expensive. Seed may have low germination and viability and should be tested if sourced from a reseller. However, the initial costs can be amortised over the life of the planting and they may provide multiple ecosystem benefits.

Minimising disruption in the vineyard

Optimal biological control in vineyards is achieved by minimising the use of broad-spectrum insecticides that can also kill predators (Bernard et al. 2007). The use of non-selective pesticides should be eliminated if insectary habitat is to be established nearby (Winkler, 2005). The overuse of pesticides may result in a range of unintended consequences including the development of resistance in some arthropod pests (Whalon et al. 2008). Ideally pest control is achieved using biological control, with the targeted application of selective insecticides used to reduce pest populations to below damaging levels, only if they are required. Agricultural systems are typically difficult environments for predatory arthropods to thrive because of the high level of disruption. Greater stability of arthropod populations (van Emden and Williams, 1974; Landis et al. 2005) is likely in vineyards where tillage and chemical inputs are minimised (McLaughlin and Mineau, 1995; Nash et al. 2008) and a greater diversity and complexity of insectary plants is promoted.

Assumptions about native insectary

Based on the information above, I suggest the following hypotheses regarding the interactions of arthropods with native insectary plants.

1. Predators will naturally occur in remnant vegetation and vineyards in different abundances and diversities.
2. Natural enemies will benefit from the provision of insectary plantings.
3. Native plant species will vary in their capacity to offer provisioning services to different predatory arthropods.
4. Insectary plantings will attract different natural enemies at different times of the year, and this will depend on their capacity to provide the required provisioning services.
5. The strategic use of native insectary plantings, both spatially and temporally is important to deliver these services when they are needed.
6. Natural enemy abundance will decline with greater distance away from insectary plantings.
7. The capacity of insectary plants to provide provisioning services will increase as they reach maturity.
8. The capacity of natural enemies to control LBAM and other vineyard pests will differ, and will be dependent on host and prey densities.
9. Multi-species interactions will occur between natural enemies and prey species.
10. The biological control provided by generalist predators will differ depending on the resources available, vineyard management practices employed and the seasonal conditions experienced.

References for the literature review are presented at the end of Chapter 7.

CHAPTER THREE

Which species of Tortricidae leafrollers are key insect pests in South Australian vineyards?

Statement of authorship

Which species of Tortricidae leafrollers are key insect pests in South Australian vineyards?

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Published as:

Retallack, M. J., Mackay, D., Thomson, L. J., and Keller, M. A. (2018). Which species of Tortricidae leafrollers are key insect pests in South Australian vineyards? *Transactions of the Royal Society of South Australia* 142, 130-142. https://doi.org/10.1080/03721426.2018.1510656

MR collected samples, conducted molecular laboratory analysis, interpreted data and wrote the manuscript. DM, LT and MK contributed to the manuscript production. MK helped in developing the idea, provided guidance throughout and assisted with statistical analysis. All authors have contributed significantly and agree with the manuscript.

Data availability

The datasets generated and analysed during the current study are available in the GenBank repository (Accession Numbers: MG851725 - MG851793).

Disclosure statement

The authors declare there are no conflicts of interest.

Abstract

Light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae) is regarded as the key insect pest in Australian vineyards and it is also an important pest of apples and citrus. *Epiphyas postvittana* is indigenous to Australia and has a wide geographical distribution. Recent observations suggest that leafroller species other than *E. postvittana* may be causing damage in grapevine canopies. A study of tortricids was undertaken in Adelaide Hills and McLaren Vale vineyards, South Australia. Four hundred and seven specimens of Tortricidae were collected from grapevine canopies. Molecular techniques were used to identify species. The mean prevalence of *E. postvittana* per sample was 91.0% in 2014/15 and 96.2% in 2015/16. Larval *Acropolitis rudisana*, lucerne leafroller, *Merophyas divulsana* and cotton tipworm, *Crocidosema plebejana* were also found in the grapevine canopy at much lower densities for the first time. The presence of leafroller species *A. rudisana*, *M. divulsana* and *C. plebejana* on grapevines, confirms these species of Tortricidae may also be present in South Australian vineyards. This study confirms that *E. postvittana* is the most common tortricid pest in Adelaide Hills and McLaren Vale vineyards and also illustrates the utility of molecular methods in determining with confidence the species identity of larval Tortricidae.

Key words

Acropolitis rudisana, *Crocidosema plebejana*, *Epiphyas postvittana*, light brown apple moth, *Merophyas divulsana*, tortricid, vineyard

Key messages

- Light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae) is regarded as the key insect pest in Australian vineyards.
- However, larvae of Tortricidae have no defining morphological features and molecular methods are required to determine with confidence the species identity of larval Tortricidae.
- A study of tortricids was undertaken in South Australian vineyards.
- In addition to *E. postvittana*, larval tortricids *Acropolitis rudisana*, *Merophyas divulsana* and *Crocidosema plebejana* were also found on the grapevine canopy at much lower densities for the first time.

Introduction

Light brown apple moth (LBAM), *Epiphyas postvittana* (Lepidoptera: Tortricidae) is the key insect pest that causes economic damage in Australian vineyards and it is also an important pest of apples and citrus (Johnston, 1963; Mo et al. 2006). *Epiphyas postvittana* is indigenous to Australia and has a wide geographical distribution including New Zealand, USA, UK, Ireland and parts of Europe (Suckling and Brockerhoff, 2010). Larval *E. postvittana* damage leaves, flower clusters and berry skins. Damaged skins provide infection sites for *Botrytis cinerea* and other bunch moulds, which result in a reduction in fruit quality and yield losses (Ferguson, 1995). Bunch rots can be caused by filamentous fungi, yeast and bacteria (Steel et al. 2013). Annual national losses from *E. postvittana* and related bunch rots, were estimated to be $70 million per year in Australia (Scholefield and Morison, 2010).

Recent observations by Feng et al. (2016) suggest that species of Tortricidae other than *E. postvittana* may be present in Australian vineyards. They found *Acropolitis rudisana* present in woody habitats adjacent to vineyards and *Merophyas divulsana* present on cover plants in vineyards.

Given the cryptic nature of the larvae of Tortricidae having no defining morphological features; this raises the question, are other species of previously unnoticed tortricids present in grapevine canopies? If so, what is the likely impact on wine producing grapevines and/or existing integrated pest management (IPM) practices?

The role of Tortricidae has not been fully elucidated in Australian vineyards. Better species identification will provide a better understanding of tortricid activity and an improved understanding of the horticultural risk posed by each species to ensure effective IPM control strategies for all species present (Bernard et al. 2007).

Lepidoptera: Tortricidae

Tortricidae is a diverse family of moths, which includes more than 10,000 described species worldwide (Gilligan et al. 2014) and at least 249 named species in Australia (Horak, 2006). Larval Tortricidae are called leafrollers because they commonly build protective feeding shelters, by folding leaves over their bodies and use webbing to secure these structures. Tortricidae have a wide host range of woody and herbaceous plants (Brown et al. 2010). The larvae of Tortricidae have a similar appearance, which makes it impossible to identify species without a microscope or other laboratory technique such as DNA analysis (Barr et al. 2011; Feng et al. 2016).

E. postvittana

Epiphyas postvittana is an Australian native leafroller which was first described in 1863 (Geier and Briese, 1981) (Figure 1a). It is a damaging pest of grapevines in Australia (Buchanan et al. 1991; Glenn and Hoffmann, 1997). It has been recorded from more than 500 plant species in 121 families and 363 genera (Brown et al. 2010), including a range of broad leaved weeds often found in vineyards, such as capeweed, *Arctotheca calendula* and plantain, *Plantago lanceolata*.

The larva passes through six instars (Danthanarayana, 1975) and grows up to 20 mm in length. In the field, early instars of *E. postvittana* selectively feed on the undersides of grapevines leaves within a silk refuge. They are often found in the developing leaves at the apical meristem. Older larvae can be found on older leaves, or within the developing inflorescences, or bunches of grapes (Brown et al. 2010). *Epiphyas postvittana* typically completes three to four generations annually in Australia (Magarey et al. 1994).

A. rudisana

Acropolitis rudisana (Lepidoptera: Tortricidae) is a native leafroller and is widespread in eastern Australia (Figure 1b). Hosts of *A. rudisana* include weed species often found in Australian vineyards such as clover, *Trifolium sp*., capeweed, *A. calendula*, and grapevines, *Vitis sp*., but not specifically *Vitis vinifera* L. (Brown et al. 2008). There is a scarcity of published information about the biology of *A. rudisana*.

M. divulsana

The lucerne leafroller, *M. divulsana* (Lepidoptera: Tortricidae), is a significant pest of cultivated lucerne, *Medicago sativa* (Allsopp et al. 1983b; Whittle et al. 1991b), and is a native Australian species (Figure 1c). Hosts of *M. divulsana* include weed species often found in Australian vineyards such as plantain, *Plantago sp*., clover, *Trifolium sp*., and capeweed, *A. calendula* (Brown et al. 2008). Little is known about the presence of *M. divulsana* in perennial horticultural crops and *V. vinifera* has not previously been regarded as a host species. When field conditions are conducive, successive discrete generations of *M. divulsana* occur during summer and autumn approximately five weeks apart (Whittle et al. 1991b).

Crocidosema plebejana

The cotton tipworm, *Crocidosema plebejana* (Lepidoptera: Tortricidae) is an introduced pest of cotton in Australia (Bishop and Blood, 1978) (Figure 1d). Outbreaks are associated with the growth of its main host marshmallow, *Malva parviflora* (Hamilton and Zalucki, 1993; Williams et al. 2011) which is often found in vineyards. *Crocidosema plebejana* has not been found previously on *V. vinifera*.

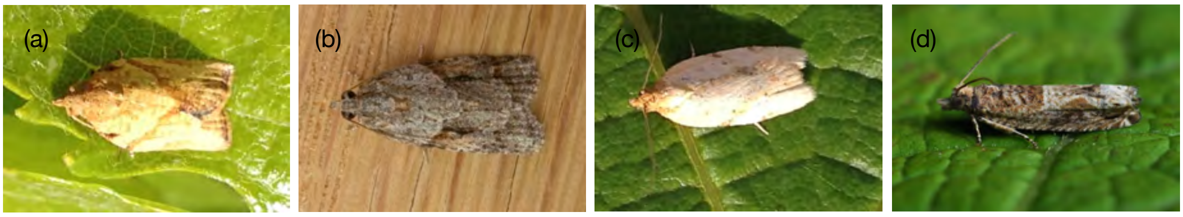

Figure 5. light brown apple moth, *E. postvittana* (a), *A. rudisana* (b), lucerne leafroller, *M. divulsana* (c), cotton tipworm, *C. plebejana* (d).

Images 1(a) and 1(c) by Mary Retallack. Image 1(b) *Acropolitis rudisana* by Hobern (2008) is licensed under the Creative Commons Attribution 2.0 Generic license. Image 1(d) by uncredited at http://revtangen.blogspot.com.au/2016/09/

Aims

We sought to ask which tortricids are present in South Australian vineyards, and does the diversity of tortricids vary significantly among vineyards? If species of tortricids other than *E. postvittana* are present and have different behavioural characteristics, then this may change the management approaches adopted for leafroller control both in vineyards and other horticultural crops such as apples and citrus. To answer these questions, we used molecular methods to determine the species of Tortricidae present on the canopies of *V. vinifera* in Adelaide Hills and McLaren Vale vineyards in the 2014/15 and 2015/16 growing seasons.

Materials and methods

Arthropod collection in the field

Lepidopteran larvae were collected from grapevine canopies during periods of peak activity from mid- to late-October until mid-December, over two successive seasons. Samples were collected weekly from 30 October 2014 to 11 December 2014 (season 2014/15) and from 16 October 2015 to 9 December 2015 (season 2015/16).

A total of 18 sample sites were assessed during 2014/15 and 2015/16. Larval samples were collected from seven vineyards in the Adelaide Hills near Mount Torrens (Site 1: 34°53'38.23"S 138°55'55.45"E), Mount Barker (Site 2: 35°4'11.46"S 138°54'15.18"E, Site 3: 35°4'13.50"S 138°54'14.68"E), Nairne (Site 9: 35°3'9.55"S 138°54'48.54"E), Lenswood (Site 10: 34°53'31.56"S 138°50'5.01"E), Ashton (Site 17: 34°56'54.93"S 138°43'45.70"E), The Range (Site 18: 35°14'34.34"S 138°38'29.03"E); and eleven in the McLaren Vale wine region near McLaren Vale (Site 4: 35°11'18.58"S 138°31'0.72"E, Site 5: 35°11'18.30"S 138°31'4.38"E, Site 6: 35°11'25.21"S 138°30'54.28"E, Site 8: 35°12'28.58"S 138°32'47.70"E, Site 11: 35°12'31.80"S 138°31'47.20"E, Site 13: 35°17'9.54"S 138°31'20.94"E, Site 14: 35°17'12.54"S 138°31'21.25"E), McLaren Flat (Site 7: 35°11'48.23"S 138°34'21.87"E, Site

12: 35°13'9.85"S 138°33'27.03"E), Chapel Hill (Site 15: 35°10'18.23"S 138°33'0.98"E, Site 16: 35°10'6.38"S 138°33'58.33"E), where tortricids were reported to be present by local vignerons and via CropWatch bulletins (Hamilton, 2014). Typically, two to four pairs of rows were assessed per site. These vineyards grew a range of varieties including Chardonnay (Sites 2 and 10), Viognier (Site 9), Pinot Noir (Sites 1 and 17), Grenache (Site 7), Shiraz (Sites 3, 5, 8, 11, 12, 14, 15, 16 and 18), Cabernet Franc (Site 4), Sangiovese (Site 6), and Mataro (Site 13). The sampling techniques used did not lend themselves to making extensive comparisons between the sample sites (see map in the Addendum to Chapter 3).

Season 2014/15

A random sampling technique was used in 2014/15. Each sub-sample was collected by firmly striking the grapevine cordon five times with a rubber mallet, over a beat net measuring 700 mm x 700 mm that held a 250 ml collection container. This process was repeated five times for each composite sample, alternating between each side of a pair of vine rows. A total of ten composite samples (replicates) were collected from each vineyard per sampling date. Arthropods were killed in the field using ethyl acetate vapour. The larvae of Tortricidae were removed and placed in 95% ethyl alcohol (EtOH) and stored in a refrigerator at 4 °C. At the end of the season, larval samples were stored at -80 °C prior to processing in April 2015. Sixty-four specimens comprising six pupae, one moth and 57 larvae were collected in season 2014/15.

Season 2015/16

Due to the low number of larval samples collected in 2014/15 as a result of the method employed, a targeted sampling technique was used in season 2015/16 to ensure maximal capture. Grapevine shoots were systematically scanned over a 30 minute period to find larvae, which were deposited in a 10 ml tube containing 95% EtOH in the field. Samples were stored in a refrigerator at 4 °C, prior to PCR based analysis of DNA gene barcodes in January 2016. Three hundred and sixty-nine larvae were collected in season 2015/16.

Molecular analysis

DNA extraction

The DNA extraction protocol followed the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The samples were placed in tubes, macerated using individual grinding sticks and left to incubate for two hours at 56 °C following the manufacturer's protocol. The concentrations of DNA samples were estimated using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Extracted DNA was stored at -20 °C.

PCR protocol and Sanger sequencing

The specimens were characterised through amplification of the mitochondrial cytochrome oxidase 1 (MT-CO1) gene using a PCR-based protocol to determine each species. The selected larvae were PCR amplified and sequenced in both directions for the barcode region of CO1 using the universal primer pairs LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') which targeted the full-length 658 bp DNA barcode fragment (Hajibabaei et al. 2006; Rougerie et al. 2011). PCR was carried out in a 50 µL reaction volume, containing 5 µL of 10X PCR buffer minus Mg, 1.5 µL 50 mM $MgCl_2$, 1 µL of primer mix (10 µM each), 1 µL of 10 mM dNTP mixture, 2 µL of template DNA, 0.2 µL of Taq DNA polymerase (Platinum^{TM} Taq DNA polymerase; Invitrogen^{TM}) and 38.3 µL nuclease-free water up to 50 µL volume.

When sequence results were inconclusive or the sample of Tortricidae had been parasitised by a braconid wasp, *Dolichogenidea* ssp. in the field prior to collection and its DNA dominated the sequence, then Lepidoptera-specific primers LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') and MH-MR1 (5'-CCTGTTCCAGCTCCATTTTC-3') were used to sequence the partial DNA barcode fragment of 311 bp (Hajibabaei et al. 2006; Rougerie et al. 2011) to confirm the species. The thermal profile used for both barcoding reactions consisted of an initial denaturing step of 1 min at 94 °C, followed by five cycles of 40 s at 94 °C, 40 s at 45 °C and 1 min at 72 °C, followed by 35 cycles of 40 s at 94 °C, 40 s at 51 °C and 1 min at 72 °C, with a final extension step at 72 °C for 5 min (Hajibabaei et al. 2006; Rougerie et al. 2011). The PCR products were run in 2% agarose (LE Analytical grade, Promega) via electrophoresis at 120 volts for 30 min to check for single amplicons of the expected size and visualised in UV light. Samples of unpurified PCR product showing strong bands were sent to the Australian Genomic Research Facility (AGRF), Adelaide, South Australia for Sanger sequencing. Dual-direction sequencing using the LepF and LepR (or subsequently LepF and MH-MR1) primers was carried out in 20 µL reaction volumes.

Data analysis

CO1 DNA sequences were obtained from the PCR amplicons. The quality of the forward and reverse sequences was confirmed by the number of Q20 bases detected. These sequences were trimmed and aligned using the program Geneious® then matched with partial CO1 sequences in the GenBank public database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) via a BLAST search. Key GenBank accession numbers used to confirm the identity of each species of tortricid, included HM346472.1 (*E. postvittana*), KF402639.1 (*A. rudisana*), KF153775.1 (*M. divulsana*) and KC315445.1 (*C. plebejana*). The corresponding GenBank accession numbers for isolates of Tortricidae generated in this study are MG851725 - MG851793.

At one site *A. rudisana* was apparently found more frequently than at the other sites. The Fisher Exact Test (http://www.quantitativeskills.com/sisa/statistics/fisher.htm) was used to test if this was an exceptionally high incidence (= occurrence in a sample). The incidence frequency at this site and at all other sites were cast in a contingency table. The probability for this table and all others more extreme were calculated and the sum indicated the overall probability of this observation.

Results and discussion

Prevalence of *E. postvittana*

Epiphyas postvittana was consistently the dominant species of Tortricidae found in Adelaide Hills and McLaren Vale vineyards. A total of 433 larval Lepidoptera was collected from grapevine canopies and identified using PCR based analysis of DNA gene barcodes. Of these, 407 were larval Tortricidae (*n* = 43 in 2014/15 and *n* = 364 in 2015/16). The difference in the number of larvae collected was a result of the two different collection methods (random versus targeted). The mean prevalence of *E. postvittana* per sample of moth larvae was 91.0% in season 2014/15 and 96.2% in season 2015/16 (Table 1). These results confirm *E. postvittana* (*n* = 389 specimens) is the most common tortricid pest in Adelaide Hills and McLaren Vale vineyards. *Acropolitis rudisana* (*n* = 16 specimens), *M. divulsana* (*n* = 1 specimen) and *C. plebejana* (*n* = 1 specimen) larvae were found for the first time in a grapevine canopy, but at much lower densities. Other species of Lepidoptera found included apple looper, *Phrissogonus laticostata* (Lepidoptera: Geometridae) (*n* = 18), native budworm, *Helicoverpa punctigera* (Lepidoptera: Noctuidae) (*n* = 2), diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae (*n* = 1), and five undetermined specimens which could not be identified due to the low number of Q20 bases detected.

Prevalence of *A. rudisana* at key vineyard sites

Of the 15 larval *A. rudisana* collected in season 2015/16 in Adelaide Hills and McLaren Vale vineyards, nine specimens (60%) were collected from the #16 vineyard site, which is located adjacent to a large area of remnant bushland. Furthermore, of the 25 unique visits to sample sites, *A. rudisana* was present at the #16 vineyard 100% of the time, versus 23% for the remaining pooled data of the sites sampled. This indicates that *A. rudisana* was arguably more likely to be found at the #16 vineyard site (*P* = 0.055, Fisher's Exact test). Other vineyards in South Australia may also have *A. rudisana* present and this may warrant investigation on a vineyard-by-vineyard basis.

Table 1. Mean prevalence of Tortricidae per sample in 2014/15 and 2015/16

Season	***n***		***Epiphyas postvittana***	***Acropolitis rudisana***	***Merophyas divulsana***	***Crocidosema plebejana***
	(Unique visits to sample sites)	**(Tortricid specimens)**	**Mean (± 95% CI)**	**Mean (± 95% CI)**	**Mean (± 95% CI)**	**Mean (± 95% CI)**
2014/15	13	43	91.0% (74.2 to 100%)	7.7% (-9.1 to 24.5%)	0% (NA)	1.3% (-1.5 to 4.2)
2015/16	25	364	96.2% (93.5 to 99.0%)	3.7% (1.0 to 6.5%)	0.04% (0.0 to 0.1%)	0% (NA)

This is the first time a complex of leafroller larvae present on grapevine canopies have been characterised using molecular biological techniques. The presence of *E. postvittana* has been well documented in vineyards. However, the presence of *A. rudisana, M. divulsana* and *C. plebejana* on *V. vinifera* canopies has not been described previously.

Pheromones for mating disruption

Pheromone infused twist ties have been used successfully in large scale mating disruption trials in south-eastern Australia (Mo et al. 2006). Pheromone traps have also been used successfully to survey the distribution of *E. postvittana* in California (Brown et al. 2010) and control *M. divulsana* in lucerne crops in Australia (Whittle et al. 1991b; Bishop, 1993). However, they are not currently, widely employed by vignerons in Australia.

New specialised pheromone and lure application technology provides an alternative to existing pheromone application. This technology provides a similar efficacy to disrupt the mating of light brown apple moth when compared to pheromone infused twist ties, while streamlining the application of pheromones, via manual or mechanical application (Suckling et al. 2012a; Suckling et al. 2012b). Growers may wish to try this ‘next generation’ pheromone application method in the future. However, the use of synthetic pheromones is highly target specific (Brockerhoff et al. 2012), and the effectiveness of mating disruption will fail if non-target species of Tortricidae are present.

Similarly, if pheromone traps specific to *E. postvittana* are used to indicate leafroller activity, other species of Tortricidae such as *A. rudisana*, *M. divulsana* and *C. plebejana* will not be detected. This emphasises the importance of knowing the species of Lepidoptera present prior to implementing an IPM plan.

Overwintering moth larvae

Australian vineyard managers often scout broadleaf weeds in the mid-row for the presence of moth larvae, to provide an indication of leafroller activity early in the growing season (Brockerhoff et al. 2011). Given the impossibility of identifying larval tortricids in the field to species, if the larvae are all assumed to be *E. postvittana* the abundance of damaging species of tortricids may be overestimated, leading to unnecessary waste of time and resources.

Alternative prey for predator arthropods

Merophyas divulsana is found on mid-row cover plants in the vineyard (Feng et al. 2016), but has not been previously described on grapevine canopies. A single *M. divulsana* and *C. plebejana* larva was each found in a grapevine canopy over the two sampling seasons, suggesting that it is unlikely *M. divulsana* or *C. plebejana* frequently migrates into the grapevine canopy. This may be due to *M. divulsana* and *C. plebejana* not preferring the physical cues or the foliar chemistry of grapevines (Rizvi and Raman, 2016). If *M. divulsana* or *C. plebejana* is present on grapevines, then it is likely to be in very low abundance and of insignificant impact and risk.

However, larval *M. divulsana* and *C. plebejana* may provide a source of alternative prey or hosts, to boost the presence of predators and parasitoids of *E. postvittana* when insectary food (nectar and pollen) sources are low early in the growing season (Hassell and May, 1986; Gurr et al. 2004; Barnes et al. 2010). This decoupling of reliance on early-flowering insectary plants, potentially allows predators of *E. postvittana* to colonise and provide natural biological control in vineyards more quickly. Similarly, larval *A. rudisana*, *M. divulsana* and *C. plebejana* provide diversified host options for parasitoids of *E. postvittana*, such as *Dolichogenidea tasmanica*, *Therophilus unimaculatus* and the commercially available *Trichogramma carverae* in vineyards (Yazdani et al. 2015; Feng et al. 2016).

Biosecurity

This research provides a benchmark for four species of Tortricidae and provides a possible methodology for avoiding the challenge of identifying species of Lepidoptera from immature life stages correctly in the field, if species are represented in reference databases. These findings also reinforce the need for robust molecular based protocols for the rapid identification of exotic pests, to enable the deployment of early intervention management options following a pest incursion.

Critically, accurate identifications would enable a thorough understanding of a pests' host preferences and distribution which amongst other things is needed to determine the capacity of an introduced pest species to displace current species populations.

If there was an incursion of omnivorous leafroller, *Platynota stultana*, European grapevine moth, *Lobesia botrana*, or American berry moth, *Polychrosis viteana* into Australia, it is conceivable that they could invade and remain undetected in vineyards for a prolonged period, as has been the case with identifying the presence of *A. rudisana*, *M. divulsana* and *C. plebejana* on *V. vinifera*.

Conclusion

This research has demonstrated that light brown apple moth, *E. postvittana* is a key tortricid pest of South Australian vineyards. Low densities of *A. rudisana*, *M. divulsana* and *C. plebejana* have been found on the canopies of *V. vinifera* for the first time. As they are closely related to *E. postvittana*, it is anticipated *A. rudisana, M. divulsana* and *C. plebejana* can be managed through existing IPM strategies.

Acropolitis rudisana, M. divulsana and *C. plebejana* may also provide a valuable source of alternative hosts for parasitoids and alternative prey for predators, when they are located in vineyard mid-rows. This is especially important during the winter period and early in the growing season, when alternative prey is needed to boost the presence of key predators of *E. postvittana*, so they can provide natural biological control before light brown apple moth populations reach damaging levels in grapevine canopies. This study highlights the importance of using molecular methods to determine the species of Tortricidae at the larval stage with confidence.

The role of Tortricidae should be elucidated in Australian vineyards. First, the level of damage that *A. rudisana*, *M. divulsana* and *C. plebejana* can make should be studied. Then, if these species aren't important economically, they might be used as an alternative host for *D. tasmanica* the key parasitoid of *E. postvittana*. *Trichogramma carverae* is available commercially as a biological control option for *E. postvittana*. It is not known if they will also parasitise *A. rudisana*, *M. divulsana* and *C. plebejana*.

Acknowledgements

This research was made possible through scholarships to Mary Retallack awarded by the Australian Government Research Training Program Stipend, The University of Adelaide C. J. Everard Supplementary Scholarship, SARDI Women's Suffrage Centenary Bursary, Grape and Wine Research and Development Corporation PhD Supplementary Scholarship [GWR Ph1209]. Project operating support was provided via the Grape and Wine Research and Development Corporation and via the Adelaide Hills Wine Region Post Graduate Biodiversity Study Grant. We thank managers who allowed us to collect data in their vineyards: Darren Arney, John Good, Anton Groffen, Matt Hatwell, Prue Henschke, Janet and Erinn Klein, Craig Markby, David Paxton, Edd Perkins, and Fiona Wood. We thank Dr. Kelly Hill, and Dr. Fung Yi who assisted with molecular methods in the laboratory.

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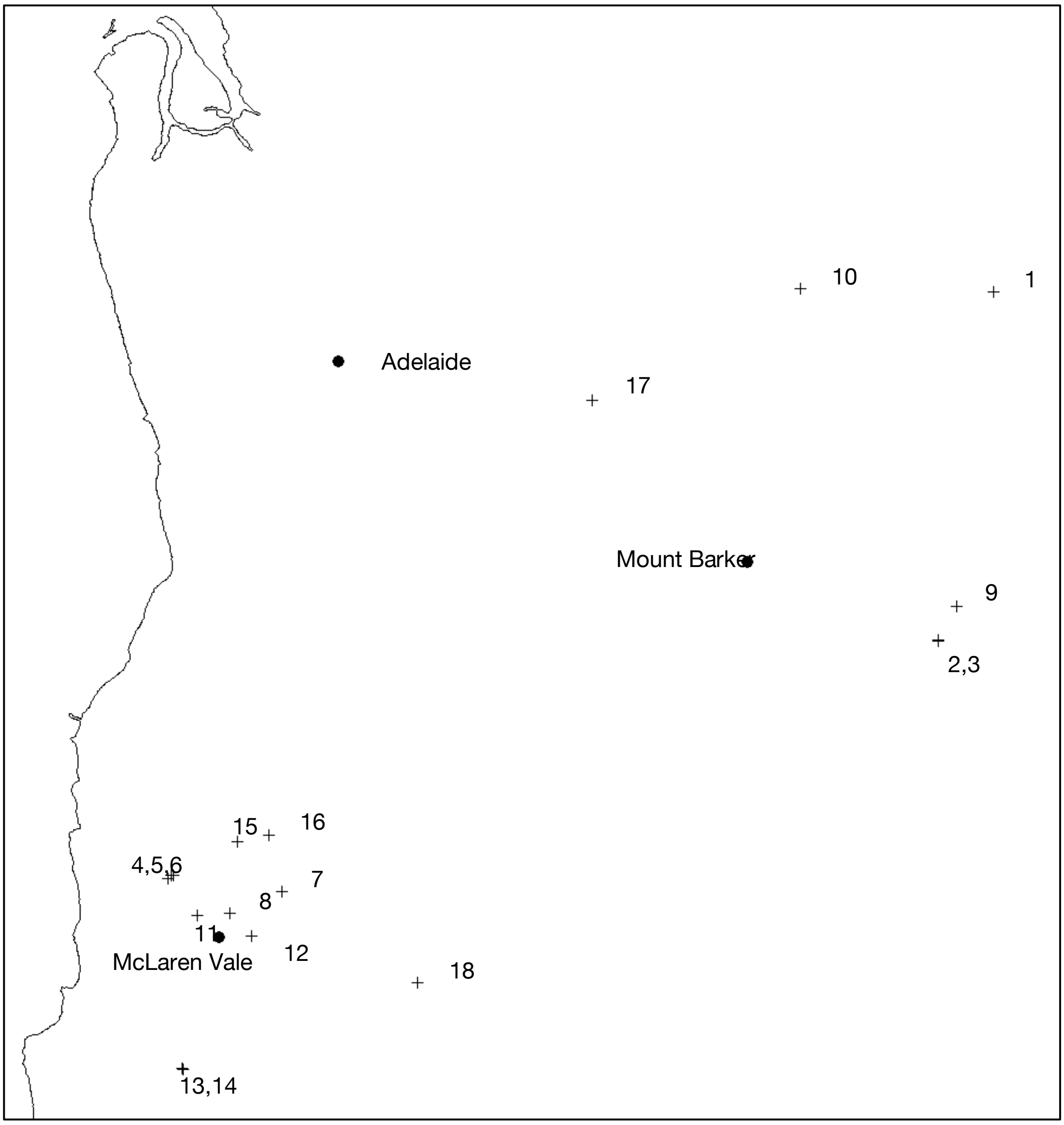
Addendum to Chapter 3

A map of the study sites is presented here.



Addendum Figure. Map showing locations of 18 sites where tortricids were sampled from grapevines in vineyards (+). Major towns (●) are shown for reference.

CHAPTER FOUR

Predatory arthropods associated with potential native insectary plants for Australian vineyards

Statement of authorship

Predatory arthropods associated with potential native insectary plants for Australian vineyards

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Published as:

Retallack, M.J., Thomson, L.J, and Keller, M.A. (2019). Predatory arthropods associated with potential native insectary plants for Australian vineyards. *Australian Journal of Grape and Wine Research,* doi: 10.1111/ajgw.12383.

MR collected samples, identified arthropods, interpreted data and wrote the manuscript. LT and MK contributed to the manuscript production. MK helped in developing the idea, provided guidance throughout and assisted with statistical analysis. All authors have contributed significantly and agree with the manuscript.

Data availability

The datasets generated and analysed during the study along with supporting documentation are available in The University of Adelaide Figshare online digital repository (DOI 10.4225/55/5b0a3a2e6b2e9, 10.4225/55/5b0a3a5d6f519, 10.25909/5bda753f80027, 10.25909/5bda767197447).

Disclosure statement

The authors declare there are no conflicts of interest.

Abstract

Background and Aims: We evaluated three native plants to determine their capacity to provide insectary benefits to predatory arthropods in vineyards, and thereby to enhance biological control of insect pests. Native plants are preferred as supplementary flora, as they are naturally adapted to Australia's climatic conditions.

Methods and Results: Stands of mature *Bursaria spinosa*, *Leptospermum continentale* and *Rytidosperma* ssp. located adjacent to or in the mid-rows of Adelaide Hills, Barossa Valley and Eden Valley vineyards were sampled for arthropods in 2013/14. *Vitis vinifera* was also sampled. Twenty seven thousand and ninety-one individual invertebrate specimens were collected, comprising 20 orders and 287 morphospecies. Eight thousand, eight hundred and eighty predators, 6,790 herbivores and 11,421 other specimens were collected. Predatory arthropods dominated the diversity of morphospecies present on each plant. Out of a total of 98 predatory morphospecies, 67 were found on *B. spinosa,* 63 on *L. continentale*, 56 on *V. vinifera* and 38 in association with *Rytidosperma* ssp*.* The difference between predatory and herbivore morphospecies was highest on *Rytidosperma* ssp. (2:1 predators: herbivores), followed by *L. continentale*, *V. vinifera* and *B. spinosa*.

Conclusion: *Bursaria spinosa* and *L. continentale* have the potential to be used as insectary plants in association with Australian vineyards, as they support populations of predatory arthropods throughout the year. *Rytidosperma* ssp. could provide insectary benefits on sites where the black Portuguese millipede, *Ommatoiulus moreleti* is not considered to be a problem.

Significance of the Study: This study confirms associations between predatory arthropods and three native plants. The opportunity to plant selected native insectary species could help wine grape growers save time and resources by producing fruit with lower pest incidence, while enhancing biodiversity of their vineyards.

Key words: *Bursaria spinosa, Leptospermum continentale,* native insectary*, Rytidosperma* ssp., vineyard.

Introduction

Grapevines, *Vitis vinifera* (L.), in Australia suffer varying levels of damage by pest species depending on the climatic zone (Scholefield and Morison, 2010). Light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) is the dominant insect pest causing damage to flower clusters and berry skins in Australian vineyards. Damaged skins provide infection sites for *Botrytis cinerea* (Pers.) and other bunch moulds, which result in a reduction in fruit quality and yield losses (Ferguson, 1995). Annual national losses from *E. postvittana* and related bunch rots were estimated to be \$70 million per year in Australia in 2010, in addition to \$0.5 million per year caused by garden weevils, grape phylloxera, mealybugs, scales and trunk boring insects (Scholefield and Morison, 2010). Other vineyard pests include Australian grapevine moth, elephant weevil and mites (Bernard et al. 2007; Thomson et al. 2007).

Biological control is a key component of arthropod-mediated ecosystem services, which are used to manage pests in production landscapes (Isaacs et al. 2009). Biocontrol is estimated to provide five to ten times more control of pests than pesticides (Pimentel et al. 1992). A range of generalist predators and host specific parasitic wasps contribute to the biocontrol of *E. postvittana* and other vineyard pests (Bernard et al. 2006a; Paull and Austin, 2006; Feng et al. 2015b, a; Yazdani et al. 2015) and up to 90% of newly hatched leafroller larvae may be killed by predators in the absence of toxic chemicals (Helson, 1939; Waterhouse and Sands, 2001). Predatory arthropods such as spiders, lacewings, predatory bugs, ladybird and carabid beetles are commonly found in vineyards (Bernard et al. 2007; Thomson and Hoffmann, 2009a). The majority of predators that attack crop pests are native (Gagic et al. 2018). Their presence in the vineyard can be boosted by incorporating native insectary plants (Thomson and Hoffmann, 2008).

Ecosystem services that plants provide include provisioning, regulating, cultural, and supporting services (Mace et al. 2012; Schellhorn et al. 2015). Arthropod 'provisioning' services from insectary plants provide 'SNAP', an acronym that refers to shelter, nectar, alternative prey and pollen (Barnes et al. 2010; Gurr et al. 2017), which nourish predatory arthropods and can extend their presence in production landscapes (Gurr et al. 1998). In turn, predators provide 'regulating' ecosystem services, which involve biological suppression of vineyard pests. Stands of native vegetation adjacent to vineyards have been associated with increased biodiversity (Thomson and Hoffmann, 2010; Smith et al. 2015) and provide season-long benefits to boost the activity of predators and parasitoids (Thomson and Hoffmann, 2013; Zemenick et al. 2018). Much emphasis has been put on the role of parasitoid wasps by Australian research in recent years (Bernard et al. 2006a; Paull and Austin, 2006; Perovic and Gurr, 2012; Feng et al. 2015a, b; Yazdani et al. 2015; Feng et al. 2016). The relationship, however, between predators and individual species of native insectary plants has largely been neglected. We chose to address this gap in knowledge.

Biodiversity and provision of ecosystem services can be improved by at least 20% in vineyards by retaining inter-row vegetation cover in preference to intensive soil tillage and herbicide use (Winter et al. 2018) and can also lead to greater resilience within the system (Altieri, 1991; Andow, 1991; Stamps and Linit, 1997; Schellhorn et al. 2015; Gagic et al. 2018). Exotic insectary species, such as buckwheat, alyssum, and phacelia have been trialed in Australia with varying degrees of success, but they are not easy to establish and maintain in the dry Australian environment (Bone et al. 2009; Thomson et al. 2010a). In contrast, native plants are naturally adapted to Australian conditions (Pandey et al. 2018) and are consistently reported as having low occurrence of pests (Parry et al. 2015) and high occurrence of natural enemies (Gurr et al. 2017; Gagic et al. 2018). Thus, native plants may be better insectary plants in Australia.

Careful screening of candidate insectary plants is important to ensure success. Increasing biodiversity in general is no guarantee of pest suppression (Gurr et al. 2003; Karp et al. 2018) as planting incompatible plants may have unintended consequences by enhancing pest populations (Baggen and Gurr, 1998; Gurr et al. 1998; Winkler, 2005; Ambrosino et al. 2006). Insectary plants need to be attractive to predators but not to pests, and be easy to establish and maintain, without actively competing with grapevines.

Increased biodiversity is often promoted as an important indicator of vineyard health but it can be difficult to measure (Altieri, 1999; Winter et al. 2018). Thomson et al. (2007) suggest that a surrogate indicator such as the diversity of predatory arthropods, which have a direct impact on pest abundance, can be used as one way to assess the benefits of enhancing biodiversity. This study aimed to broaden our understanding of the ecosystem services that are provided by native flora in vineyards.

A detailed understanding of the associations between native insectary plants and predatory arthropods has not been identified previously in association with Australian vineyards. We aim to determine if selected candidate insectary plants have the capacity to support populations of predators throughout the year, and could provide habitat for economically damaging vineyards pests. The observed versus estimated morphospecies richness was used to demonstrate the adequacy of the sampling program (Gotelli and Colwell, 2011).

We collected arthropods on the canopies of Christmas bush, *Bursaria spinosa* (Cav.), prickly tea-tree, *Leptospermum continentale (*Forst. and G.Forst) and wallaby grass, *Rytidosperma* ssp. (DC) adjacent to or in the mid-rows of Adelaide Hills, Barossa Valley and Eden Valley vineyards during 2013/14. *Vitis vinifera* (L.) (Vitales: Vitaceae) was also sampled.

Materials and methods

Native insectary plant selection

Plants were selected from local, native plant community lists and screened for their potential attractiveness to predators, and likelihood of providing habitat for herbivorous pests (Fiedler and Landis, 2007b; Fiedler et al. 2008; Isaacs et al. 2009). The three native candidate insectary plants were selected for assessment based on their attributes and the availability of established plants adjacent to or in vineyards (Table 1).

Table 2. Plant species, common name, family, plant type and food resources provided by each plant.

Plant species	Common name	Family	Plant type	Food resources
Vitis vinifera	Grapevine	Vitaceae	Deciduous vine fruit crop	Pollen and nectar
Bursaria spinosa	Christmas bush	Pittosporaceae	Native evergreen shrub	Pollen and nectar
Leptospermum continentale	Prickly tea-tree	Myrtaceae	Native evergreen shrub	Pollen and nectar
Rytidosperma ssp.	Wallaby grass	Poaceae	Native perennial grass	Pollen

Bursaria spinosa (Figure 1a) and *L. continentale* (Figure 1b) are erect, evergreen, shrubs. They produce pollen and nectar, which attracts a wide range of arthropods. *Rytidosperma* ssp. are erect, perennial grasses (Figure 1c). They grow actively during the spring and enter dormancy when soils dry out in summer (Penfold and McCarthy, 2010). *Rytidosperma* ssp. are associated with a range of predatory arthropods (Danne et al. 2010; Penfold and McCarthy, 2010; Wood et al. 2011) and can be selected for specific tolerance to site characteristics and low growing habit for use under perennial crops (Prescott, 2016). Mixed plantings of wallaby grasses were included in this study comprising common wallaby grass, *R. caespitosum*, brown-back wallaby grass, *R. duttonianum*, copper-awned wallaby grass, *R. fulvum*, hairy wallaby grass, *R. pilosum*, slender wallaby grass, *R. racemosum*, and small-flowered wallaby grass, *R. setaceum*. Each species is endemic to southern and eastern Australia.

Figure 6. Australian native insectary plants, *Bursaria spinosa* (a), *Leptospermum continentale* (b), and *Rytidosperma* ssp. (c). Photos: Mary Retallack

Arthropod sampling survey and methods

Surveys were conducted at six locations in South Australia (Figure 2). Four sites were in the Adelaide Hills and one each in the Barossa Valley and Eden Valley. Fungal disease pressure on each vineyard was managed using low inputs of fungicide sulfur and/or copper sprays. No broad-spectrum insecticides were applied.

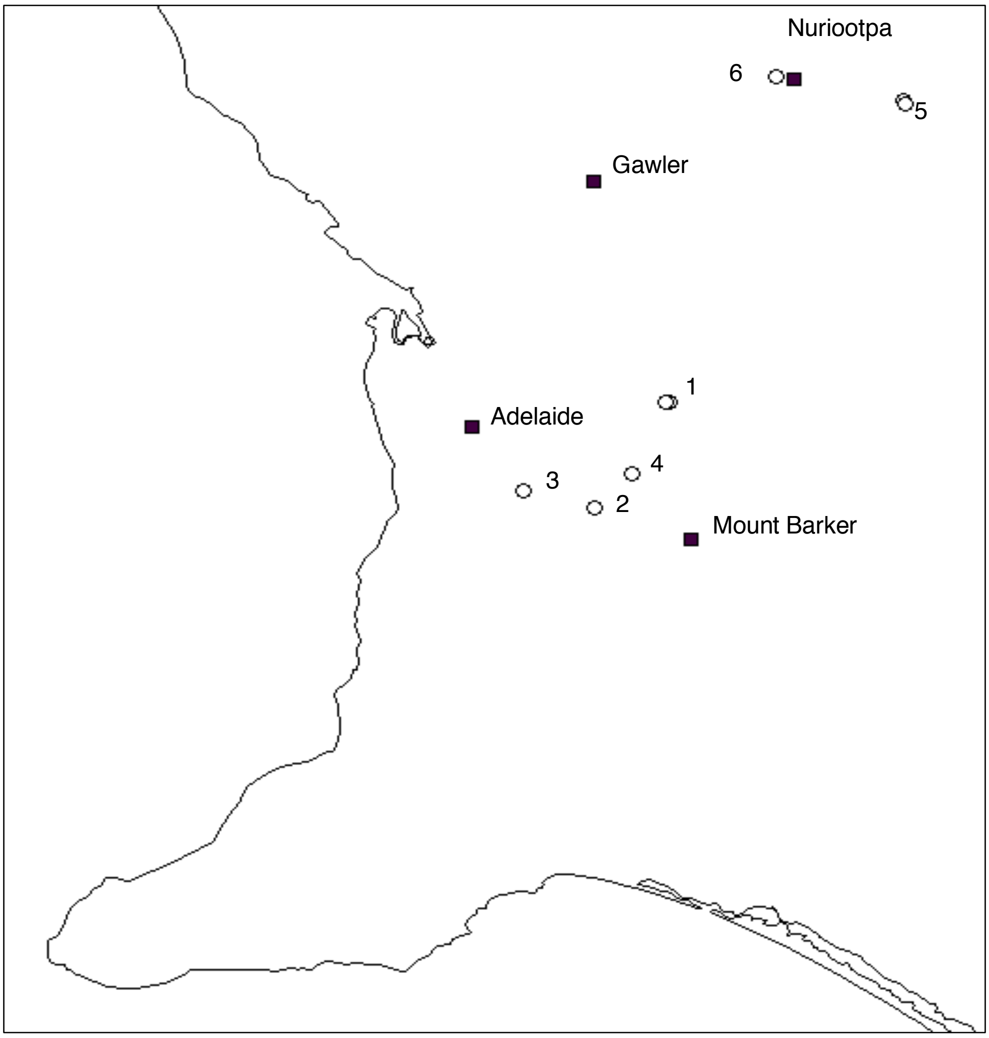

Figure 7. Location of data collection sites in South Australia. 1. Lenswood, (a) *Bursaria spinosa*, (b) *Leptospermum continentale*, (c*) Vitis vinifera* cv. Chardonnay; 2. Aldgate, *B. spinosa*; 3. Belair, *B. spinosa and L. continentale*; 4. Balhannah, *B. spinosa*; 5. Keyneton, (a) *Rytidosperma* spp., (b) *Rytidosperma* spp. and *V. vinifera* cv. Shiraz; 6. Nuriootpa, *Rytidosperma* spp. and *V. vinifera* cv. Shiraz.

Three sampling methods were employed, tailored to each plant species. A modified sweep net was used to sample the native woody vegetation. Each sample from *B. spinosa* or *L. continentale* was collected by firmly shaking the foliage five times, inside an insect sweep net (370 mm in diameter), modified to hold a funnel and a 250 mL collection container. This process was repeated five times to form a composite sample. Samples from grapevines were collected by firmly striking the cordons five times with a rubber mallet, over a beat net (700 x 700 mm) that held a 250 mL collection container. This process was repeated five times for each composite sample, alternating between each side of a pair of vine rows. This provided an effective method of sampling non-flying arthropods from the grapevine canopy. Ten composite samples were collected per sampling date at each site with the modified sweep net and beat net capture techniques. Arthropods were killed in the field using ethyl acetate. Plants of *Rytidosperma* ssp. were sampled using ten pitfall traps at each site. Round plastic 850 mL containers with a diameter of 120 mm were placed in a PVC plastic sleeve, flush with the soil surface. Wire covers, 1 mm thick with 25 mm hexagonal gaps were used to limit non-target catch. The pitfall traps were charged with propylene glycol to a height of approximately 30 mm. Plastic shields, 250 x 250 mm, were placed above the traps to exclude rainfall. Pitfall traps were exposed for two weeks prior to collection.

Sampling period and focus

Samples were collected fortnightly from September to December, and monthly in March, May and August. Arthropods were extracted, sorted to order, family and/or morphospecies and then stored in 80% EtOH in 100 mL plastic containers. Further identification was determined using Naumann (1991) arthropod identification keys. The sampling focus was on ground and canopy based predatory arthropods with prominent chewing and/or piercing mouthparts and/or herbivore pest species. Springtails (Collembola) and other tiny arthropods were not assessed due to their high abundance and lack of relevance to this study. Parasitoids were not considered in detail as they were not part of the focal group, the complexity of identifying each specimen to family, and the trapping methods utilised were not suited to capturing a representative sample of flying arthropods. Canopy samples were collected at the same time of day for each trapping method to minimise bias. Because of the differences among sampling methods only qualitative, rather than quantitative, comparisons can be made between plant species. Six hundred and eighty-one reference specimens were either mounted or preserved in 95% EtOH and transferred to the Waite Insect and Nematode Collection.

Analytical methods

Due to zero inflated data the ten sweep net samples, ten beat net samples, and ten pitfall samples were each pooled to give a single sample for each vegetation type, each of the nine sample sites, and each sampling date. Replicate dates were subsequently pooled by month, so a single monthly data point resulted for each vegetation type. Replicated incidence data were assessed to determine observed versus estimated species richness and percentage of morphospecies found in samples following Chao's bias corrected method for replicated samples (Gotelli and Colwell, 2011). Data were cast in contingency tables and analysed with the Fisher Exact Test to determine the proportions of elephant weevil, *Orthorhinus cylindrirostris* (Schönherr) (Coleoptera: Curculionidae) present on *L. continentale* compared to the other plants sampled (pooled). The larvae of Tortricidae have no defining morphological features, so they were identified to species using molecular methods during an associated study (Retallack et al. 2018).

Results

Arthropods

Twenty-seven thousand and ninety-one individual specimens were collected, comprising 20 orders and 287 morphospecies on *Rytidosperma* ssp. (*n* = 9,927), *B. spinosa* (*n* = 6,798), *V. vinifera* (*n* = 6,026)*,* and *L. continentale* (*n* = 4,340) (Table 2). Of the 20 orders found the most abundant, in order were Coleoptera, Diplopoda, Araneae, Dermaptera, Hemiptera, Neuroptera, and Hymenoptera which made up 92% (*n* = 24,921) individuals and 198 morphospecies. Thirteen orders made up the remaining 8% (*n* = 2,170) comprising 89 morphospecies.

Arthropods were categorised into different functional groups, as either predators (including parasitoids) (*n* = 8,880), herbivores (*n* = 6,790), or other (alternative prey, scavenger, seed or pollen feeders, detritivores) (*n* = 11,421), following the feeding habits outlined in Naumann (1991). Captures on *B. spinosa* resulted in the greatest number of arthropod morphospecies (*n* = 185), followed by *L. continentale* (*n* = 147), *V. vinifera* (*n* = 127), and *Rytidosperma* ssp. (*n* = 100).

Arthropod morphospecies richness

The majority of arthropod species present associated with each plant was estimated to have been collected in samples (Table 3), which indicates sampling protocols were adequate. A range between 68.4% of total morphospecies was collected on *V. vinifera* to 79.5% in association with *Rytidosperma* ssp. The lower proportion of observed versus estimated other morphospecies, can be attributed to a higher abundance of singletons and undetermined specimens, which are considered to be transient and inconsequential to this study.

Table 3. Predator, herbivore and other arthropod taxa, number of morphospecies and individuals associated with each plant species.

Predator taxa	*Bursaria spinosa*		*Leptospermum continentale*		*Vitis vinifera*		*Rytidosperma* ssp.	
	spp.	In	spp.	In	spp.	In	spp.	In
INSECTA								
ODONATA	1	1	0	0	0	0	0	0
DERMAPTERA								
Forficulidae ⊗	1	1	1	14	1	893	1	1547
MANTODEA	1	15	1	6	1	1	0	0
HEMIPTERA	7	122	7	121	7	137	7	140
Anthocoridae	1	1	1	4	0	0	0	0
Nabidae	1	5	1	9	1	9	1	4
Pentatomidae	2	47	2	33	2	65	1	100
Reduviidae	2	38	2	7	3	3	4	35
HYMENOPTERA ▲	10	172	7	121	7	137	7	140
Formicidae ⊗	1	4		70	1	65	1	29
NEUROPTERA	3	308	3	138	2	482	2	245
Chrysopidae	1	95	1	19	1	22	1	3
Hemerobiidae	1	208	1	118	1	460	1	242
Mantispidae	1	5	1	1	0	0	0	0
COLEOPTERA	16	179	14	234	10	946	6	90
Anthicidae	1	3	0	0	0	0	0	0
Cantharidae	1	12	1	85	0	0	0	0
Carabidae ⊗	1	16	1	27	1	6	4	87
Cleridae ⊗	7	27	6	26	2	2	0	0
Coccinellidae	5	69	5	77	6	898	1	2
Melyridae ⊗	1	52	1	19	1	40	0	0
Staphylinidae	0	0	0	0	0	0	1	1
DIPTERA	0	0	1	3	0	0	1	2
Syrphidae	0	0	0	0	0	0	1	2
Asilidae	0	0	1	3	0	0	0	0
ARACHNIDA								
ACARI	0	0	0	0	1	8	0	0
ARANEAE	27	834	27	508	30	488	13	828
Araneidae	7	158	5	95	6	136	2	18
Deinopidae	1	10	1	1	1	2	1	2
Dysderidae	1	5	0	0	1	15	0	0
Gnaphosidae	2	13	2	16	3	29	3	6
Linyphiidae	0	0	0	0	1	2	1	19
Lycosidae	1	8	1	21	1	15	1	766
Oxyopidae	1	3	1	1	1	3	0	0
Philodromidae	1	36	1	2	1	8	0	0
Pholcidae	1	4	1	2	0	0	0	0
Salticidae	3	156	4	77	4	87	1	3
Sparassidae	1	20	1	24	1	18	0	0
Tetragnathidae	2	117	2	123	2	124	0	0
Theridiidae	0	0	0	0	0	0	1	4
Thomisidae	3	165	4	94	4	13	1	1
Zodariidae	1	2	1	1	1	1	1	7
PSEUDOSCORPIONES	0	0	0	0	1	3	0	0
CHILOPODA ▼	1	1	0	0	0	0	3	106

Herbivore taxa	*Bursaria spinosa*		*Leptospermum continentale*		*Vitis vinifera*		*Rytidosperma* ssp.	
	spp.	In	spp.	In	spp.	In	spp.	In
INSECTA								
ORTHOPTERA	8	11	3	9	3	18	2	10
Acrididae	1	1	0	0	1	10	0	0
HEMIPTERA	17	365	11	208	10	111	6	18
Alydidae ⊗	0	0	1	5	1	4	2	7
Cicadellidae	4	137	3	20	2	2	1	3
Coccidae	1	3	0	0	2	37	1	2
Miridae	3	151	2	94	2	11	0	0
Lygaeidae ⊗	1	54	1	61	1	6	1	1
Pentatomidae	7	19	3	25	2	51	1	5
Tingidae	1	1	1	3	0	0	0	0
COLEOPTERA	12	51	13	200	12	137	6	43
Cerambycidae	3	12	1	1	0	0	0	0
Curculionidae	9	39	12	199	12	137	6	43
LEPIDOPTERA	4	21	3	39	4	16	2	318
Geometridae	1	13	1	32	1	2	0	0
Noctuidae	0	0	0	0	1	12	2	318
Tortricidae	2	8	2	7	2	2	0	0
DIPLOPODA								
JULIDA								
Julidae ▼	1	347	1	62	1	2177	1	2629

Other taxa	*Bursaria spinosa*		*Leptospermum continentale*		*Vitis vinifera*		*Rytidosperma* ssp.	
	spp.	In	spp.	In	spp.	In	spp.	In
INSECTA								
ORTHOPTERA	1	1	0	0	1	2	2	16
PHASMATODEA								
Phasmatidae	0	0	1	6	0	0	0	0
BLATTODEA ▼	1	1	0	0	3	14	6	347
HEMIPTERA	19	349	10	673	9	88	4	16
Miridae ‡	5	224	4	598	4	88	2	2
Pentatomidae ‡	7	66	3	61	3	27	1	1
THYSANOPTERA	3	94	1	42	0	0	0	0
PSOCOPTERA	0	0	0	0	1	1	0	0
HYMENOPTERA	2	3	3	234	1	1	1	9
Apidae	1	1	1	1	1	1	1	9
COLEOPTERA	23	3570	19	1624	14	391	17	2881
Buprestidae ▼	2	6	2	5	1	1	1	4
Chrysomelidae ‡	2	22	2	7	0	0	0	0
Elateridae ▼	1	4	1	1	1	13	1	103
Latridiidae ▼	1	3354	1	1136	2	352	0	0
Mordellidae	2	104	1	11	0	0	0	0
Scarabaeidae ▼	3	7	4	339	1	1	5	537
Tenebrionidae ▼	1	10	0	0	4	15	6	2230
LEPIDOPTERA	5	31	6	16	1	6	2	9
Psychidae	0	0	2	2	0	0	0	0
DIPTERA	10	107	5	21	2	2	3	8
ARACHNIDA								
ACARI	1	1	0	0	0	0	1	5
MALACOSTRACA								
ISOPODA								
Armadillidiidae ▼	0	0	0	0	2	5	3	529
Singletons: undetermined	11	213	8	79	7	14	6	12

▲ = parasitoid, ‡ = herbivore, ⊗ = scavenger, omnivore, seed or pollen feeder, ▼= detritivore. spp., number of morphospecies. In, number of individuals;

Table 1. Estimated morphospecies richness based on Chao's species richness estimator equation.

Functional group	***Bursaria spinosa***			***Leptospermum continentale***			***Vitis vinifera***			***Rytidosperma* ssp.**		
	Obs.	**Est.**	%	**Obs.**	**Est.**	%	**Obs.**	**Est.**	%	**Obs.**	**Est.**	%
All	185	261	70.8%	147	215	68.5%	127	186	68.4%	100	126	79.5%
Predators	67	80	83.5%	63	81	77.5%	55	65	85.7%	38	47	84.8%
Herbivores	42	61	69.2%	31	35	89.1%	30	39	76.5%	17	18	93.6%
Other	76	115	66.0%	53	108	49.0%	41	90	45.7%	45	58	73.6%

%, proportion of the estimated total number of arthropod species observed; Est., estimated total number of arthropod species; Obs., observed number of arthropod species.

Morphospecies and individual arthropods

Predatory arthropods dominated the morphospecies present on each plant (Figure 3). The ratio of predator to herbivore morphospecies on each plant (pooled for all sites) was highest for *Rytidosperma* ssp. (2:1 predators: herbivores). Out of a total of 98 predatory morphospecies, 67 were found on *B. spinosa,* 63 on *L. continentale*, 56 on *V. vinifera* and 38 in association with *Rytidosperma* ssp.

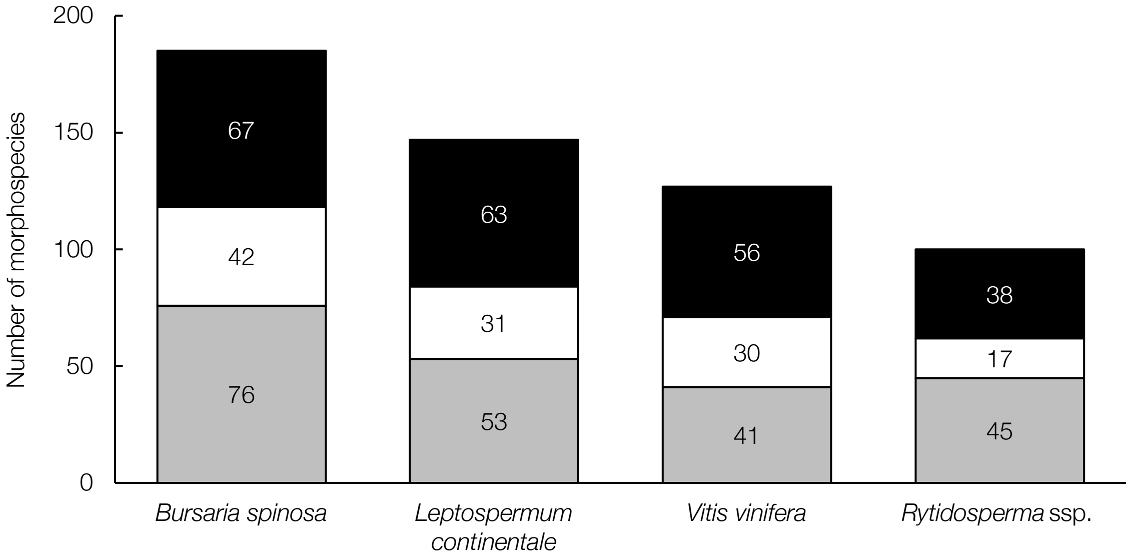

Figure 8. Number of predator (■), herbivore (□) and other arthropod (▨) morphospecies recorded over a 12-month period and the ratio of predator to herbivore morphospecies (pooled for all sites) on *Bursaria spinosa* (1.6:1), *Leptospermum continentale* (2.0:1), *Vitis vinifera* (1.9:1) and *Rytidosperma* spp. (2.2:1).

Insectary plants

Flowering phenology was assessed for each plant. The flowering period of *V. vinifera* occurred from 30 November to 13 December 2013 and the fruit was picked on 3 April 2014 in the Adelaide Hills. The flowering period of *V. vinifera* occurred from 11 November to 18 November 2013 and the fruit was picked on 14 March in the Barossa Valley and 7 April 2014 in the Eden Valley. *Leptospermum continentale* flowered from August to December, *Rytidosperma* ssp. flowered in December and *B. spinosa* flowered from December to February (Table 4). The insectary plants flowered at times which overlapped with the flowering and fruit set period of *V. vinifera*, which is a critical time for *E. postvittana* activity and potential crop damage.

Table 5. Flowering phenology of *Bursaria spinosa, Leptospermum continentale*, *Rytidosperma* ssp. and *Vitis vinifera* from August 2013 to February 2014.

Plant species	Common name	Family	Flowering period (month)						
			A	S	O	N	D	J	F
Vitis vinifera	Grapevine	Vitaceae				▨	▨		
Bursaria spinosa	Christmas bush	Pittosporaceae					▨	▨	▨
Leptospermum continentale	Prickly tea-tree	Myrtaceae	▨	▨	▨	▨	▨		
Rytidosperma ssp.	Wallaby grass	Poaceae					▨		

Predatory arthropods showed a clear pattern of seasonal abundance on all plants. Natural enemies were most abundant from October to January on *B. spinosa*, *L. continentale* and *V. vinifera* and from October to December on *Rytidosperma* ssp. (Figure 4). This period coincides with the peak time that predators are needed for crop protection during flowering and in the lead up to harvest. The presence of predatory arthropods reduced as weather conditions became less favourable and access to floral resources diminished.

Functional group: predators

Araneae (spiders)

Araneae were the highest number of predatory specimens (*n* = 2,658). The most abundant families were the Lycosidae, Araneidae, Tetragnathidae, Salticidae, and Thomisidae, comprising 82% of all spiders and 19 morphospecies. The most speciose of these was Araneidae with eight morphospecies, followed by Salticidae, and Thomisidae (four morphospecies each). Among the Araneae, 69% (*n* = 1,841 individuals) were active hunters or ambush spiders, while the remainder were sedentary or web dwelling.

Dermaptera (earwigs)

A single species of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae) was the most abundant predatory morphospecies and was present throughout the year. It was primarily found in association with *Rytidosperma* ssp. (*n* = 1,547) and *V. vinifera (n* = 893).

Coleoptera (beetles)

The Coleoptera were the most speciose order with 85 morphospecies for all trap types. The most abundant predatory groups of beetles were the Coccinellidae, Carabidae (some species are also seed predators), and Melyridae. Transverse ladybird beetle, *Coccinella transversalis* (Fabricius) (Coleoptera: Coccinellidae) was the most abundant ladybird), followed by *Scymnus* ssp., and common spotted ladybird, *Harmonia conformis* (Boisduval). Other species included minute two-spotted ladybeetle, *Diomus notescens* (Blackburn), and *Cryptolaemus montrouzieri* (Mulsant). Coccinellidae were present throughout the year.

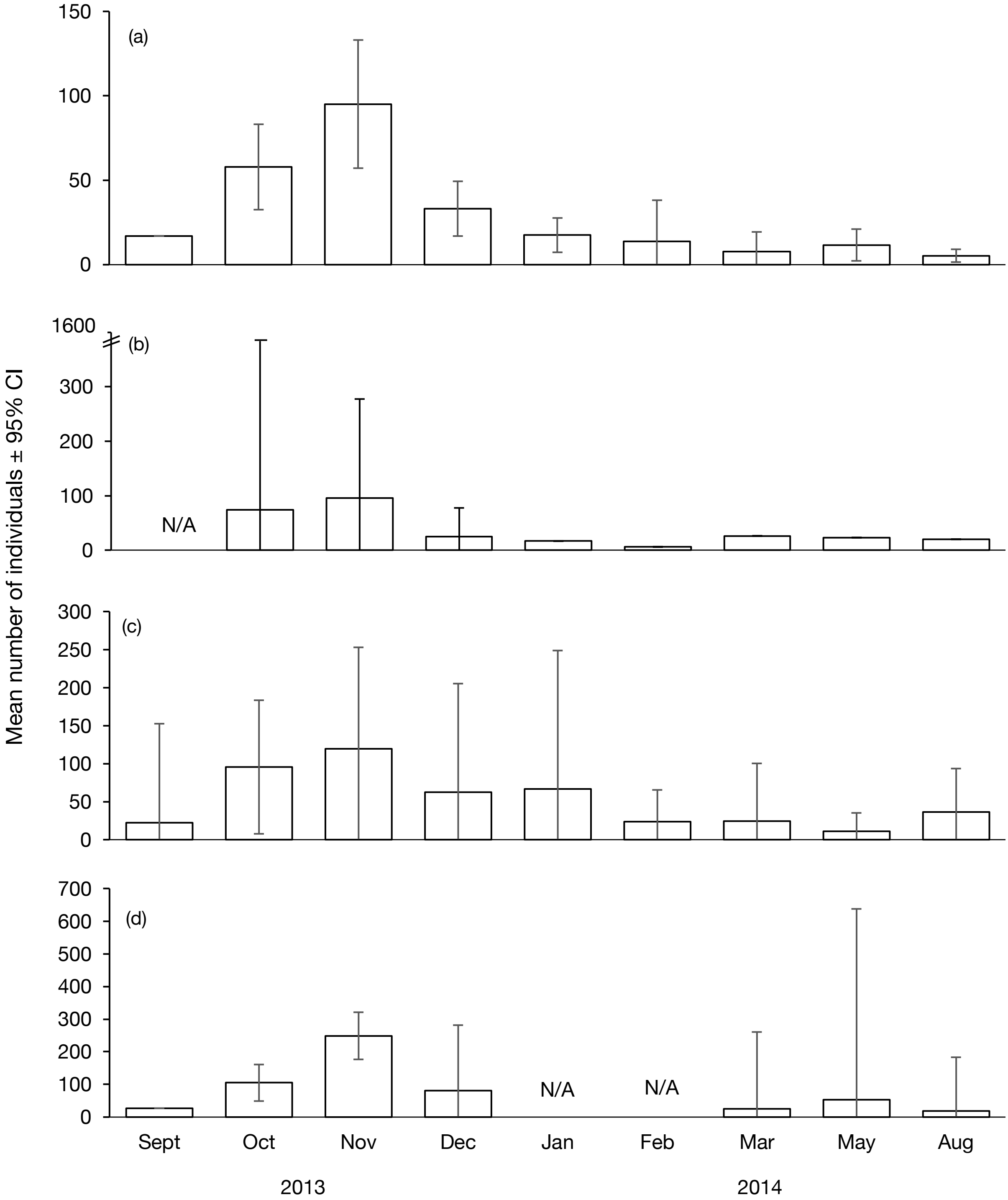

Figure 9. Temporal abundance of predator arthropods pooled across all (a) *Bursaria spinosa*; (b) *Leptospermum continentale*; (c) *Vitis vinifera*; and (d) *Rytidosperma* spp. sites over a 12-month period (mean ± 95% confidence interval per month). Means per sampling date were pooled for each month. N/A, no data collected. Note: the maxima on the vertical axes vary.

Neuroptera (lacewings)

The brown lacewing, *Micromus tasmaniae* (Rambur) (Neuroptera: Hemerobiidae) was the most abundant morphospecies of Neuroptera, followed by the green lacewing, *Mallada signata* (Schneider) (Neuroptera: Chrysopidae), and a mantid lacewing (Neuroptera: Mantispidae).

Hymenoptera (wasps and ants)

The predatory Hymenoptera were represented by 13 morphospecies (*n* = 479) on all vegetation types. The most abundant groups of predatory wasps were Sphecidae and Vespoidea, and the parasitic wasps super-families of Chalcidoidea, Ichneumonoidea, Proctotrupoidea, and Tiphioidea. Ants (Formicidae) were grouped together. Hymenoptera was not a key focus of this study and no further details are provided.

Hemiptera (true bugs)

The Hemiptera were the second most speciose order with 55 morphospecies pooled for all plant types. The most abundant predatory groups within Hemiptera were the Pentatomidae, Geocoridae, Reduviidae, and Nabidae. Two predatory morphospecies of Pentatomidae were observed. The glossy shield bug, *Cermatulus nasalis* (Westwood) (Hemiptera: Pentatomidae) had the highest abundance and was present at all sites, followed by the predatory shield bug, *Oechalia schellenbergii* (Guérin) (Hemiptera: Pentatomidae) which were found on *B. spinosa* and *L. continentale*. Both *C. nasalis* and *O. schellenbergii* were present throughout the entire season. The big-eyed bug, *Geocoris* spp. (Fallén) (Hemiptera: Geocoridae) was was captured on all plant species. The orange assassin bug, *Gminatus australis* (Erichson) (Hemiptera: Reduviidae) was the most abundant morphospecies of Reduviidae and was found on the *B. spinosa and L. continentale*. Other morphospecies of Reduviidae included the black ground assassin bug, *Peirates* ssp. (Serville) (Hemiptera: Reduviidae), brown assassin bug, *Coranus* ssp. (Curtis) (Hemiptera: Reduviidae), *Coranus granosus* (Stål) (Hemiptera: Reduviidae), and *Pnirsus cinctipes* (Stål) (Hemiptera: Reduviidae). The Pacific damsel bug, *Nabis kinbergii* (Reuter) (Hemiptera: Nabidae) was captured on all plant species. The minute pirate bug, *Orius* ssp. (Wolff) (Hemiptera: Anthocoridae) was found on *B. spinosa* and *L. continentale* only.

Functional group: herbivores

Julida (millipedes)

The invasive black Portuguese millipede, *Ommatoiulus moreleti* (Julida: Julidae) was the common species found across all sites. Populations of *O. moreleti* were highest in association with *Rytidosperma* ssp. (*n* = 2,629) and on *V. vinifera* (*n* = 2,177). Their abundance on grapevines was highest early in the growing season, declining during the warmer months of

December to February. Following rainfall events in February, millipedes dominated 92% of all individual arthropods found (n = 1,335) on *V. vinifera* in March 2014.

Coleoptera (weevils)

Four hundred and eighteen individual specimens and 18 morphospecies of Curculionidae were recorded, including 17 specimens of elephant weevil, *O. cylindrirostris* on all species except *Rytidosperma* ssp. *Orthorhinus cylindrirostris* was only present on *L. continantale* 10% of the time over the 20 sampling dates, at low abundance (n = 11). This indicates that *O. cylindrirostris* was no more likely to be found on *L. continentale* than on the other plants sampled (P = 0.66, Fisher's Exact test).

Lepidoptera (moths)

Four hundred and fifty-six specimens and 16 morphospecies of Lepidoptera were found comprising the families of Noctuidae, Geometridae, Tortricidae, and Psychidae. Three morphospecies of Noctuidae were found comprising Australian grapevine moth, *Phalaenoides glycinae* (Lewin) (Lepidoptera: Noctuidae), herringbone cut worm*, Agrotis* ssp. (Ochsenheimer) (Lepidoptera: Noctuidae), and common armyworm, *Leucania convecta* (Walker) (Lepidoptera: Noctuidae). Australian grapevine moth is a native pest of grapevines (Magarey et al. 1994) and was found in low abundance. *Agrotis* ssp. and *L. convecta* were found exclusively in association with *Rytidosperma* ssp., in relatively high abundance compared to other arthropods during August, September and October. A single morphospecies of apple looper, *Phrissogonus laticostata* (Walker) (Lepidoptera: Geometridae), two species of Tortricidae, *E. postvittana* and *Acropolitis rudisana* (Walker) and two morphospecies of Psychidae were also found.

Discussion

Associations between insectary plants and predatory arthropods

This study identified associations between three native Australian plants, *B. spinosa*, *L. continentale, Rytidosperma* ssp. and predatory arthropods that could support biocontrol of pests in vineyards. *Bursaria spinosa* and *L. continentale* were associated with a wide range of spiders, lacewings, predatory bugs and beetles. Wolf spiders, earwigs, brown lacewings, and predatory beetles were found abundantly in association with *Rytidosperma* ssp.

Seasonal synchrony and overwintering

Native plants are naturally adapted and can provide insectary benefits throughout the year. The species selected provide floral resources, which presence increases the potential of predators to provide control during the critical spring/summer period when pests typically move into the vineyard. Grapevines are deciduous. The reduction in insectary resources during dormancy may result in a resource bottleneck, resulting in an interruption in the presence of predators and parasitoids that may otherwise breed continuously (Schellhorn et al. 2015). In contrast, *B. spinosa* and *L. continentale* are both evergreen plants and have the capacity to support populations of mobile predators throughout the year that can passively populate vineyards and provide continuity of resources. The seasonality of ecosystem services could be extended by planting a range of suitable native perennial plants such as*. B. spinosa, L. continentale* and *Rytidosperma* ssp., to ensure habitat permanency and synchrony of provisioning services is continuous. Furthermore, wallaby grasses appear to have unique attributes that create strong associations with wolf spiders, brown lacewings and glossy shield bugs. This indicates that *Rytidosperma* ssp. offers complementarity and is an attractive component to a mix of native, woody plants to enhance insectary benefits.

Arthropods

Vineyard predators

We found a range of predators that were abundant. High abundance values are often associated with species adapted to site characteristics and are regarded as being present in a ‘suitable’ habitat (VanDerWal et al. 2009). We were able to determine the common predators relevant to Australian vineyards. Predators with a range of functionalities and habitat preferences, which are present at the same time, and/or succeed one another are needed to optimise complementarity benefits, enhancing their capacity to attack different life stages of the pest simultaneously (Holt and Lawton, 1994; Losey and Denno, 1999; Cardinale et al. 2003).

Spiders were found in abundance from a range of functional groups. Some species exhibited separate ground or canopy locale preferences (Costello and Daane, 1995). For example, wolf spiders were found abundantly in association with *Rytidosperma* ssp. but more rarely on the other plants. This is important as spiders overwinter in vineyards, albeit in low abundance (Costello and Daane, 1999; Thomson and Hoffmann, 2007b) and are likely to feed on larvae of Lepidoptera and other pests that often shelter on broad leafed weeds early in the growing season (D'Alberto et al. 2012). D'Alberto et al. (2012) found that there were limited benefits of non-crop woody vegetation on spiders in Australian vineyards. Conversely, individuals of Salticidae and Thomisidae were found abundantly on *B. spinosa* and *L. continentale*. Another tea-tree species, *L. aevigatum* (Gaertn.) F. Muell (Myrtaceae) is reported to boost the presence of two hunting spiders in California, measurably reducing the numbers of *E. postvittana* larvae (Hogg et al. 2014). Hogg and Daane (2011) also reported that natural habitat is a key source of spiders in Californian vineyards.

European earwig, *F. auricularia* is an important omnivorous predator of *E. postvittana* in vineyards (Danthanarayana, 1980; Bernard et al. 2007; Frank et al. 2007; Kehrli et al. 2012) but may cause minor, isolated damage to newly emerging growth just after budburst (Magarey et al. 1994). Any risk is likely to be offset, however, by the biocontrol benefits *F. auricularia* provides in vineyards especially in the period leading up to harvest, when chemical control options are limited (Frank et al. 2007). The brown lacewing was predominantly found in association with *Rytidosperma* ssp. Wood et al. (2011) found that brown lacewings most likely breed on wallaby grass, *R. bipartitum* (previously *Austrodanthonia linkii*). Daane et al. (2018) also found that native grasses provide good resources and habitat for natural enemies. This association may be important for manipulating populations of brown lacewings at the property scale. It is anticipated that by incorporating *B. spinosa* and *L. continental* into vineyard landscapes this will help support the presence of a multitude of predatory arthropods.

Vineyard pests

The incidence of herbivores on the native insectary plants assessed was negligible. These plants were not found to be breeding sites for vineyard herbivores and are not considered a threat when planting them in and around mature vineyards. A very low abundance of leafrollers was found on the woody plants only. The benefits of planting *Rytidosperma* ssp. between vines are supported with direct evidence of increased predation of *E. postvittana* eggs where *Rytidosperma* ssp. and windmill grass, *Chloris truncata* were present (Thomson and Hoffmann, 2009a; Danne et al. 2010).

Populations of black Portuguese millipede, *O. moreleti* are not as susceptible to predation as they eject highly repellent chemical compounds called benzoquinones from their defensive glands when attacked, rendering them inedible (Sekulic et al. 2014; Vujisic et al. 2014; Shear, 2015; Makarov et al. 2017). They are widespread in southern Australia and tend to be more abundant where leaf litter and soil moisture are present (Paoletti et al. 2007). Damage to the skins of wine grapes may result in a quality downgrade or rejection of fruit in the vineyard. Their presence in picking bins often results in wine taint in grape ferments (Stankovic et al. 2016).

Elephant weevil, *O. cylindrirostris* is a wood boring pest of grapevines (Retallack, 2003; Coventry et al. 2004; Bernard et al. 2007; Scholefield and Morison, 2010). Despite adult elephant weevil being observed briefly on *L. continentale* it is not considered a larval host plant. There is no evidence to suggest it poses a threat as weevils may transiently acquire pollen and nectar from a range of flowering plants. While individual species of herbivores were observed in this study, little is known about how different habitats and plant communities influence the dynamics of multiple herbivore species and their potential impact on fruit production (Perez-Alvarez et al. 2018).

Conclusion

This study identified associations between native insectary plants *B. spinosa, L. continentale* and *Rytidosperma* ssp., and predatory arthropods in and around vineyards, which have not been identified before in Australia. The sampling program recovered the majority of estimated species diversity in the samples, which indicates sampling protocols were adequate. Each plant species supports diverse predatory species, which should attack a range of other arthropods across their life stages. The native insectary plants assessed in this study were not found to be breeding sites for vineyard herbivores and are not considered a threat when planting them in or around mature vineyards. The native plants are naturally adapted and can provide insectary benefits throughout the year, especially during spring and summer to boost the activity of predators and reduce pest pressure on developing fruit. Vineyard managers are encouraged to explore the use of *B. spinosa, L. continentale* and *Rytidosperma* ssp. as insectary plants in their vineyards. This information should help wine grape growers save time and resources by producing fruit with lower pest incidence, while enhancing biodiversity of their vineyards. In addition, the results of this study may be applicable to a range of Australian production systems.

Future research

Further research is required to elucidate the potential of a broader suite of native insectary plants to support the diversity of predatory arthropods in vineyards. Assessment of the optimal size, layout and composition of insectary plantings is required, as well as the anticipated benefit of boosting natural biocontrol, with minimal intervention required in the vineyard. This research identified the morphospecies present in native vegetation and vineyards and their temporal abundance throughout the year. An outstanding issue is that we are not sure that all predators will readily move between native vegetation and vineyards. To build on this research further work is required to quantify the movement of predators from insectary plants into the vineyard. An integrated approach is required to assess the capacity of predatory arthropods and parasitic wasps to contribute to the biocontrol of economically damaging pests and the individual contributions each predator makes. Similarly, it is anticipated that a multi-species insectary plant community is preferable to single species planted in strategic locations. This assumption needs to be tested.

Acknowledgements

This research was made possible through scholarships to Mary Retallack awarded by the Australian Government Research Training Program Stipend, The University of Adelaide C. J. Everard Supplementary Scholarship, SARDI Women's Suffrage Centenary Bursary, Grape and Wine Research and Development Corporation PhD Supplementary Scholarship [GWR Ph1209]. Project operating support was provided via the Grape and Wine Research and Development Corporation and via the Adelaide Hills Wine Region Post Graduate Biodiversity Study Grant.

We thank managers who allowed us to collect data in their vineyards: Prue Henschke, Craig Markby, and Dan Falkenberg. We also thank Associate Prof. Duncan Mackay, Dr. Michael A Nash, and Dr. Maarten van Helden who provided feedback on the manuscript and Glenys M Wood who assisted with arthropod identification.

Supplementary information

Arthropod survey locations

Surveys were conducted at six locations in South Australia. Four were in the Adelaide Hills near the townships of Lenswood (Site 1: a, b, c. 34°53'29.85"S 138°50'6.15"E), Aldgate (Site 2: 35°1'55.47"S 138°44'58.85"E), Belair (Site 3: 35°0'36.60"S 138°39'49.63"E), and Balhannah (Site 4: 34°59'15.46"S 138°47'43.53"E). Two Barossa survey sites were located near the townships of Keyneton (Site 5: a. 34°29'33.97"S 139° 7'16.79"E, b. 34°29'49.76"S 139° 7'28.32"E), and Nuriootpa (Site 6: 34°27'41.72"S 138°58'4.31"E). Data were collected on *B. spinosa* at sites 1a, 2, and 4; on *L. continantale* at sites 1b, and 3; on *Rytidosperma* ssp. at sites 5a, 5b, and 6; and on *V. vinifera* varieties Chardonnay (Site 1c) and Shiraz (Sites 5b, and 6).

Supplementary info. Table 6. Diversity of Araneae collected for all traps showing mode of predation (behaviour), habitat and % presence by plant. Undetermined morphospecies comprised juveniles that could not be identified with confidence to family but resembled Gnaphosidae or Salticidae.

Family	Behaviour	Niche	*B. spinosa*	*L. continentale*	*V. vinifera*	*Rytidosperma* ssp.
		n	834	508	488	828
Lycosidae	Hunter (active)	Ground	1%	4%	3%	93%
Salticidae	Hunter (active)	Canopy	19%	15%	18%	0%
Thomisidae	Ambush (active)	Canopy	20%	19%	3%	0%
Gnaphosidae	Hunter (active)	Ground	2%	3%	6%	1%
Dysderidae	Hunter (active)	Ground	1%	0%	3%	0%
Zodariidae	Hunter (active)	Ground	0%	0%	0%	1%
Sparassidae	Hunter (active)	Ground/Canopy	2%	5%	4%	0%
Philodromidae	Ambush (active)	Canopy	4%	0%	2%	0%
Oxyopidae	Ambush (active)	Canopy	0%	0%	1%	0%
Undetermined	Ambush (active)	Canopy	17%	11%	8%	0%
Araneidae	Web (sedentary)	Canopy	19%	19%	28%	2%
Tetragnathidae	Web (sedentary)	Canopy	14%	24%	25%	0%
Linyphiidae	Web (sedentary)	Canopy	0%	0%	0%	2%
Deinopidae	Web (sedentary)	Canopy	1%	0%	0%	0%
Pholcidae	Web (sedentary)	Canopy	0%	0%	0%	0%
Theridiidae	Web (sedentary)	Ground/Canopy	0%	0%	0%	0%
			100%	**100%**	**100%**	**100%**

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Addendum to Chapter 4

The peer reviewed paper presented in Chapter 4 was published in mid-February 2019. However, several queries were raised by the thesis examiners regarding Chapter 4 and 5, so I have added the following points of clarification to ensure completeness. The version of Chapter that appears here includes corrections that are not included in the published paper.

Functional group descriptors

Arthropods were categorised into different functional groups, as either predators (including parasitoids), herbivores or other throughout the entirety of the thesis. The treatment for each functional group was the same throughout the thesis. Parasitoids are included in the functional group of predators, as they have the capacity to provide biocontrol of pests.

A review of species revealed that six out of the 287 morphospecies were incorrectly categorised resulting in a net change in n = 156 (1.7%) individual specimens being moved from predator to other, and a corresponding increase of n = 156 (1.4%) in other. These changes are reflected here in Chapter 4 for completeness and did not create a material change in the application of the results.

The predatory Hymenoptera were represented by 13 morphospecies (n = 479) on all vegetation types. The most abundant groups of predatory wasps were Sphecidae and Vespoidea, and the parasitic wasp super-families of Chalcidoidea, Ichneumonoidea, Proctotrupoidea, and Tiphioidea. Ants (Formicoidea) were grouped together. Hymenoptera was not a key focus of this study and no further details are provided.

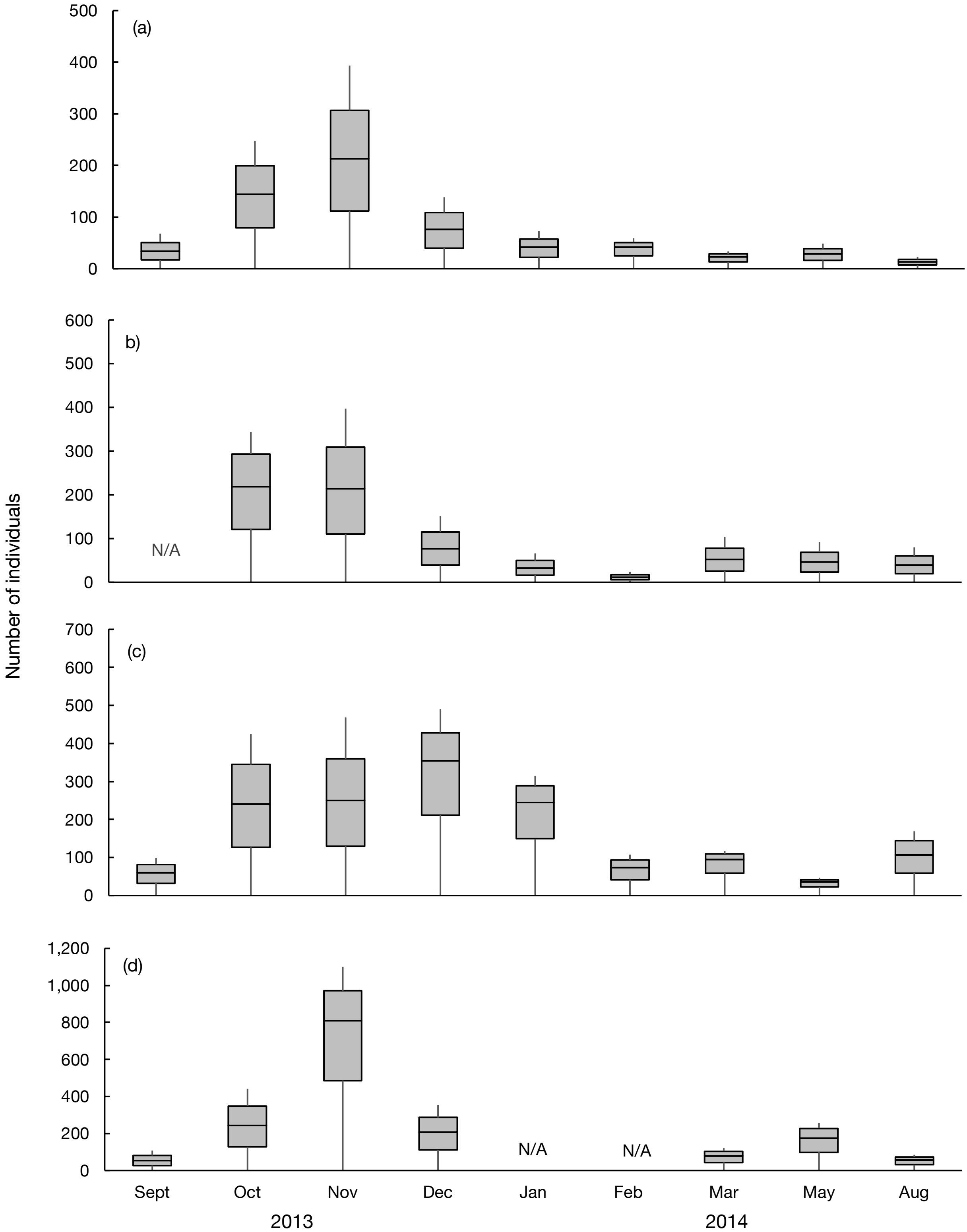
The use of morphospecies as surrogates for taxonomic species has been proposed as an alternative to overcome the identification difficulties associated with many invertebrate studies (Derraik et al. 2010). This is the approach adopted here. Hymenoptera including wasps were included as morphospecies but were not analysed further, as they were not part of the focal group and pose serious challenges to accurate identification even by expert taxonomists.

Inclusion of a focal crop

Grapevines were included as the focal horticultural crop for comparison with the locally-adapted native insectary plants. Vineyards are a managed habitat where pest insects may cause substantial damage. The management of pests is required to minimise the economic damage caused by reduced fruit quality and yield losses (Scholefield and Morison, 2010). The area in and around the vineyard is also a place where insectary plants could be incorporated to support predators and contribute towards the biocontrol of economically damaging pests.

Use of statistical analysis

Chao’s bias corrected method for replicated samples was used to demonstrate the adequacy of sampling effort in Chapter 4. In Chapter 5, species accumulation curves were used to reinforce this conclusion. Both demonstrated that the sampling effort was adequate.



Addendum Figure. Temporal abundance of predator arthropods pooled across all (a) *Bursaria spinosa* (*n* = 26); (b) *Leptospermum continentale* (*n* = 11); (c) *Vitis vinifera* (*n* = 26); and (d) *Rytidosperma* spp. (*n* = 16) sites over a 12-month period. The box plots represent the median (central line), first and third quartiles (grey box), and the whiskers the total range. N/A, no data collected.

CHAPTER FIVE

The potential biological and functional diversity offered by native insectary plants when planted in and around Australian vineyards

Statement of authorship

The potential biological and functional diversity offered by native insectary plants when planted in and around Australian vineyards

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MR collected samples, identified arthropods, interpreted data and wrote the manuscript. DM, LT and MK contributed to the manuscript production. MK and DM helped in developing the idea, provided guidance throughout and assisted with statistical analysis. All authors have contributed significantly and agree with the manuscript.

Data availability

The datasets generated and analysed during the study along with supporting documentation are available in The University of Adelaide Figshare online digital repository (DOI 10.25909/5bdbfdb8a98a4)

Disclosure statement

The authors declare there are no conflicts of interest.

Abstract

Background and Aims: We evaluated the arthropod communities found in association with three native plant species that have attributes suitable for insectary planting. Insectary plants provide food, shelter and alternative prey/hosts, which nourish and support the presence of predatory arthropods. We sought to determine which species have the capacity to provide enhanced biodiversity and effective functional diversity, when incorporated in and around Australian vineyards. The incorporation of native plants in association with vines, may enhance the diversity and abundance of predatory arthropods. They have the potential to be a key component of biological control programs used to manage pests in production systems.

Methods and Results: We used data on arthropods that were collected in association with two native shrubs, *Bursaria spinosa* (Cav.) (Apiales: Pittosporaceae) and *Leptospermum continentale* (Forst. and G.Forst) (Myrtales: Myrtaceae), the perennial grasses, *Rytidosperma* ssp. (DC) (Poales: Poaceae), and a selected horticultural crop, *Vitis vinifera* (L.) (Vitales: Vitaceae), to explore relationships between each plant and associated arthropods using common diversity indices. Results showed that by incorporating insectary plants in and around vineyards, it may be possible to increase the functional diversity of predatory arthropods by more than 3x when either *B. spinosa* or *L. continentale* is present versus grapevines only. The richness of predator morphospecies across all plant types (S = 98) was nearly double the number found in association with grapevines (S = 56). The arthropod faunas of the two native, perennial, evergreen plants exhibited the greatest similarity. Conversely, the assemblages of *L. continentale* and *Rytidosperma* ssp. had the lowest number of morphospecies in common. The grasses *Rytidosperma* ssp. appear to provide a valuable complementarity habitat for arthropod species other than those commonly found in association with the woody perennial plants. It may increase the net number of predator morphospecies by around 27% when planted in combination with the woody perennial plants.

Conclusion: The incorporation of native insectary plants *B. spinosa*, *L. continentale* and *Rytidosperma* ssp. has the potential to enhance biodiversity and effective conservation biological control efforts. They provide a suitable habitat to support diverse and functional populations of predatory arthropods.

Significance of the Study: This study broadens our understanding of the biological and functional diversity offered by three native insectary species as model plants. They could be incorporated in association with vineyards to boost the presence of predatory arthropods, which contribute towards biocontrol of economically damaging vineyard pests.

Key words: native insectary plants*, B. spinosa, L. continentale, Rytidosperma* ssp.*,* vineyards

Introduction

Biodiversity within a production system represents the variety and heterogeneity of organisms present (Cardinale et al. 2012). Although the term ‘biodiversity’ was first used in the late 1980s (Wilson and Peter, 1988), the concept of ‘biological diversity’ had been discussed since the nineteenth century and continues to be widely used. A measure of functional diversity is often used within biological communities and ecosystems to refer to the variety and number of species that fulfil different functional roles (Colwell, 2009), including biological control of pests by predators. A lack of biodiversity can lead to instability within a production system (Altieri, 1999; Gurr et al. 2004) such as a vineyard. The problem with intensifying crop production and the production of larger monocultures, with little natural habitat and diversity, is that landscape simplification often results in an increase in pest pressure (Altieri and Letourneau, 1982) and insecticide use to combat outbreaks of pests (Meehan et al. 2011).

Enhanced biodiversity is often promoted as an important indicator of vineyard health (Altieri, 1999; Gurr et al. 2003; Barnes et al. 2010; Winter et al. 2018). Non-crop plants may have the capacity to maintain and enhance biodiversity (van Emden, 1965, 2003). Structurally poor agricultural landscapes may benefit from the creation of perennial boundary vegetation (Tscharntke et al. 2007). It is thought that diversified landscapes hold potential for the conservation of biodiversity and sustenance of pest control functions (Landis et al. 2000a; Bianchi et al. 2006; Letourneau et al. 2011). There is current interest in biodiversity loss due to crop production and the consequent alteration in ecosystem services provision. The presence of non-crop vegetation, including native insectary plants, may be an important contributor to effective functional diversity and the associated provision of ecosystem services (Close et al. 2009; Mace et al. 2012; Schellhorn et al. 2015). Functional diversity is reflected by the diversity and abundance of predatory arthropods. The contribution made by non-crop habitat will depend on its structural diversity, both complexity and heterogeneity, or degree of disturbance (Gurr et al. 2004; Shields et al. 2016).

Native plants are preferred as supplementary flora, as they are naturally adapted to Australia’s dry and hot climatic conditions (Danne et al. 2010; Pandey et al. 2018). They are consistently reported as having a low occurrence of pests (Parry et al. 2015), a high occurrence of natural enemies (Gurr et al. 2017; Gagic et al. 2018) and they have the capacity to contribute towards CBC (Danne et al. 2010; Thomson and Hoffmann, 2010; Wood et al. 2011; Pandey et al. 2018). Native plants may also provide ‘SNAP’ resources, i.e. shelter, nectar, alternative prey and pollen (Barnes et al. 2010; Gurr et al. 2017), and may nourish natural enemies and extend their presence in production landscapes (Gurr et al. 1998). The use of predatory arthropods as biocontrol agents to manage pests in production systems is an example of an arthropod-mediated ecosystem service (Isaacs et al. 2009). The presence of predatory arthropods such as spiders, lacewings, predatory beetles and bugs in a vineyard can be boosted by incorporating native insectary plants (Thomson and Hoffmann, 2008). Many predators that attack crop pests are native (Gagic et al. 2018). An increase in predators has been reported where there are stands of native vegetation adjacent to crop production areas (Landis et al. 2000b; Landis et al. 2005; Parry et al. 2015).

Objective measures of biodiversity can be difficult to evaluate because of the many indices available. There is no consensus on which are most appropriate or informative when applied to different natural or disturbed production systems (Morris et al. 2014). Thomson et al. (2007) suggest that a surrogate indicator, such as the diversity of predatory invertebrates that have a direct impact on pest abundance, can be used as one way to assess the benefits of enhancing biodiversity. Given the relationship between the diversity and abundance of arthropod predators and the presence of individual native insectary plant species has largely been neglected in Australia, we chose to address this gap in the research.

Biodiversity indices generally fall into two main groups, basic and compound indices. Typically, the primary focus is on morphospecies diversity (Morris et al. 2014). Richness (S), or the number of morphospecies present, is the simplest metric used to represent diversity (Whittaker, 1972). However, this measure can be imprecise as it is subject to random variation in natural systems which can skew results and is therefore limited in its capacity to provide informative comparisons. Problems associated with comparing morphospecies richness among communities that have been collated using different sampling methods and/or effort were discussed by Moreno and Halffter (2000) and Willott (2001). Moreno and Halffter (2001) conclude that morphospecies accumulation curves are a practical tool for inventory assessment, even for very diverse groups. Species abundance, the number of individuals of each morphospecies found in samples, is also important for assessing diversity (Jost, 2006). The proportional abundance of the most abundant single morphospecies in a population can be expressed using the Berger-Parker dominance (BP) calculation (Berger and Parker, 1970). Evenness (E) represents the degree to which relatively equal numbers of individuals belong to each morphospecies (Morris, et al. 2014).

Various compound indices that attempt to combine measures of richness and abundance include Shannon's diversity (H') and Simpson's dominance (or inverse) diversity (D_2) indices (Simpson, 1949). H' (also known as the Shannon entropy index) represents the uncertainty about the identity of an unknown individual (Shannon, 1948). In a highly diverse and evenly distributed system, an unknown individual could belong equally to any morphospecies, leading to a high uncertainty in predictions of its identity. H' increases as both the richness and the evenness of a community increases (Magurran, 1988). D_2 is the inverse of Simpson's original index. It provides a measure of diversity that is less sensitive to morphospecies richness (Simpson, 1949). H' is regarded by Jost (2006) as the most useful of all diversity indices, but also notes that its calculated value provides a measure of the uncertainty rather than diversity. Other measures have limitations. S is sensitive to the number of rare morphospecies, D_2 is sensitive to the presence of abundant morphospecies and BP is only sensitive to the most abundant morphospecies (Morris et al. 2014). The effective number of equally common morphospecies can be calculated from H' to estimate a true indicator of the diversity of a community, p_i, which can be directly compared among communities (Jost, 2006).

Numerous abundance-based similarity indices that compare two assemblages based on morphospecies incidence of presence/absence have been proposed (Chao et al. 2006). The Jaccard index is commonly used to assess the similarity of presence/absence (incidence) data and is based on simple counts, comprising the number of morphospecies shared by two assemblages and those that are unique to each (Jaccard, 1902). This similarity measure has a defined maximum of 1.0 where communities are all identical and 1/N when the communities are dissimilar (Jost, 2006).

Another similarity index, Bray-Curtis, is widely used to generate distance matrices in plant habitat ordination studies (Gotelli and Ellison, 2004). Non-metric multidimensional scaling (NMDS) is a method of ordination, which utilises the Bray-Curtis index for analysing ecological data. This scaling method does not assume linear relationships and can be used with data that are non-normally distributed, arbitrary, or discontinuous, or that contain numerous samples with a value of zero (McCune and Grace, 2002).

This study aims to build on earlier work carried out by Retallack et al. (2019) in an associated study, where arthropods associated with three locally-adapted native plants, Christmas bush, *Bursaria spinosa* (Cav.), prickly tea-tree, *Leptospermum continentale (*Forst. & G.Forst) and wallaby grass, *Rytidosperma* ssp. (DC) were characterised (Table 1). They concluded that each native plant had the potential to be used as insectary in association with Australian vineyards, as the plants support populations of predatory arthropods throughout the year. *Vitis vinifera* was included as the focal horticultural crop for comparison with the locally-adapted native insectary plants because vineyards are a managed habitat where pest insects may cause substantial damage (Scholefield and Morison, 2010). The areas in and around vineyards are also where insectary plants could be incorporated to support predators and contribute towards the biocontrol of economically damaging pests.

Here we report on further analyses that aim to broaden our understanding of biological and functional diversity offered by these native insectary plants as model species that can be incorporated in and around Australian vineyards to boost the presence of predatory arthropods. The term functional diversity was conceived as a way to further measure the ecological importance of morphospecies within a community, as a way to better understand how biodiversity affects different ecosystem functions (Petchey and Gaston, 2006; Laureto et al. 2015). The richness and abundance of predator arthropods has been adopted as an objective measure of functional diversity. In this study we consider the overall diversity of arthropod species and focus on the contribution made by a group of predatory arthropods, as they have functional traits that contribute to pest regulation within the production ecosystem.

We set out to answer the following questions about selected native candidate insectary plants: 1. What is the biological and functional diversity associated with each plant species? This will highlight the potential contribution of each plant; 2. What are the features of an effective, functional native insectary plant assemblage for use in and around vineyards? This will highlight the number of plants needed for effective function. 3. What is the level of similarity and dissimilarity between the arthropod faunas of each plant species? This will provide an indication of the versatility of each plant. To address these aims we used arthropod data collected in association with the selected native insectary plants. Adjacent plantings of the selected horticultural crop, *Vitis vinifera* (L.) (Vitales: Vitaceae) was also sampled in Adelaide Hills, Barossa Valley and Eden Valley vineyards during 2013/14.

Table 7. Plant species, common name, family, plant type and food provided by each plant.

Plant species	Common name	Family	Plant type	Food resources
Vitis vinifera	Grapevine	Vitaceae	Deciduous vine fruit crop	Pollen and nectar
Bursaria spinosa	Christmas bush	Pittosporaceae	Native evergreen shrub	Pollen and nectar
Leptospermum continentale	Prickly tea-tree	Myrtaceae	Native evergreen shrub	Pollen and nectar
Rytidosperma ssp.	Wallaby grass	Poaceae	Native perennial grass	Pollen

Materials and methods

Arthropod survey and sampling methods

Surveys were conducted at six locations in South Australia, four were in the Adelaide Hills and one each in the Barossa Valley and Eden Valley (Retallack et al. 2019). Three sampling methods were employed. A modified sweep net was used to sample the native woody vegetation. Each sample from *B. spinosa* or *L. continentale* was collected by firmly shaking the foliage five times, inside an insect sweep net (370 mm in diameter), modified to hold a funnel and a 250 mL collection container. This process was repeated five times to form a composite sample. Samples from grapevines were collected by firmly striking the cordons five times with a rubber mallet, over a beat net (700 x 700 mm) that held a 250 mL collection container. This process was repeated five times for each composite sample, alternating between each side of a pair of vine rows. A total of ten composite samples were collected per sampling date at each site with the modified sweep net and beat net capture techniques. Arthropods were killed in the field using ethyl acetate. Plants of *Rytidosperma* ssp. were sampled using ten pitfall traps at each site, exposed for two weeks prior to collection. Samples were collected fortnightly from September to December, and monthly in March, May and August during 2013/14. Arthropods were extracted, sorted to order, family and/or morphospecies. Refer to Retallack (2019) for a full description of the sampling methods.

Data analysis

Due to zero inflated data the ten sweep net samples, ten beat net samples, and ten pitfall samples from the respective vegetation types were each pooled to give a single sample for each of the nine sample sites, and each sampling date. Replicated incidence data were assessed to determine observed morphospecies richness for each plant and to calculate associated diversity indices. To determine the influence of each plant species on the total sampled arthropod community, basic and compound diversity indices were calculated for each. Basic indices comprised S, a measure of the number of morphospecies, and BP to calculate the proportional abundance of the most abundant morphospecies (P_{max}; (Berger and Parker, 1970). The compound indices H' ("Shannon") and D_2 ("Inverse Simpson") were calculated using the R package 'Vegan' (Oksanen et al. 2018). While a number of different indices are available and show different aspects of diversity, H' was chosen as it clearly demonstrates the diversity of arthropods associated with each plant when morphospecies are considered together in total and in functional groups. It is regarded by Jost (2006) as the most useful of all diversity indices. H' (x) was converted to a true diversity p_i for each plant following Jost (2006). The value of p_i takes into account the 'effective number of species' as defined by Macarthur (1965), who introduced a method for transforming diversity indices to a species richness scale. True diversity is also known as the 'numbers equivalent' of diversity (Adelman, 1969; Patil and Taillie, 1982). Evenness was calculated as D_2 ("Inverse Simpson") and richness D_2/S following Morris et al. (2014). Randomised morphospecies accumulation curves were constructed for each plant and their associated arthropod communities, using the R package 'Vegan' (Oksanen et al. 2018). These curves show the number of species that you would expect to see if you collected a designated number of samples from each site.

Data were cast in contingency tables and the Jaccard similarity index was calculated (Gardener, 2017) to determine the similarity of arthropods found between each combination of plants. To analyse variation in arthropod communities in association with each plant species, NMDS was employed using the metaMDS function in the R package ‘Vegan’ (Oksanen et al. 2018) to plot ordinations based upon Bray-Curtis dissimilarities. This scaling method can be used to determine if arthropod communities differed based on the habitat offered by each plant species.

Results

Richness, dominance and evenness comparisons

Twenty seven thousand and ninety-one arthropods were categorised into different functional groups, as either predators (including parasitoids), herbivores, or other (alternative prey, scavenger, seed or pollen feeders, detritivores), following the feeding habits outlined in Naumann (1991) arthropod identification keys (Table 2). The complete dataset is presented in Retallack et al. (2019).

Captures on the woody, evergreen shrubs, *B. spinosa* and *L. continentale* resulted in the highest richness of arthropod morphospecies, followed by the deciduous, grapevine *V. vinifera* and native perennial grasses, *Rytidosperma* ssp. (Table 2). Captures on *L. continentale* resulted in the highest evenness followed by *Rytidosperma* ssp., *V. vinifera* and *B. spinosa.* Analysis based upon the BP dominance index indicated *B. spinosa* and *L. continentale* were dominated by individuals of minute brown scavenger beetle (Coleoptera: Latridiidae). The arthropod fauna on *V. vinifera* and *Rytidosperma* ssp. were dominated by the invasive, black Portuguese millipede, *Ommatoiulus moreleti* (Lucas) (Julida: Julidae).

Captures on the woody, evergreen shrubs *B. spinosa* and *L. continentale* also resulted in the highest richness of predator morphospecies, followed by *V. vinifera* and native perennial grasses, *Rytidosperma* ssp. (Table 2). The richness of predator morphospecies across all plant types (S = 98) was nearly double the number found in association with grapevines (S = 56). Abundant predatory morphospecies included the brown lacewing, *Micromus tasmaniae* (Rambur) (Neuroptera: Hemerobiidae), as well as (i) various morphospecies of active hunters or ambush spiders from the families of Salticidae, and Thomisidae on *B. spinosa*; and (ii) wasps, spiders, and brown lacewings on *L. continentale.* The most abundant morphospecies on *V. vinifera* and *Rytidosperma* ssp., was the European earwig, *Forficula auricularia* (Linnaeus) (Dermaptera: Forficulidae), followed by the transverse ladybird beetle, *Coccinella transversalis* (Fabricius) (Coleoptera: Coccinellidae) and the brown lacewing on *V. vinifera*. Morphospecies of wolf spiders and the brown lacewing were also abundant on *Rytidosperma* ssp. The highest evenness of predators was on *B. spinosa* and *L. continentale,* followed by *Rytidosperma* ssp., and *V. vinifera.*

Table 8. Richness, dominance and diversity of arthropods for each plant species (all morphospecies and predator morphospecies)

[1] P_{max} is the proportion of individuals belonging to the most abundant morphospecies. Formulas from McCune and Grace (2002), Shannon (1948), Simpson (1949) and (Hill, 1973).

Diversity index ***n* = sample date-site combination**	**Traditional formula**[1]	***Bursaria spinosa*** 40		***Leptospermum continentale*** 20		***Vitis vinifera*** 41		***Rytidosperma* ssp.** 28	
		All	**Predators**	**All**	**Predators**	**All**	**Predators**	**All**	**Predators**
Individuals		6,798	1,633	4,340	1,127	6,026	3,043	9,927	3,077
Richness (S)	# of morphospecies	185	67	147	63	127	56	100	38
Berger-Parker dominance (BP)	P_{max}	0.49	0.13	0.26	0.11	0.29	0.30	0.50	0.50
Simpson's dominance (D_2)	$1/\Sigma^{Pi_2}$	3.82	19.14	11.23	19.98	5.31	3.09	6.50	5.39
Simpson's evenness (E)	D_2/S	0.02	0.29	0.08	0.32	0.04	0.06	0.06	0.14

Morphospecies accumulation curves

Randomised morphospecies accumulation curves are clearly approaching an upper asymptote in every case that reflects the estimated total numbers of arthropod species that are associated with each plant. Species accumulation curves for all arthropod species showed two groups comprising the arthropods associated with *B. spinosa* and *L. continentale,* and then *V. vinifera* and *Rytidosperma* ssp. (Figure 1). There was little difference in the number of morphospecies found within each pairing at the standardised sample size of 20 sites. The first pair is clearly associated with more diverse communities of arthropods than the second.

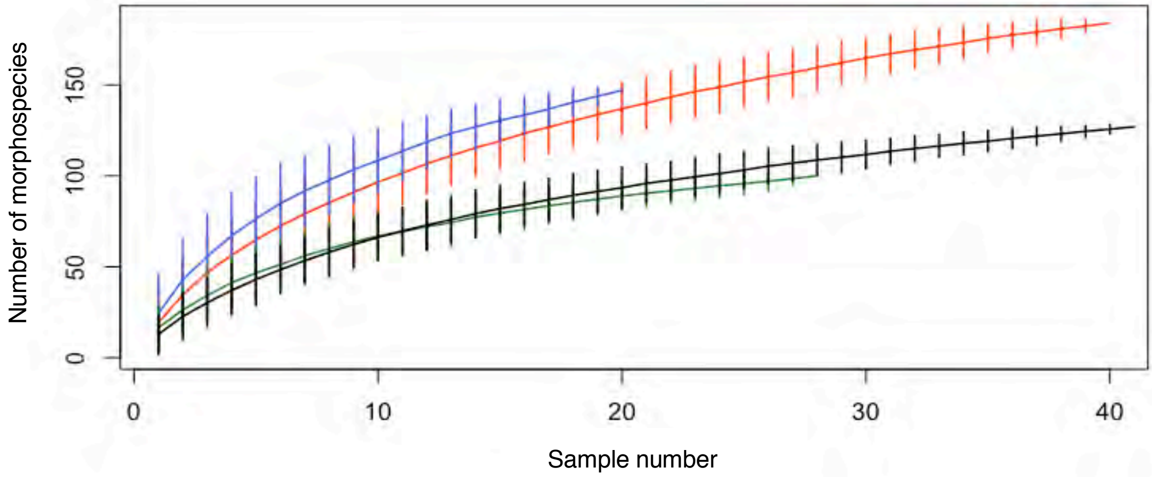

Figure 10. Randomised species accumulation curves generated using the total number of arthropod morphospecies found in association with *Bursaria spinosa* (red line)*, Leptospermum continentale* (blue line)*, Vitis vinifera* (black line)*,* and *Rytidosperma* ssp. (green line).

This trend was also shown by the predator communities, but they showed greater differences in the number of morphospecies found between each vegetation type (Figure 2). Species accumulation curves for spider communities showed two groups comprising *B. spinosa*, *L. continentale* and *V. vinifera*, and then *Rytidosperma* ssp. (Figure 3).

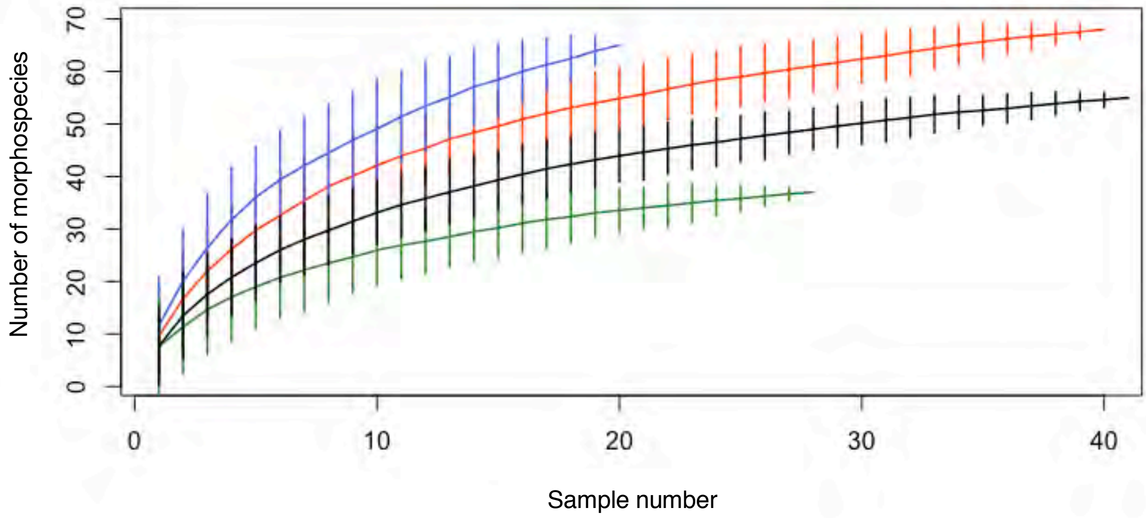

Figure 11. Randomised species accumulation curves generated using the total number of predatory morphospecies found in association with *Bursaria spinosa* (red line), *Leptospermum continentale* (blue line), *Vitis vinifera* (black line), and *Rytidosperma* ssp. (green line).

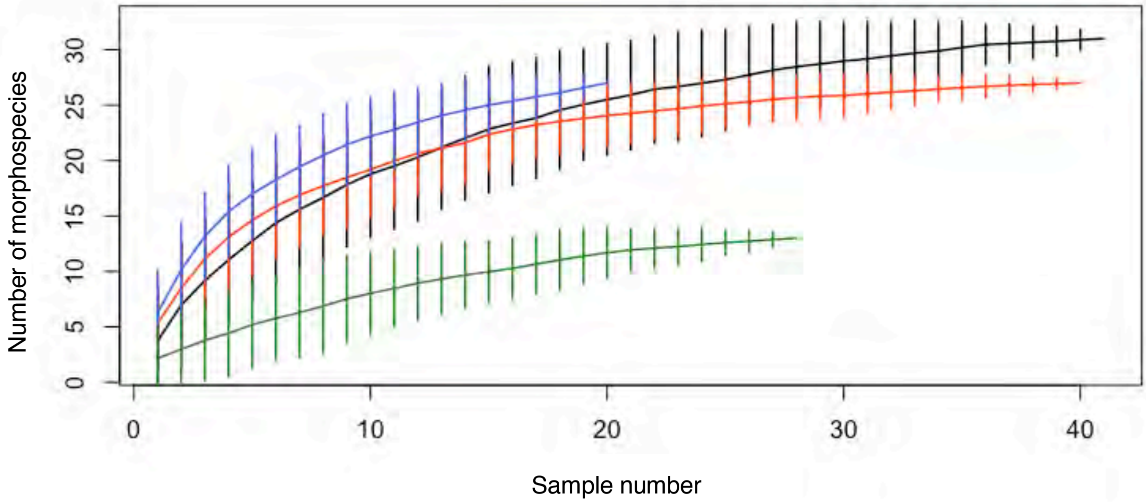

Figure 12. Randomised species accumulation curves generated using the total number of spider morphospecies found in association with *Bursaria spinosa* (red line), *Leptospermum continentale* (blue line), *Vitis vinifera* (black line), and *Rytidosperma* ssp. (green line).

True diversity index (p_i)

Based on comparison of Shannon's true diversity associated with each plant of H', the greatest diversity of arthropods (p_i) was detected on *L. continentale,* followed by *B. spinosa*. *Vitis vinifera* and *Rytidosperma* ssp. had similar values of true diversity (Figure 4). When predators were assessed using the same index, *B. spinosa* and *L. continentale* were closely aligned and exhibited the greatest predator diversity, followed by *V. vinifera* and *Rytidosperma* ssp. with a clear gap present between these two pairs of plants (Figure 5). This suggests incorporating each of these locally-adapted native plants in and around vineyards it may be possible to increase the functional diversity offered by predatory arthropods more than three times when *B. spinosa* or *L. continentale* is incorporated into a landscape containing vineyards. The highest number of spider morphospecies was observed on *B. spinosa*, *L. continentale* and *V. vinifera* compared to *Rytidosperma* ssp. (Figure 6).

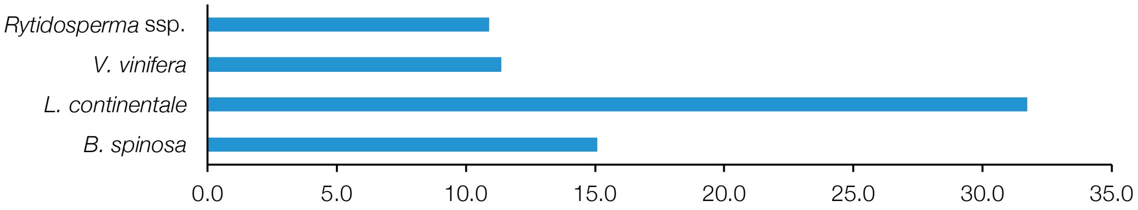

Figure 13. Overall arthropod morphospecies diversity (p_i) based on Shannon's index for each plant.

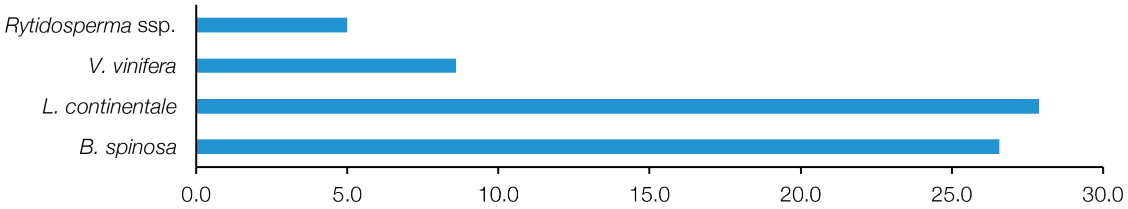

Figure 14. Predatory arthropod morphospecies diversity (p_i) based on Shannon's index for each plant.

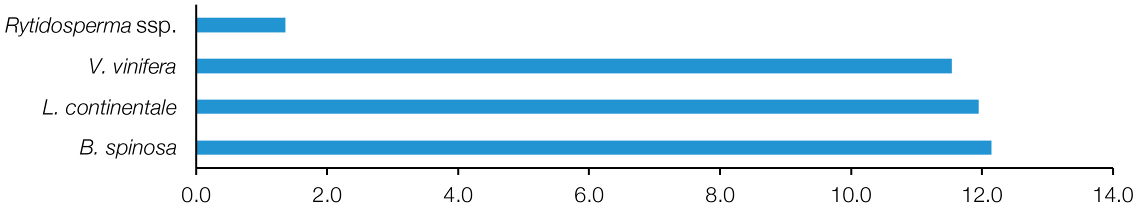

Figure 15. Spider morphospecies diversity based (p_i) on Shannon's index for each plant.

Similarity

The Jaccard index of similarity for all morphospecies (Table 3a), predators (Table 3b) and spiders (Table 3c) was highest between *B. spinosa* and *L. continentale*. The proportional emphasis afforded by the index to singletons versus no singletons (removed) skewed the numbers both ways, but did not change the overall hierarchy of results from highest to lowest assemblage similarity. Conversely, *Rytidosperma* ssp. consistently exhibited the lowest number of shared morphospecies of each of the plant pairs. However, wallaby grasses provide valuable habitat for morphospecies other than those commonly found in association with the woody perennial plants, such as wolf spiders, rove beetles, carabid beetles, and assassin bugs. When *Rytidosperma* ssp. is included in a plant assemblage with each woody plant species, this could result in a net increase in predator morphospecies of 25% to 30% (Table 4). Morphospecies of spiders, ladybird beetles, carabid beetles, earwigs, shield bugs, and lacewings were found across all four plant species.

Table 9. Estimated similarity of morphospecies (Jaccard's similarity coefficient) associated with each plant with and without singletons. (a) All arthropod morphospecies (b) predator morphospecies and (c) spider morphospecies.

Focal plant species	**Comparative plant species**							
	***n* = morphospecies**		***Bursaria spinosa***		***Leptospermum continentale***		***Vitis vinifera***	
	All	**No singletons**	**All**	**No singletons**	**All**	**No singletons**	**All**	**No singletons**
(a) all morphospecies								
B. spinosa	185	134						
L. continentale	147	110	0.52	0.57				
V. vinifera	127	90	0.37	0.33	0.39	0.35		
Rytidosperma ssp.	100	79	0.21	0.15	0.20	0.15	0.29	0.24
(b) predators								
B. spinosa	67	54						
L. continentale	63	53	0.67	0.70				
V. vinifera	56	43	0.52	0.56	0.59	0.52		
Rytidosperma ssp.	38	31	0.25	0.20	0.23	0.18	0.32	0.28
(c) spiders								
B. spinosa	27	26						
L. continentale	27	21	0.74	0.74				
V. vinifera	30	25	0.68	0.70	0.73	0.64		
Rytidosperma ssp.	13	10	0.29	0.24	0.33	0.19	0.34	0.30

Table 10. Estimated number of shared and unique morphospecies (Jaccard's similarity coefficient) associated with each and with one another. (a) All arthropod morphospecies (b) predators and (c) spider morphospecies.

Focal plant species	**Comparative plant species**								
	Bursaria spinosa			***Leptospermum continentale***			***Vitis vinifera***		
	Shared	**Unique**		**Shared**	**Unique**		**Shared**	**Unique**	
		Sp. 1	**Sp. *n***		**Sp. 2**	**Sp. *n***		**Sp. 3**	**Sp. *n***
(a) all morphospecies									
B. spinosa (Sp.1)									
L. continentale (Sp.2)	113	72	34						
V. vinifera (Sp.3)	84	101	43	77	70	50			
Rytidosperma ssp. (Sp.4)	49	136	51	41	106	59	51	76	49
(b) predators									
B. spinosa (Sp.1)									
L. continentale (Sp.2)	52	15	11						
V. vinifera (Sp.3)	42	25	14	44	19	12			
Rytidosperma ssp. (Sp.4)	21	46	17	19	44	19	23	33	15
(c) spiders									
B. spinosa (Sp.1)									
L. continentale (Sp.2)	23	4	4						
V. vinifera (Sp.3)	23	4	7	24	3	6			
Rytidosperma ssp. (Sp.4)	9	18	4	10	17	3	11	19	2

Ordination (dissimilarity) of arthropod communities

The NMDS-ordination (stress value = 0.13) revealed broad clusters for each plant species with substantial overlap among them (Figure 7). This indicates the dissimilarity distances among *V. vinifera* versus *B. spinosa, L. continentale* and *Rytidosperma* ssp. are low according to the Bray-Curtis dissimilarity index. *Vitis vinifera* and *B. spinosa* had a high level of variation among sample sites and dates in the diversity associated with these plants. This is demonstrated by the larger size of each envelope of data points associated with each species. The dissimilarity was greatest between *L. continentale* and *Rytidosperma* ssp. but these plants had less variation in diversity associated with samples. This is demonstrated by the smaller size of each envelope of points.

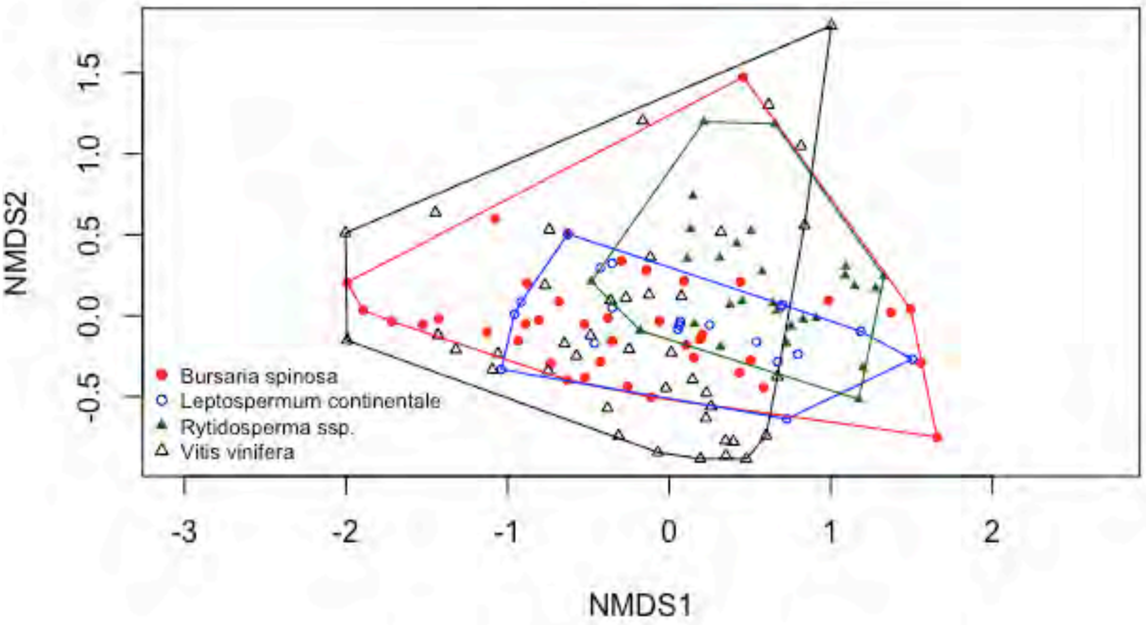

Figure 16. Ordination scatter plot showing the scaled distribution of arthropod diversity dissimilarity associated with sites and sampling dates when the arthropod community was sampled on each plant species. Community structure was extracted by NMDS ordination using the Bray-Curtis dissimilarity index for arthropod morphospecies mean abundance data. Lines represent envelopes enclosing the data points associated with each plant.

Discussion

A functional diversity of predatory arthropods is desirable to target different life stages of economically damaging pests to enhance pest suppression (Hogg and Daane, 2014). Top down control of pests can be strengthened when a range of predators complement each other (Gurr et al. 2003; Letourneau et al. 2009). The analyses reveals that each of the three insectary plant species has the capacity to contribute to an increase in functional diversity and enhance the diversity and abundance of predatory arthropods when planted in association with a vineyard. A comparison of diversity indices among them shows that the woody native plants are associated with a higher number of arthropod morphospecies overall, as well as a higher number of predatory morphospecies, when compared to grapevines or wallaby grass. *Rytidosperma* ssp. is associated with dissimilar predatory arthropods, which could provide complementarity functional diversity (Montoya et al. 2012). Diverse assemblages of predatory arthropods are preferred as they are frequently more effective in reducing the density of herbivore pests (Cardinale et al. 2003).

Morphospecies accumulation curves can provide a useful measure to predict the richness of arthropods on each plant by using a standard number of sampling units (Moreno and Halffter, 2001). Each curve approached an upper asymptote, which suggests that the most common species are included in the species inventories and sampling has exceeded the minimum effort required for adequate completeness of the inventories (Willott, 2001). *Bursaria spinosa* and *L. continentale* are associated with a higher richness of arthropod morphospecies. Greater numbers of species are important requirement for ecosystem multifunctionality (Hooper et al. 2005; Hector and Bagchi, 2007; Byrnes et al. 2014; Lefcheck et al. 2015). The similar number of morphospecies found on the woody plants indicates they may provide comparable habitat features. This is likely partly due to the evergreen nature of these shrubs.

Additional measures of diversity also provide a more comprehensive picture of the communities associated with each plant. Shannon's transformed measure (pi), clearly estimates the true diversity of arthropods associated with each plant when they are considered together in total and in functional groups (Jost, 2006). A comparison of the indices suggests that *L. continentale* and *B. spinosa* have a greater capacity to enhance functional biodiversity. Both plants have the capacity to enhance the functional diversity of the system by more than three times when they are planted in association with grapevines (Figure 5). Similarly, the inclusion of all three insectary plants in and around vineyards could nearly double the predatory morphospecies richness. If such an increase can be realised, then the overall effectiveness of biological pest control could be enhanced by adding functional redundancy (Letourneau et al. 2015).

The ordination of plant species by sample and location indicated that the community structure in the vineyard overlapped to some extent. Some of the same arthropods were found on all of the insectary plants. But this analysis showed that there is complementarity among the different morphospecies that are not found on grapevines. Ordination reveled a higher level of variation in arthropod diversity associated with *B. spinosa* and *V. vinifera,* which is also reflected in the lower evenness scores for each. This suggests that there are likely to be site specific factors that affect which predatory species are found in association with each plant.

Conversely, the smaller size of each envelope of points for *L. continentale* and *Rytidosperma* ssp. is reflected in the higher evenness scores of each plant species. This suggests that the fauna associated with these plants is more consistent from site to site.

A resource bottleneck may result in an interruption in the presence of predators and parasitoids that may otherwise breed continuously in the presence of nourishing sources of food and prey (Schellhorn et al. 2015). Due to the seasonality of foliage growth of grapevines and wallaby grass, the reduction in 'SNAP' resources during dormancy may result in a decline in provisioning resources available to predators. However, it should be possible to fill these resource gaps. The flowering period of *L. continentale* is typically from August to December while *B. spinosa* flowers from November to February (Retallack et al. 2019). Therefore, it is possible to have plants that flower over seven months of the growing season or more, if the appropriate species are planted in combination with vineyards. This would extend the period when floral resources are available to natural enemies. These plants could also serve as a refuge. Spray application against fungal pathogens and insects, as well as some soil management practices, can adversely affect arthropod populations (Yachi and Loreau, 1999). The presence of insectary plants planted nearby should facilitate recolonisation of vineyards by predator populations after disturbances (Tscharntke et al. 2005).

The dissimilarities of morphospecies diversities between grapevines and each prospective insectary plant suggest that they are associated with arthropods that may have different traits that provide complementary functions compared to those that are more common on grapevines (Loreau, 2000). For example, perennial cover crops can function as an 'ecological turn-table', which has the capacity to activate and influence key processes and components of an agroecosystem (Altieri, 1999). Wallaby grasses provide habitat for predatory morphospecies that are not commonly found in association with woody plants. European earwigs, wolf spiders and brown lacewings were species that were more commonly found in association with wallaby grass. Each of these predators are likely to contribute to biological pest control, as they are reported to feed on larvae of *E. postvittana* and other Lepidoptera that cause damage in vineyards (Bernard et al. 2006b; Frank et al. 2007; Paull, 2007; Thomson and Hoffmann, 2009b; Thomson and Hoffmann, 2010; D'Alberto et al. 2012; Hogg et al. 2014). The lower degree of predatory species overlap with the other plant species implies that *Rytidosperma* ssp. should add different and potentially complementary diversity to a vineyard ecosystem. This is consistent with the hypothesis that more species are needed to assure function (Montoya et al. 2012). The growth habit of *Rytidosperma* ssp. makes these grasses conducive to being planted under-vine and in the mid-row areas where woody plants are unsuitable.

A greater complexity of habitat structure can contribute to greater arthropod diversity, as has been demonstrated in previous studies on spider communities (Costello and Daane, 1997; D'Alberto et al. 2012; Hogg and Daane, 2015; Rosas-Ramos et al. 2018). Species rich plantings are preferred to support multiple trophic levels of arthropods (Soliveres et al. 2016). Plant diversification promotes diverse arthropod communities that may provide greater stability of ecosystem provisioning (Lichtenberg et al. 2017). Larger natural areas of vegetation are favoured but the vegetation that remains is often fragmented in production systems. The conservation of small patches may present a good strategy to maximise diversity within the landscape, especially for plants and arthropods that require smaller habitats in association with simple production landscapes (Tscharntke et al. 2002a).

Research indicates that growers are willing to adopt appropriate advice about the use of non-crop insectary plants based on sound research (Shields et al. 2016). The three plant species considered here could contribute to such a strategy for vineyards. The different arthropod communities found in association with perennial grass strips, native evergreen plants and deciduous vines could combine in a landscape that provides more consistent biological control of damaging arthropods.

Conclusion

Our findings indicate that the native, perennial, evergreen plants *B. spinosa* and *L. continentale* have the capacity to support a higher diversity of predators than grapevines alone, and hence could increase the abundance and diversity of predators in the associated grapevines. By incorporating each of these native plant assemblage in and around vineyards it may be possible to increase the functional diversity offered by predatory arthropods, by more than three times when *B. spinosa* and *L. continentale* are incorporated versus grapevines only. *Rytidosperma* ssp. should provide complementarity through its association with dissimilar predatory arthropods. When *Rytidosperma* ssp. is included in a plant assemblage with of each woody plant species and grapevine, this could result in a further net increase in predator morphospecies richness in the order of 27%.

The incorporation of native insectary plants *B. spinosa*, *L. continentale* and *Rytidosperma* ssp. has the potential to enhance biodiversity in a vineyard. This could improve biological control by providing a suitable habitat to support diverse and functional populations of predatory arthropods. Vineyard managers are encouraged to explore the use of insectary plants in association with vineyards.

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CHAPTER SIX

Distribution modelling of three native insectary plants

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MR collected samples, identified arthropods, interpreted data and wrote the manuscript. DM, LT and MK contributed to the manuscript production. MK helped in developing the idea, provided guidance throughout and assisted with modelling analysis.

Data availability

The datasets generated and analysed during the study along with supporting documentation are available in The University of Adelaide Figshare online digital repository (DOI 10.4225/55/5b0a3a8155f39, 10.4225/55/5b0a3a9e772db, 10.4225/55/5b0a3ab69fdac).

Abstract

Background and Aims: I evaluated the existing and potential geographic distribution of two native woody insectary plants and six species of native insectary grasses. This was done to determine suitability for planting them in association with grapevines under different climatic conditions around Australia.

Methods and Results: The existing geographic distributions of the two locally-adapted native plants *B. spinosa and L. continentale, and the six native grasses R. caespitosum, R. duttonianum, R. fulvum, R. pilosum, R. racemosum,* and *R. setaceum* were determined using currently available geographical distribution records. Climatic data for Australia were obtained for a historical thirty-year time period. Ecological niche models were produced for each species. A maximum entropy species distribution modelling algorithm was used to highlight the relationship of each plant to its potential niche. The results indicate that the suitable areas for these plants are south-western, south-eastern and eastern Australia, extending up to south-eastern Queensland. This broadens the potential range of each plant species to potentially take in all of the major wine growing regions within Australia.

Conclusion: The insectary plants are naturally adapted to all of the major wine growing regions within Australia and could be potentially planted wherever wine grapes are grown.

Significance of the Study: This study facilitates the testing of the contribution these plant species can potentially make towards supporting populations of natural enemies and conservation biological control efforts throughout the Australian wine sector.

Key words: native insectary plants*, B. spinosa, L. continentale, Rytidosperma* ssp.*,* vineyards

Introduction

Species distribution modelling can be used to examine the potential range of species under different climatic conditions. Ecological niche models are used to determine the potential geographic distribution of organisms, and to assess how well they are adapted to different locations (Peterson and Cohoon, 1999). This approach can also be used to assess if plants are suited to growing outside their native range (Phillips et al. 2006). In much the same way, the potential range of *E. postvittana* has been predicted and mapped to identify which coastal areas are most likely to support elevated populations of this pest (Gutierrez et al. 2010; Thomson et al. 2010a; Lozier and Mills, 2011). If the potential plant species range is broader than the current realised range, then this could extend the geographic area where insectary benefits are available to predatory arthropods. We assessed the potential geographic distribution of three selected insectary plant species in order to predict their potential suitability for planting in association with grapevines around Australia.

Prospective native insectary plants

Christmas bush, *Bursaria spinosa* (Cav.) (Apiales: Pittosporaceae) is an erect, evergreen, prickly shrub, one to four metres tall (Figure 1a). It is ubiquitous throughout southern and eastern Australia and flowers from late spring until mid to late summer. The flowers are creamy-white, fragrant, about 10 mm in diameter and grow in dense terminal clusters (Jebb and Andrews, 2001). They provide a plentiful supply of both pollen and nectar, and attract a wide range of arthropods (Webb, 1994; Retallack et al. 2019).

Prickly tea-tree, *Leptospermum continentale* (Forst. and G.Forst) (Myrtales: Myrtaceae) is an erect, evergreen, prickly leaved shrub that grows up to two metres tall and is also endemic to southern and eastern Australia (Figure 1b). Masses of white flowers 10 mm wide occur from early spring to late summer, resulting in capsular fruits that persist on older wood. Arthropods are attracted to the nectar and pollen produced by the flowers (O’Brien, 1994; Retallack et al. 2019), and these resources are reported to help to extend the longevity of parasitoid wasps (Pandey et al. 2018).

Wallaby grasses, *Rytidosperma* ssp. (DC) (Poales: Poaceae) are erect, tufted perennials, with fine leaves and distinctive white, fluffy seed heads when mature (Figure 1c). They grow from 30 to 80 cm depending on the species and growing conditions, and flowering occurs in late spring and early summer. Wallaby grasses grow actively during the spring and enter dormancy when conditions dry out in summer (Penfold and McCarthy, 2010). *Rytidosperma* ssp. are endemic in southern and eastern Australia and are known to support the presence of a range of predatory arthropods including brown lacewings, spiders, beetles, thrips and parasitoids (Danne et al. 2010; Penfold and McCarthy, 2010; Wood et al. 2011).

Different *Rytidosperma* ssp. can be selected for specific tolerance to wet sites, drought, heat, frost, acid soils, or low growing habit for use under perennial crops (Prescott, 2016). Six species of wallaby grasses were assessed in this study, comprising common wallaby grass, *R. caespitosum*, brown-back wallaby grass, *R. duttonianum*, copper-awned wallaby grass, *R. fulvum*, hairy wallaby grass, *R. pilosum*, slender wallaby grass, *R. racemosum*, and small-flowered wallaby grass, *R. setaceum* to add to previous research by Retallack et al. (2019) where a mixture of these *Rytidosperma* ssp. were assessed.

Figure 17. Prospective Australian native insectary plants, *B. spinosa* (a), *L. continentale* (b), and *Rytidosperma* ssp. (c). Photos: Mary Retallack

Methods

The potential geographic distribution of each of the plant species was modelled in order to determine its suitability for planting in association with grapevines around Australia. Known locations where each plant species occurs were obtained from the Atlas of Living Australia (https://www.ala.org.au) and visualised using DIVA-GIS (http://www.diva-gis.org) to identify and eliminate clearly invalid records, which were located in the sea. Climatic data on nine variables [annual cloud cover (%), mean annual diurnal temperature range (°C), mean annual ground-frost frequency (days), mean annual precipitation (mm), mean annual minimum temperature (°C), mean annual mean temperature (°C), mean annual maximum temperature (°C), mean annual vapour pressure (torr), and mean annual wet day frequency (days)] were obtained from the International Panel for Climate Change (IPCC: http://www.ipcc-data.org/cgi-bin/ddc_nav/dataset=cru21) for a thirty year time period (1961-1990; (Mitchell et al. 2004). I assume that these variables represent the range of conditions that are characteristic of their native range. The time period was selected to align with the historical data for each plant.

Ecological niche models were produced for each species using a maximum entropy species distribution modelling algorithm (MaxEnt V3.4.1; (Phillips et al. 2018). Environmental data layers were initially screened by the jackknife test, which was used to assess the importance of each variable in the MaxEnt model. Percent variable contribution and jackknife procedures in MaxEnt indicate the relative importance of different bioclimatic predictors. Environmental data layers with low percent contribution (below 5%) and low permutation importance (low Jackknife test gain) were removed until a 'best fit' was achieved. Variable response curves show how each environmental variable affects the MaxEnt prediction and highlight the relationship of each to a plant's potential niche.

Results

I obtained 30,209 valid geographical distribution records for *B. spinosa,* 16,403 records for *L. continentale,* 18,581 records for *R. caespitosum,* 3,630 records for *R. duttonianum,* 4,548 records for *R. fulvum*, 7,135 records for *R. pilosum,* 13,097 records for *R. racemosum,* and 18,573 records for *R. setaceum.*

Between and three and six environmental variables contributed to the to the MaxEnt models for each plant species (Table 1). The final models show the variables and the percent contribution each makes, combined with the response curves (Appendix 1). This provides the basis for the potential range predictions of each species presented in the maps, as well as the current geographical distributions (Figure 2).

A jackknife test in MaxEnt showed the variables with the greatest influence on the models. These included maximum temperature and wet day frequency for all species. For *B. spinosa* and *R. racemosum* an annual maximum temperature of approx. 12 °C degrees was associated with the highest probability of occurrence. For *L. continentale* an annual wet day frequency of approx. 9 days was associated with the highest probability of occurrence. For *R. caespitosum, R. duttonianum, R. fulvum,* and *R. pilosum* an annual maximum temperature of approx. 17 °C degrees was associated with the highest probability of occurrence. For *R. setaceum* an annual maximum temperature of approx. 14 °C degrees was associated with the highest probability of occurrence.

The results indicate that the suitable areas for these plants are south-western Western Australia, southern South Australia, Victoria, eastern New South Wales, south-eastern Queensland and eastern Tasmania, with the exception of *R. duttonianum*, which is less well adapted to south-eastern Queensland. These findings extend the potential range of each plant species to take in all of the major wine grape growing regions within Australia.

Table 11. Analysis of variable contributions to the MaxEnt model for native insectary plants.

Plant	Variable	Percent contribution	Permutation importance (%)
(a) *Bursaria spinosa*	Max temperature Ann 61-90	58.5	80.2
	Wet day freq. Ann 61-90	31.6	2.6
	Precipitation Ann 61-90	6.4	13.3
	Ground frost Ann 61-90	3.5	3.9
(b) *Leptospermum continentale*	Wet day freq. Ann 61-90	43.4	6.9
	Max temperature Ann 61-90	39.7	38.1
	Mean temperature Ann 61-90	10.9	43.3
	Precipitation Ann 61-90	6.0	11.7
(c) *Rytidosperma caespitosum*	Max temperature Ann 61-90	91.1	93.2
	Mean temperature Ann 61-90	5.5	1.7
	Precipitation Ann 61-90	3.4	5.1
(d) *Rytidosperma duttonianum*	Max temperature Ann 61-90	40.6	27.5
	Ground frost Ann 61-90	26.4	10.0
	Cloud cover Ann 61-90	11.0	32.8
	Precipitation Ann 61-90	10.0	12.1
	Mean temperature Ann 61-90	6.4	10.5
	Wet day freq. Ann 61-90	5.6	7.0
(e) *Rytidosperma fulvum*	Max temperature Ann 61-90	46.7	22.4
	Wet day freq. Ann 61-90	15.5	7.2
	Ground frost Ann 61-90	15.1	2.1
	Precipitation Ann 61-90	14.1	27.4
	Mean temperature Ann 61-90	8.5	41.0
(f) *Rytidosperma pilosum*	Max temperature Ann 61-90	53.6	10.9
	Wet day freq. Ann 61-90	20.5	2.0
	Mean temperature Ann 61-90	19.0	73.6
	Precipitation Ann 61-90	6.9	13.5
(g) *Rytidosperma racemosum*	Max temperature Ann 61-90	48.3	29.9
	Wet day freq. Ann 61-90	21.5	3.9
	Precipitation Ann 61-90	10.3	14.6
	Ground frost Ann 61-90	10.3	1.6
	Mean temperature Ann 61-90	9.6	50.0
(h) *Rytidosperma setaceum*	Max temperature Ann 61-90	87.6	83.9
	Precipitation Ann 61-90	6.7	8.9
	Mean temperature Ann 61-90	5.7	7.2

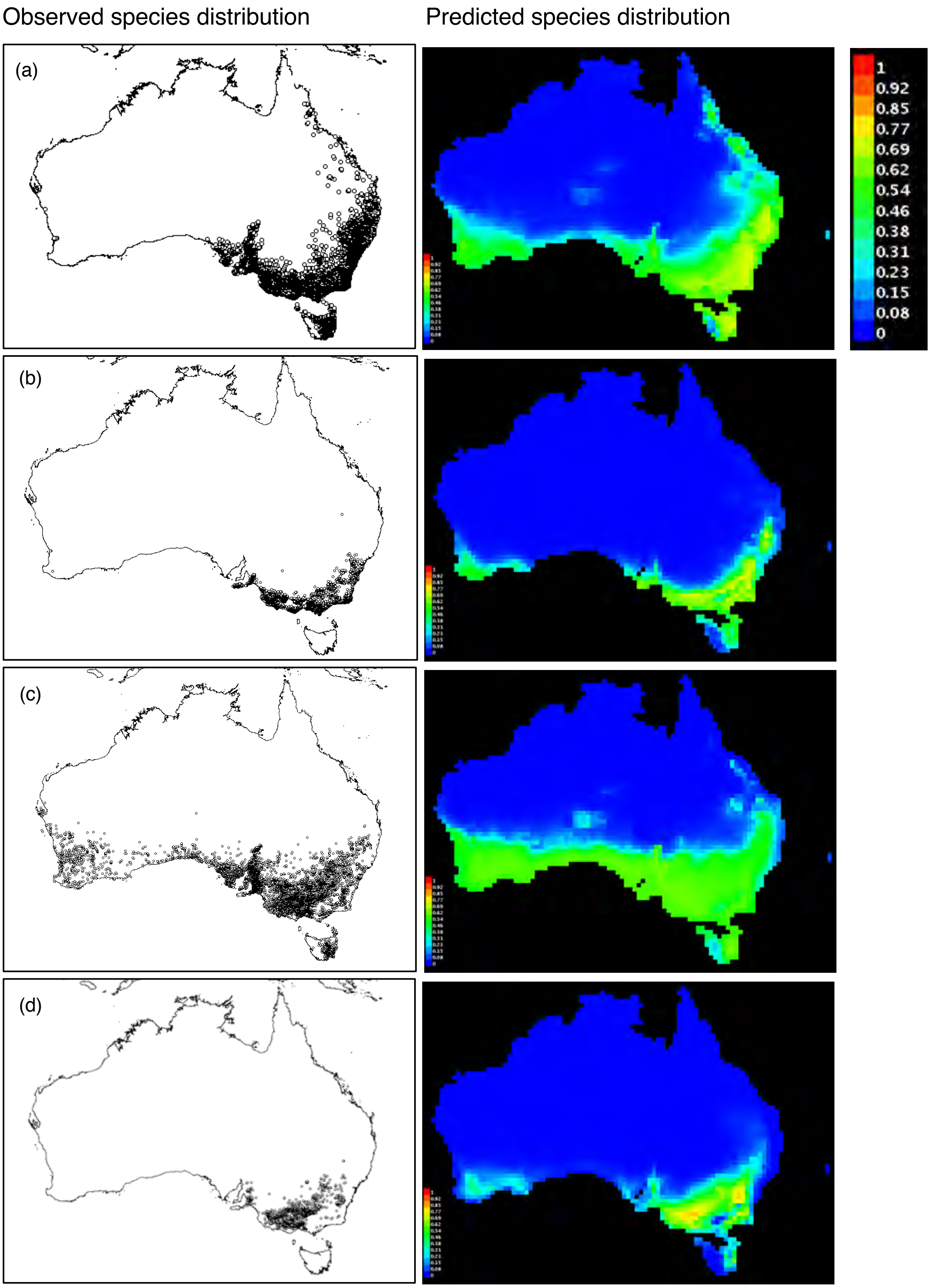

Figure 18. Observed and predicted potential distribution for native insectary plants. *Bursaria spinosa* (a), *Leptospermum continentale* (b), *Rytidosperma caespitosum* (c), *R. duttonianum* (d), *R. fulvum* (e), *R. pilosum* (f), *R. racemosum* (g), and *R. setaceum* (h). The known distribution is visualised using DIVA-GIS and the predicted potential distribution is plotted using MaxEnt. The colour scale is the estimated probability of habitat suitability (blue = unsuitable, red = most suitable).

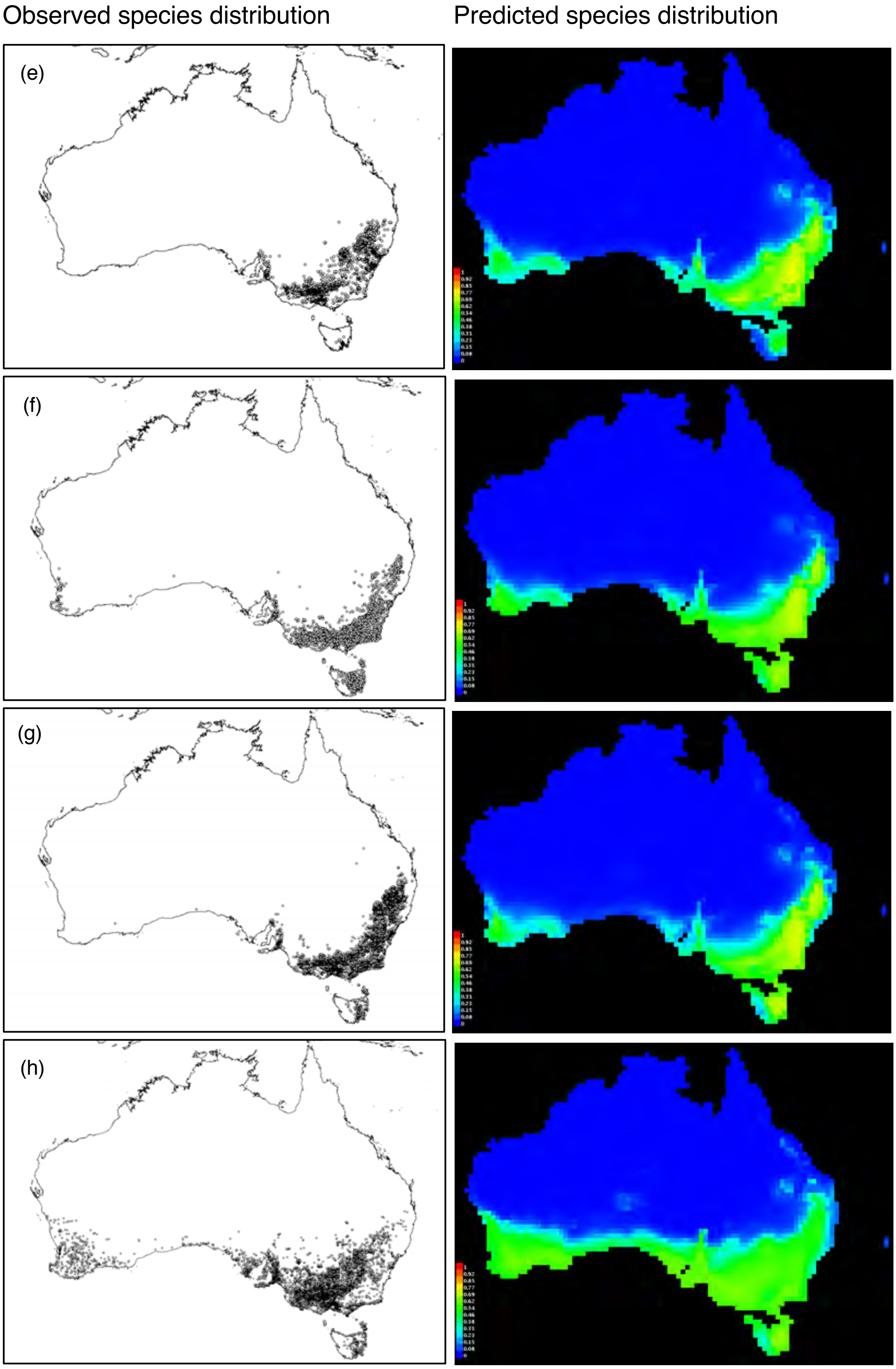

Figure 2. Observed and predicted potential distribution for native insectary plants [continued] *Bursaria spinosa* (a), *Leptospermum continentale* (b), *Rytidosperma caespitosum* (c), *R. duttonianum* (d), *R. fulvum* (e), *R. pilosum* (f), *R. racemosum* (g), and *R. setaceum* (h). The known distribution is visualised using DIVA-GIS and the predicted potential distribution is plotted using MaxEnt. The colour scale is the estimated probability of habitat suitability (blue = unsuitable, red = most suitable).

Discussion

Potential geographic distribution of native insectary plants

I found that the proposed native insectary plants have the potential to grow within all of the major wine grape growing regions of Australia (Wine Australia, 2017). The models produced for each plant species indicate that south and eastern Australia are potentially suitable for all species. It is anticipated that insectary benefits from a range of complementary locally-adapted native plants will help support the presence of predatory arthropods throughout the growing season. By broadening the range of suitable plants that can be planted in association with wine grapes, the insectary benefits available to predatory arthropods from plant resources would likely be increased. This broader range of plants in the landscape could provide additional ecosystem services benefits, by enhancing biodiversity.

In lieu of using introduced insectary species, native plants are preferred even in the event they are adapted outside their historic range. Many varieties of wine grapes are grown (Anderson and Aryal, 2013) in 65 geographically diverse regions throughout Australia (Bailey, 2016), each with its own set of climatic variables (Jarvis et al. 2017). The proposed insectary plants are readily available and can be purchased from nurseries around Australia. They would be planted into a landscape that is already being modified in places where grapevines are grown and can be irrigated. So, although these species might be grown outside their native range, the proposal to plant them as insectary species in managed landscapes should not pose any additional ecological risk.

The method used here is a useful way to predict the potential distribution of any native Australian plant. The distribution trends are similar between each plant species in the study and the model indicates that they have the potential to occur in similar environments. I have demonstrated that *B. spinosa, L. continentale*, and *Rytidosperma* ssp. each have the potential to grow successfully across a wide geographic range, including the major wine growing areas of Australia. Furthermore, these plants are known to support diverse and abundant populations of predatory arthropods (Retallack et al. 2019).

In addition to the native insectary plants that were assessed in this study, it is estimated that there are more than 21,000 flowering plants that are native to Australia (ANBC, 2015). Some of these plants have the potential to play a role as insectary plant species in each geographic range, including plants that show similar characteristics from the same genera as those studied. The climatic prediction does not forecast flowering season, synchrony with the seasonal development of grapevines or the capacity to model future distributions for these insectary plants using the same set of variables adopted here. It is proposed that this should be the focus of future research. Similarly, a diversity of insectary plants could be planted to extend the period of floral resource provision to natural enemies, which in turn provide regulating services.

Conclusion

The proposed insectary plants are naturally adapted to grow in all of the major wine grape growing regions within Australia and have the potential to be planted virtually wherever wine grapes are grown. This study facilitates testing the contribution of these plant species to conservation biological control throughout the Australian wine sector.

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Appendix 1: Response of each plant to variables and the percent contribution they make, combined with the response curves

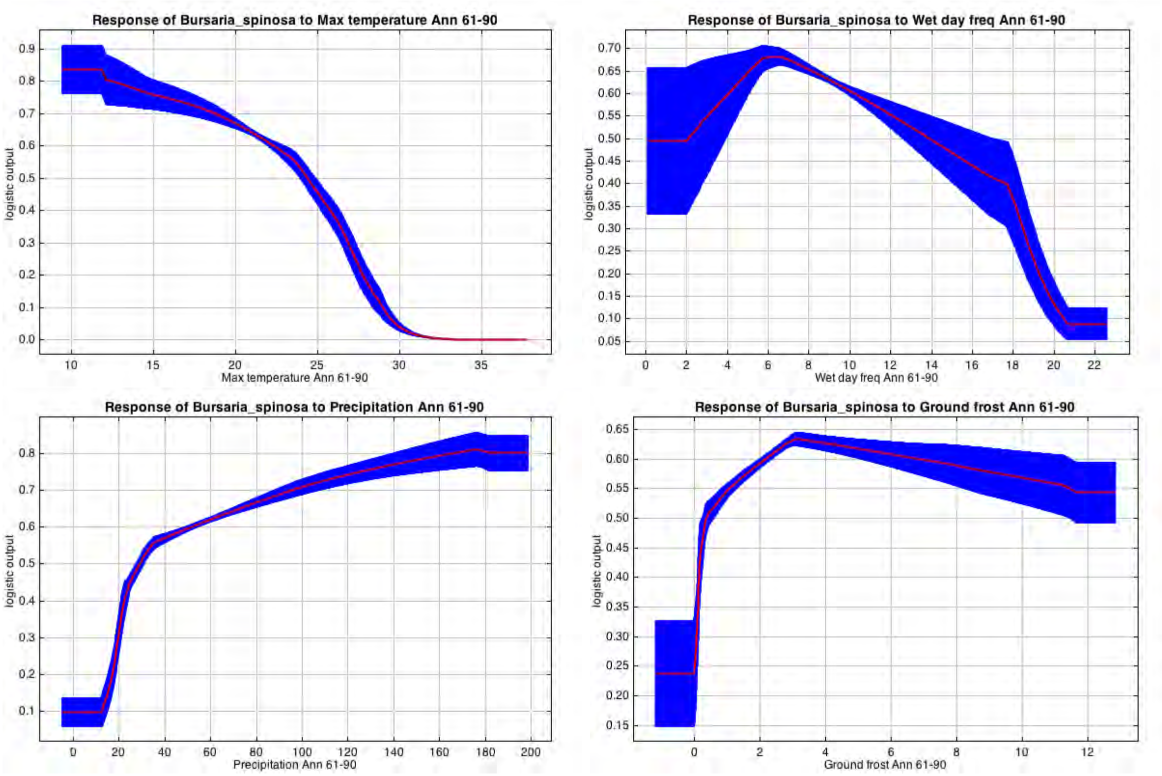

Figure 1. Response of *Bursaria spinosa* to variables and combined with the response curves

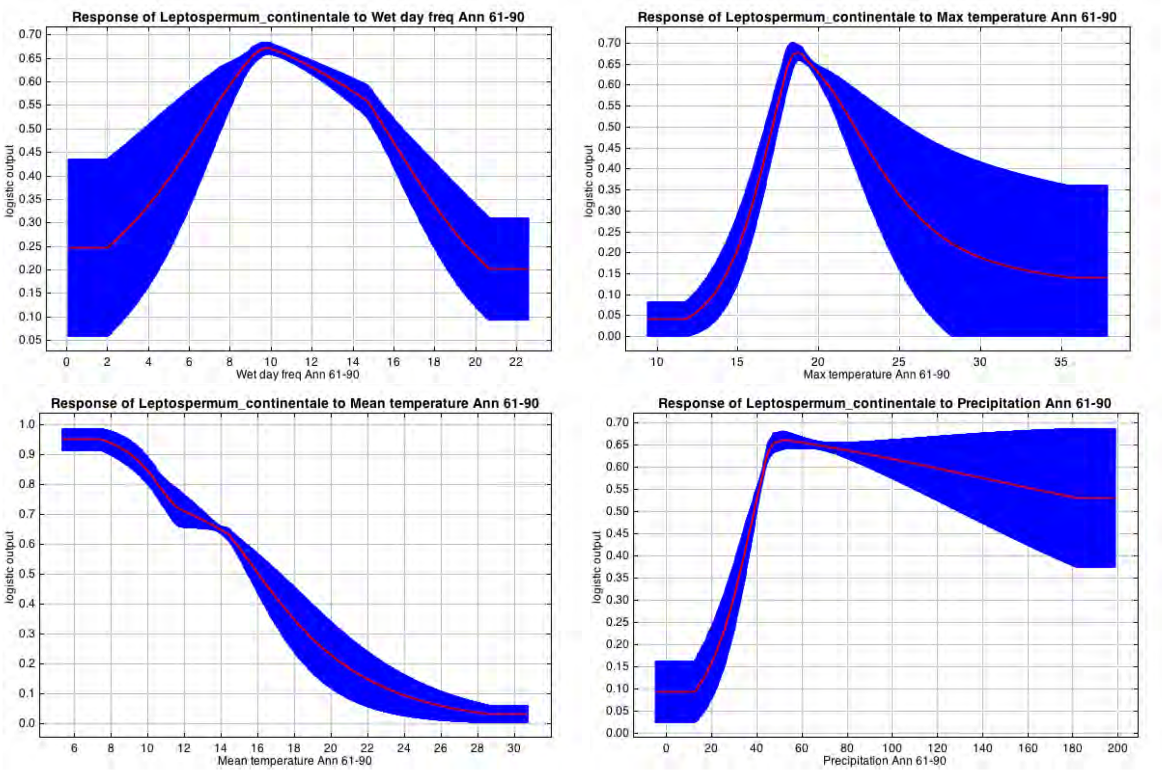

Figure 2. Response of *Leptospermum continentale* to variables and combined with the response curves

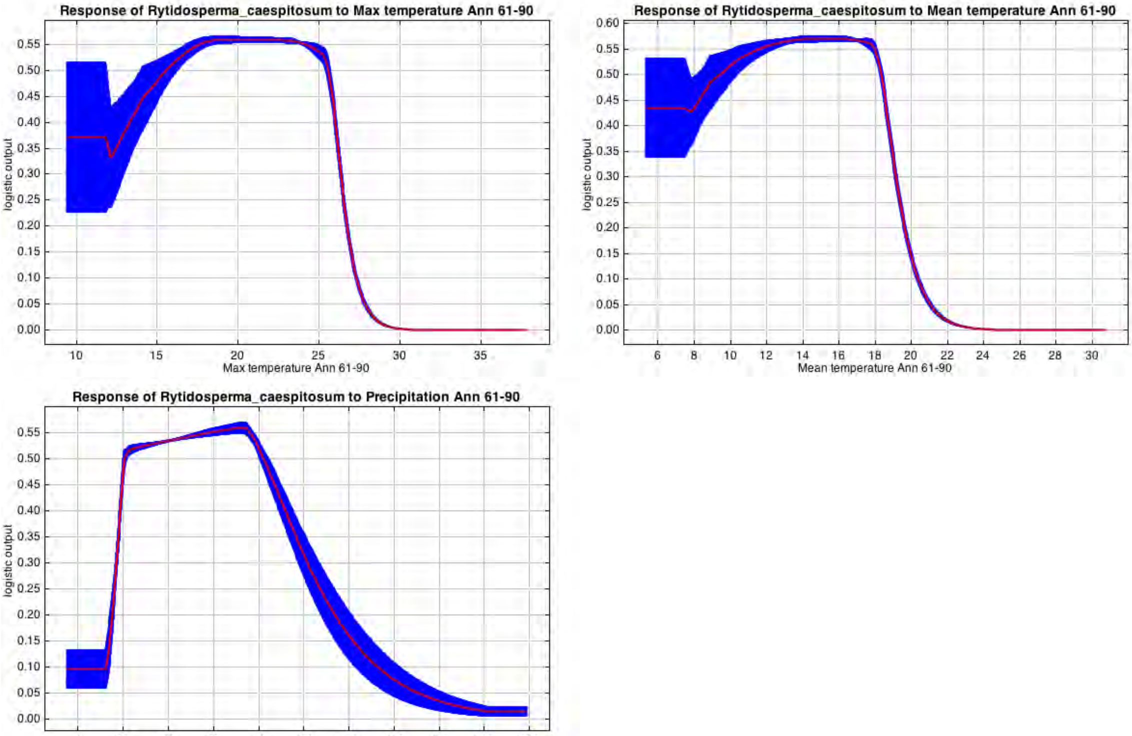

Figure 3. Response of *Rytidosperma caespitosum* to variables and combined with the response curves

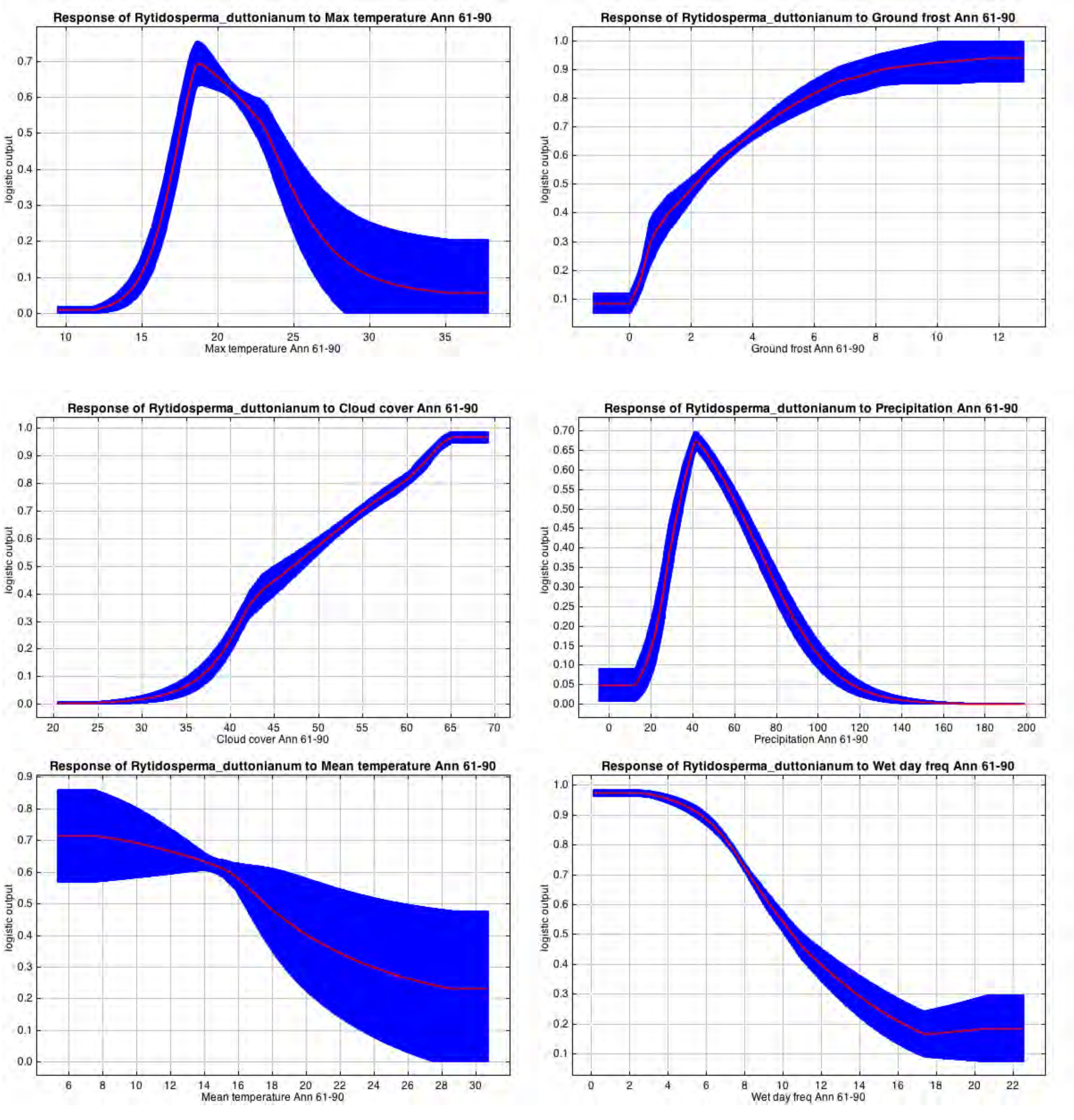

Figure 4. Response of *Rytidosperma duttonianum* to variables and combined with the response curves

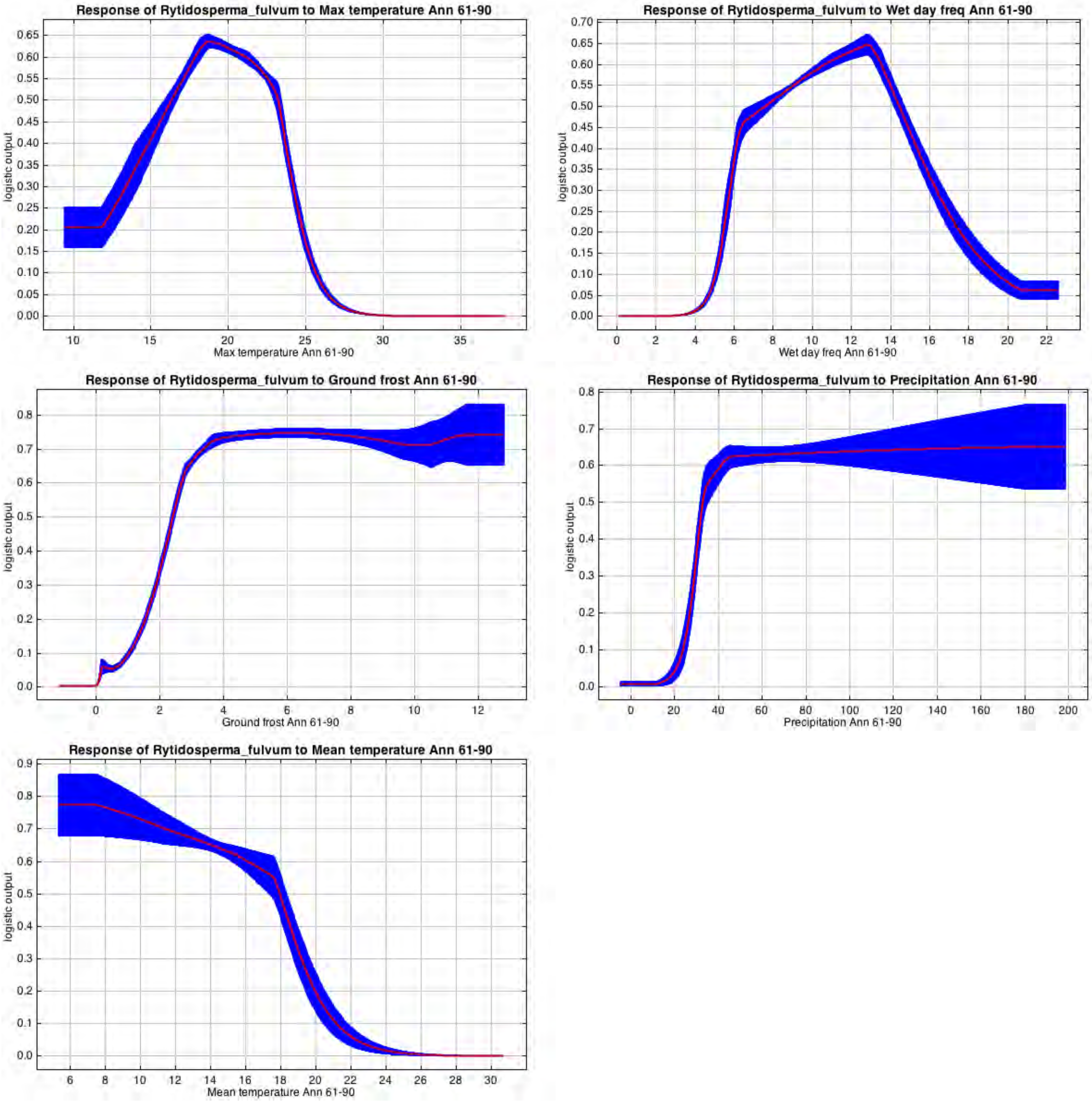

Figure 5. Response of *Rytidosperma fulvum* to variables and combined with the response curves

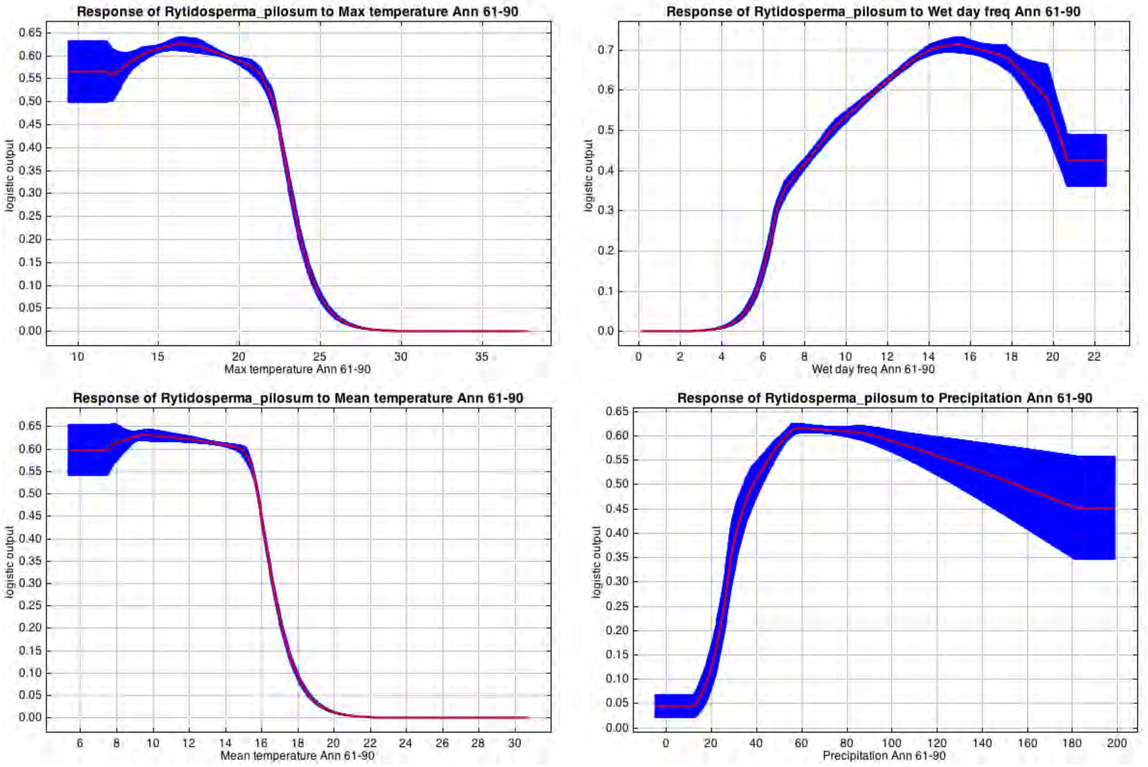

Figure 6. Response of *Rytidosperma pilosum* to variables and combined with the response curves

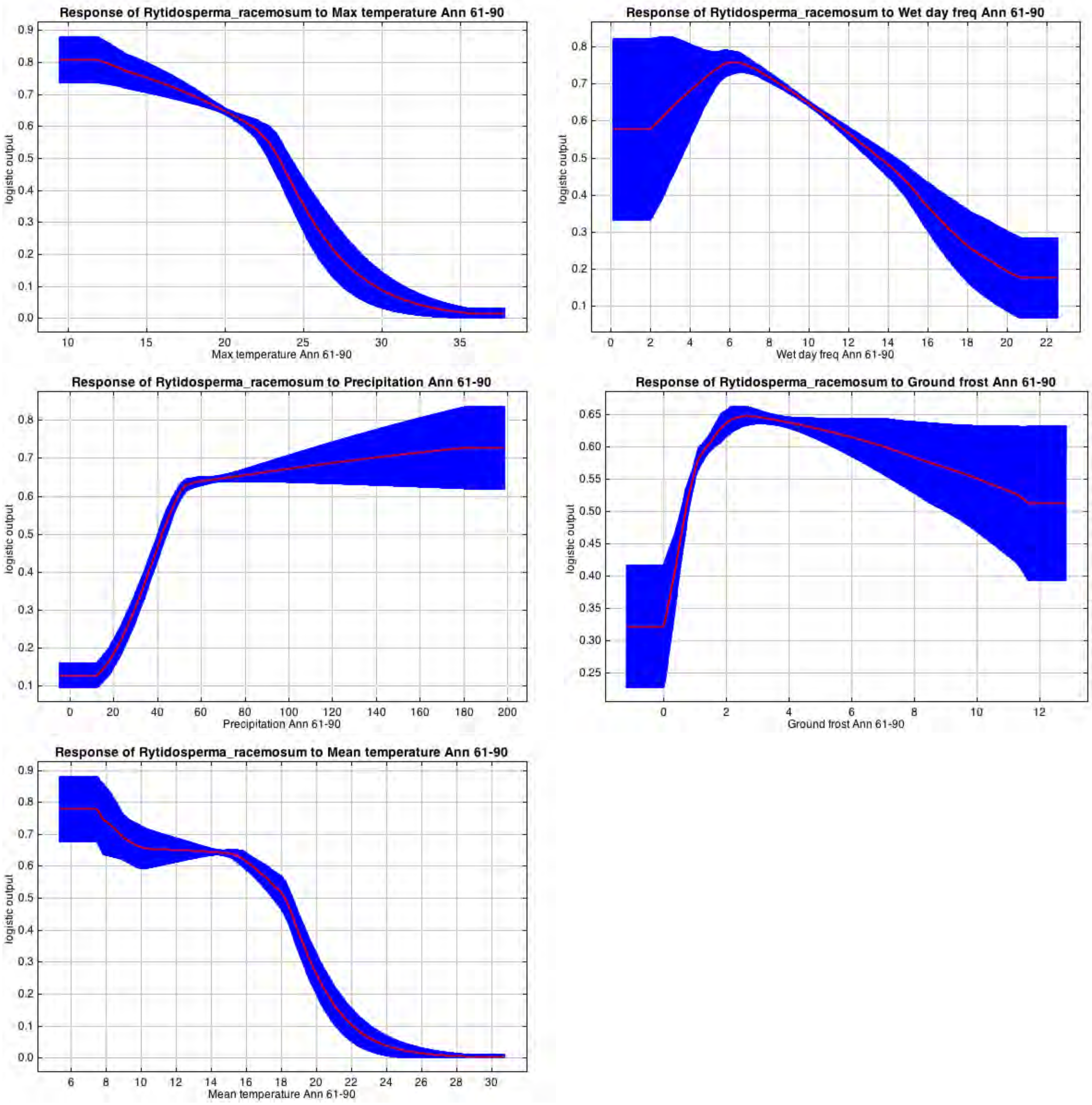

Figure 7. Response of *Rytidosperma racemosum* to variables and combined with the response curves

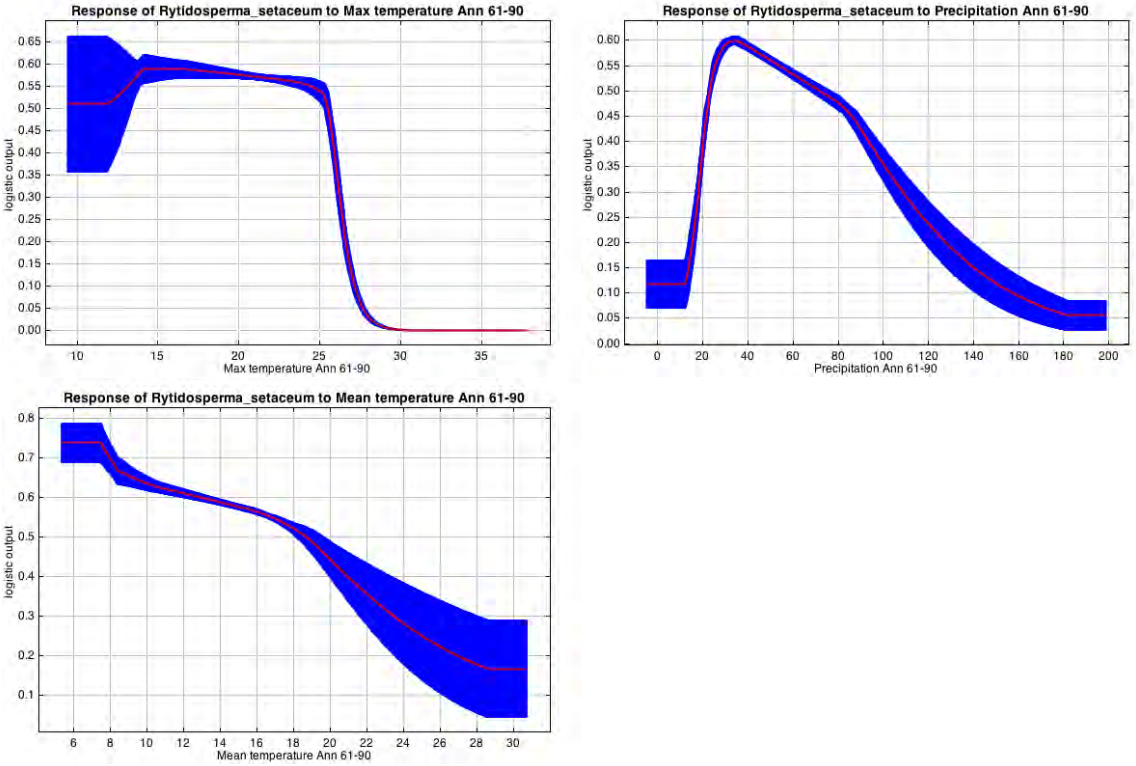

Figure 8. Response of *Rytidosperma setaceum* to variables and combined with the response curves

CHAPTER SEVEN

From theory to practice

General discussion

This section elaborates on the themes introduced in the body of the thesis and ties together the conclusions from each study. I discuss the practical significance of the research and suggest practical outcomes, so these conceptual ideas can be evaluated by wine grape growers.

Leafrollers

The first stage of this thesis (Chapter 3) was undertaken to investigate which species of leafroller moths are key insect pests in South Australian vineyards. Surprisingly, the true identity of some of the tortricids has largely gone unnoticed until now. The specialist knowledge, time and resources required to extract DNA and conduct Sanger sequencing is not accessible to grape growers. However, a practical alternative is to rear larvae in containers to adulthood (Figure 1). However, specialist knowledge is still required to ensure correct identification of adult moths, and parasitised larvae don't survive to the adult stage.

Figure 19. Growers can rear larvae to determine the species of tortricid once it emerges as an adult moth. Photos: Mary Retallack

Sampling of the larvae found in grapevine canopies indicated that *E. postvittana* is the dominant species of Tortricidae with a mean prevalence of nearly 94%. Low densities of *A. rudisana*, *M. divulsana* and *C. plebejana* were also found on foliage of *V. vinifera* for the first time. This gives wine grape growers the confidence to know they are managing a single dominant species of economically damaging leafroller, while also having a greater awareness of the diversity of leafroller species that may be present.

Of the leafrollers found, *E. postvittana*, *A. rudisana*, and *M. divulsana* are native to Australia, whereas the origin of *C. plebejana* is unknown but is suspected to be South America (Meyrick, 1917). The latter species is widely distributed throughout the world (CABI, 2018). *Acropolitis rudisana* was arguably more likely to be found at a site located adjacent to a large area of remnant bushland. *Acropolitis rudisana* may move between some native plants and vineyards habitats. This hypothesis is supported by Feng et al. (2016) who found *A. rudisana* present in woody habitats adjacent to vineyards in the Waite Conservation Reserve, Urrbrae, South

Australia. Other vineyards may also have *A. rudisana* present, which may warrant investigation on a vineyard-by-vineyard basis. This study highlights the presence of a complex of leafrollers present in Australian vineyards. It also provides insights about the potential habitat preferences and management options for control of leafroller species.

However, there are still many unknowns and future research should focus on gaining a greater understanding of the life cycle of *A. rudisana*, *M. divulsana* and *C. plebejana* in association with grapevine canopies. It is unknown if these additional species pose a similar risk, or could cause similar levels of damage as LBAM in vineyards. If they have the potential to be economically damaging to grapevines, then further research is required to determine if current IPM strategies offer sufficient control options. Correspondingly, the use of synthetic pheromones is highly target-specific. If *A. rudisana*, *M. divulsana* and *C. plebejana* are considered to be a problem, they will require specific pheromones to ensure efficacy of monitoring and/or mating disruption initiatives. This emphasises the importance of knowing the species of pests present prior to implementing an IPM plan.

Conversely, if these leafroller species do not cause economic damage, they might be promoted as an alternative host for *D. tasmanica*, the key parasitoid of *E. postvittana*. It is reported that the presence of alternative hosts could enhance the activity of parasitic wasps in crops early in the season (Pfannenstiel et al. 2010). A recent study found that *D. tasmanica* will readily parasitise (70% parasitism rate) *A. rudisana*, and *M. divulsana*, and these tortricid species are considered promising alternative hosts to support parasitoid populations in vineyards (Bui, 2018). Other parasitic wasps that parasitise *E. postvittana*, such as *T. unimaculatus,* also parasitise *A. rudisana* and *M. divulsana*. The relationships between predators and parasitoids of pests on the one hand and alternative host species on the other should be the subject of further research.

It is important to note that the presence of additional species of leafroller larvae in the midrow early in the season may provide a valuable source of alternative prey for natural enemies, if they do not readily migrate into the canopies of grapevines. An alternative source of food may help to extend populations of LBAM predators, so they can contribute to natural biological control to prevent or delay LBAM populations from reaching damaging levels in grapevine canopies. This response by predators is often predicated on their ability to be resident within the agroecosystem, or to quickly re-colonise from adjacent native vegetation. A resource bottleneck may occur during the winter period due to the absence of reliable sources of food and prey (Schellhorn et al. 2015). Pests and predators may respond to a resource disturbance by dispersing into more permanent heterogeneous and stable habitat refuges. Refuges may include mid-row and/or adjacent native vegetation, where they may delay reproduction, overwinter and then cyclically re-colonise grapevines the following spring (Wissinger, 1997). However, access to suitable sources of alternative prey may help to sustain predatory populations throughout this period, reduce the need for cyclic colonisation and help extend the response of predators early in the season. It is unknown what cascading effects the combination of these leafrollers and other herbivores may be having on grapevine yield and further research is needed to explore these relationships.

Native insectary plants

The second aim (Chapter 4) was to identify the predatory arthropods associated with potential native insectary plants for Australian vineyards. This study elucidates associations between predatory arthropods and three locally-adapted native plant species.

Manipulating the structure and habit of insectary plantings to provide multiple benefits

Growers will inevitably be interested in maximising the benefits of new management techniques. It may be possible to manipulate the flowering time, structure and habit of insectary plants to extend their benefits. For example, some woody plant species can be pruned, or hedged to manipulate the density of flower clusters, or encourage a more compact habit. If *B. spinosa* is planted at the ends of strainers, cylindrical steel mesh guards may be required to stop sheep from eating the plants during establishment (Retallack, 2018). This will support the upward growth of the plants. In addition, the sheep may ‘trim’ the sides through the guards resulting an upright and dense growth habit with a greater abundance of flowers produced (Figure 2). Both *B. spinosa* and *L. continentale* may provide a suitable alternative to roses and other introduced plants, that are often planted at the end of strainer posts in Australian vineyards and offer no benefit (Figure 3).

Figure 20. *Bursaria spinosa* planted adjacent to the strainer post (a), adjacent to the vineyard (b), and 30 cm apart to prevent dust drifting into the vineyard (c). Photos: Mary Retallack

Figure 21. Rose bushes have been traditionally used at the end of strainer posts and offer no intrinsic benefit (a), C.A. Henschke and Co. have incorporated Christmas bush at the end of their strainer posts (b,c) and it is suggested that the use of locally-adapted native plants may be a better alternative. Photos: Mary Retallack

A recent study on the use of a low-growing, kneed wallaby grass, *R. geniculatum* found that the dormancy trigger normally present is overridden when moisture is provided by irrigation (Penfold, 2018). This may render wallaby grasses unsuitable when planted undervine on water limited sites, as it may have a detrimental effect on vine vigour. However, on high vine vigour sites where the shoot growth of grapevines is consistently higher than preferred, this species may provide a good option to reduce vine vigour if necessary. Slashing the grass undervine may be an alternative way to regulate its growth. It is not anticipated wallaby grasses will present a vigour problem when it is planted in the mid-row area (Penfold, 2018).

Predators

The average ratio of predator to herbivore morphospecies on each of the selected native insectary plants was found to be double. This finding is consistent with those of Morandin et al. (2011), who found field edge plantings of native California shrubs and perennial grasses can enhance ratios of beneficial to pest insect species by about twice when compared to weedy areas. Similarly, some groups of predators such as spiders, beetles, earwigs, shield bugs, and lacewings were present on all plant types and are therefore considered more versatile and/or adaptive.

The following new analysis of the dominant functional predatory groups emerged from the earlier study presented in Chapter 4. The order Araneae was consistently in the top three most abundant predatory groups found on each plant species. Araneae (51%), Neuroptera (19%), Coleoptera (11%) and Hymenoptera (11%) dominated the counts on *B. spinosa*. Araneae (45%), Coleoptera (21%) and Neuroptera (12%) were dominant on *L. continentale*. Coleoptera (31%), Dermaptera (29%), Araneae (16%) and Neuroptera (16%) were dominant on *V. vinifera.* Dermaptera (50%), Araneae (27%) and Neuroptera (8%) were dominant in association with *Rytidosperma* ssp.

Eleven out of the 15 families of spiders found in association with grapevines comprised functional groups of active hunter/ambush, sedentary/web, ground or canopy dwelling spiders. This demonstrates the versatility and/or adaptive capacity of spiders found in production systems.

While the majority of arthropod species present associated with each plant was estimated to have been collected in this study, a list of predators of economically damaging pests is incomplete for different climatic zones. Further work utilising next-generation sequencing of predatory arthropod gut contents provides an exciting opportunity to make these important connections between predators and prey, as new cost efficient techniques are now available (Krehenwinkel et al. 2017).

Earwigs

Earwigs were present throughout the year, predominantly from October to December. This period coincides with the grapevine flowering and bunch set period. Earwigs have the capacity to provide valuable pest control (Danthanarayana, 1980; Bernard et al. 2007; Frank et al. 2007; Kehrli et al. 2012) in the period leading up to harvest. Chemical control options are limited then due to withholding periods (AWRI, 2018a) and/or efficacy of spray coverage into the developing bunches after bunch closure may be poor (Wise et al. 2010). There is some concern that if earwigs are fermented with bunches of grapes at rates of 10 or more earwigs per kilogram, they can cause taint resulting in lower quality wine with undesirable characters (Kehrli et al. 2012). However, it is not anticipated that plantings of *Rytidosperma* ssp. will elevate the presence of *F. auricularia* at harvest. The results in my study indicate that populations of earwigs decline significantly from February to May on all vegetation types.

Shield bugs

The glossy shield bug *C. nasalis,* is a predator of a range of pests including Noctuidae moths (Mensah, 1997; Gurr et al. 2004). It was predominantly found in association with *Rytidosperma* ssp. followed by *V. vinifera.* It is anticipated that the incorporation of *Rytidosperma* ssp. in the mid-row or undervine will help to support populations of *C. nasalis.* Conversely, the shield bug *O. schellenbergii* was found predominantly in association with *B. spinosa* and *L. continentale* and then grapevines. If these species are planted adjacent to vineyards, it is anticipated they will support the presence of the predatory shield bug, *O. schellenbergii* in vineyards. This distinction in habitat preferences by these two species for these plants has not been reported previously and demonstrates the complementarity of each native insectary plant when planted in combination to support the two predatory shield bug species.

Herbivores

A common concern of vineyards owners, is the potential of selected insectary plants to provide habitat for economically damaging pests. It is important that the capacity of particular plant species to harbour a pest is considered whenever insectary plants are discussed. In my study I found cutworms, millipedes and elephant weevils on insectary plants. I present a contextual framework below which outlines the potential risk posed by each and how they can be effectively managed.

Cutworms

Herringbone cut worm*, Agrotis* ssp. (Ochsenheimer) (Lepidoptera: Noctuidae) and common armyworm, *Leucania convecta* (Walker) (Lepidoptera: Noctuidae) are pests of field crops (Bugg and Waddington, 1994). They may pose a threat to the establishment grapevines, via the chewing damage they cause to developing shoots (Nicholas et al. 1994). Some caution should be taken when establishing *Rytidosperma* ssp. prior to planting a new vineyard, as this study suggests it may provide a breeding site for cutworm larvae early in early spring on some sites. Cutworm larvae can be controlled by applying *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) (Smirle et al. 2013) sprays at night, when the nocturnal cutworms are most active. Once grapevines reach maturity they are unlikely to sustain significant damage and *Agrotis* ssp. and *L. convecta* are likely to provide a source of alternative prey for predatory arthropods, including species of Lycosidae in late winter and early spring which coincides with the start of the growing season.

Millipedes

Populations of *O. moreleti* may not as susceptible to predation due to the success of its chemical defense mechanism. Highly repellent chemical compounds called benzoquinones are ejected by the millipede from its defensive glands when attacked, rendering them inedible (Sekulic et al. 2014; Vujisic et al. 2014; Shear, 2015; Makarov et al. 2017). *Ommatoiulus moreleti* are widespread in southern Australia (Baker, 1985). They tend to be more abundant where leaf litter and soil moisture are present (Paoletti et al. 2007). The following is new material collected during this study. Higher than average rainfall occurred in February 2014 with 110.8 mm (February LTA 26.0 mm Nuriootpa) falling in the Barossa Valley and 125.4 mm (February LTA 28.5 mm Lenswood) in the Adelaide Hills. The peak in *O. moreleti* activity in grapevine canopies in March 2014 (Figure 4), is consistent with the literature reporting that populations increase in Autumn after significant rainfall events (Bailey and Baker, 2016). They prefer high relative humidity and moderate temperatures (Baker, 1980), and grapevine canopies provide suitable refuge for millipedes. Correspondingly, grape berries may provide an enticing source of food, when much of the existing ground cover is lignified and dry, or conversely too wet. As with LBAM, *O. moreleti* may cause damage to the skins of berries, predisposing the bunches to *Botrytis* and other bunch rots. This may result in a quality downgrade, or rejection of fruit in the vineyard. Their presence in picking bins often results in wine taint due to the defensive excretion of benzoquinone by millipedes in grape ferments (Coulter, 2014; Stankovic et al. 2016).

It is possible to control millipede populations via the release of parasitic nematodes such as *Rhabditis necromena* (Sudhaus and Schulte, 1989) (Nematoda: Rhabditidae) (McKillup et al. 1991; Jaworska, 1994; Hensel, 1999; Bailey and Baker, 2016). They may also provide an effective long-term control option in vineyards. Exploration of effective long-term control options are needed, if millipedes are present at damaging levels in the canopy during harvest. Higher populations of *O. moreleti* were also reported by Nash et al. (2010) under high cumulative pesticide metric scores. This highlights the vigilance needed to minimise collateral damage to predatory arthropods via pesticide use in vineyards. Hence, *Rytidosperma* ssp. could provide insectary benefits on sites where *O. moreleti* is not considered to be a problem

Weevils

Elephant weevil, *O. cylindrirostris* is endemic to Australia and is a wood boring pest of grapevines (Coventry et al. 2004; Bernard et al. 2007; Scholefield and Morison, 2010). Additional introduced hosts include citrus and blueberries, and native host plants include eucalypts and acacias (Murdoch et al. 2014). Growers need not be unduly concerned at the presence of the elephant weevil on *L. continentale* and *B. spinosa.* These plants present a low biological risk, as they are not known breeding plants for *O. cylindrirostris.*

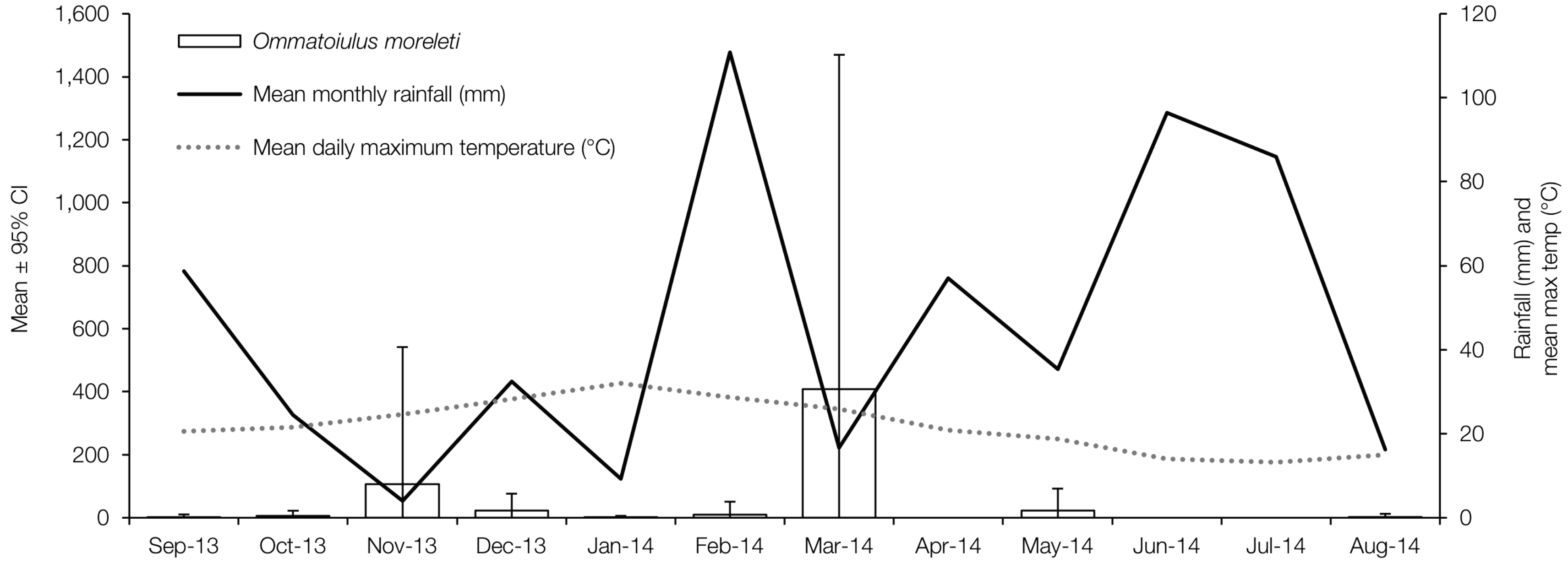

Figure 22. Temporal abundance of *O. moreleti* on *V. vinifera* pooled across all traps over a 12-month period (mean ± 95% CI per month).

Biological and functional diversity

The fourth aim (Chapter 5) was to assess the potential diversity offered by native insectary plants. Including their capacity to support populations predatory arthropods throughout the year, when planted in and around Australian vineyards. Each plant had a proportion of morphospecies composition shared with other insectary plants. This indicates that a high number of morphospecies are likely to be suited to one or more of the habitat types. Wallaby grasses have particular characteristics that create strong associations with species of predators that are not found as abundantly on woody plants. These species of native perennial grasses have the capacity to bring greater morphospecies richness to the plant assemblage, and comprise an important component of a mix of plants for insectary benefits.

The full complexity of ecosystem functionality in a production landscape isn't considered here but the provision of floral resources, as well as wetlands and/or roosting perches may also help to support higher trophic groups such as microbats (Stahlschmidt et al. 2012; Sirami et al. 2013; Froidevaux et al. 2017) and/or predatory birds (Kross et al. 2012; Benayas et al. 2017; Peisley et al. 2017), which may also have the capacity to contribute either directly or indirectly towards biological control of insect pests (Kelly et al. 2016; Thiery et al. 2018). Future research should consider the complexity of these interactions, so these components of functionality may be included.

Potential distribution of native candidate insectary plants

The third aim as outlined in Chapter 6 was to assess the existing and potential geographic distribution of each native insectary plant to determine suitability for planting each in association with grapevines around Australia. The models produced for each plant species indicated that south and eastern Australia are potentially suitable for all species. This gives growers the confidence to trial these selected plants in association with vineyards throughout Australia. The capacity to plant a diverse range of insectary plants may also help wine grape growers mitigate the effects of climate change and extreme weather events, if the current suite of plants become unsuited to their localised climatic surroundings. This is one of the reasons native insectary plants are preferred over introduced species, as they are already naturally adapted to Australia's harsh climatic conditions (Danne et al. 2010; Pandey et al. 2018). Climatic modelling and selection of grape varieties that are adapted to extremes in temperature and rainfall are two of the methods currently being used to mitigate the impacts of a changing environment in the wine sector (Anderson et al. 2008; Webb et al. 2008, 2011). If the selected insectary plants are grown outside their native range in managed landscapes, it is not considered that they would unduly threaten surrounding ecosystems and should not pose any additional ecological risk (Ewel and Putz, 2004; Grice and Martin, 2006).

Broader suite of native insectary plants

In addition to the insectary plants that were assessed in this study, it is estimated that there are more than 21,000 flowering plants that are native to Australia (ANBC, 2015), which may provide potential in each geographic range. The desktop review of plants native to South Australia (Appendix 1) identified a broader suite of locally-adapted native plants which are regarded as having the capacity to provide insectary benefits and may hold widespread appeal including wild rosemary, *Dampiera rosmarinifolia (*Schltdl.) (Asterales: Goodeniaceae), dryland tea tree 'Moonah', *Melaleuca lanceolata* (Otto) (Myrtales: Myrtaceae), clasping goodenia, *Goodenia amplexans* (F.Muell.) (Asterales: Goodeniaceae), hop goodenia, *Goodenia ovata* (Sm.), cut-leaf goodenia, *Goodenia pinnatifida* (Schltdl.), boobialla, *Myoporum insulare* (R.Br.) (Lamiales: Scrophulariaceae), *Myoporum parvifolium* (R.Br.), long-leaved bush-pea, *Pultenaea daphnoides* (J.C.Wendl.) (Fabales: Fabaceae), twiggy bush-pea, *Pultenaea largiflorens (*F.Muell. ex Benth), blue-rod, *Stemodia florulenta* (W.R.Barker) (Asteranae: Lamiales), fairy fan flower, *Scaevola aemula* (R.Br.) (Asterales: Goodeniaceae), as well as species of *Acacia* ssp., *Eucalyptus* ssp., *Lomandra* ssp. that are suited to a particular site.

It is reported that the longevity of parasitoids which predominantly feed on nectar (Gillespie et al. 2016; Gurr et al. 2017) are significantly enhanced by Australian natives including coastal rosemary, *Westringia fruticosa* (Willd.) Druce (Lamiales: Lamiaceae), creeping mint, *Mentha satureioides (*R.Br.) (Lamiales: Lamiaceae), crimson bottlebrush, *Callistemon citrinus* (Curtis) Dum.Cours. (Myrtales: Myrtaceae), tea-tree, *Leptospermum* cv. *'Rudolph' (*J.R.Forster and G.Forster) (Myrtales: Myrtaceae), grevillea, *Grevillea* cv*. 'Bronze Rambler'* (R.Br.) ex Knight (Proteales: Proteaceae), creeping boobialla, *M. parvifolium*, and austral trefoil, *Lotus australis* (Andrews) (Fabales: Fabaceae) (Pandey et al. 2018). A recent field study assessed the benefits of planting *B. spinosa*, woolly tea-tree *Leptospermum lanigerum* (Sol. ex Ait.) Sm. (Myrtales: Myrtaceae), *Hakea mitchellii* (Meisn.) (Proteales: Proteaceae), *M. lanceolata* and *Myoporum petiolatum* (R.J.Chinnock) (Lamiales: Scrophulariaceae) to benefit parasitoids adjacent to vineyards (Bui, 2018). He suggested that these plants may extend parasitoid diversity over time, and lead to better leafroller management. Other plants previously identified for their insectary benefits in vineyards include straw wallaby grass, *Rytidosperma richardsonii* (Cashmore) Connor and Edgar (Poales: Poaceae), windmill grass, *C. truncata*, creeping saltbush, *Atriplex semibaccata* (R.Br.) and lagoon saltbush, *A. suberecta* (I.Verd.) (Caryophyllales: Chenopodiaceae) (Danne et al. 2010).

What comes next?

Enhancing functional diversity and biocontrol of pests in vineyards

The incorporation of native insectary plants as supplementary flora should be used in association with production systems where SNAP resources are limited in order to support predatory arthropods (Gagic et al. 2018). By providing native perennial resources which overlap in flowering period, it may be possible to enhance the reliability and stability of AMES and pest suppression, by better supporting populations of predatory arthropods throughout the year (Isaacs et al. 2009). If *B. spinosa* and *L. continentale* are planted together, it may be possible to plant species that collectively flower over an extended period of seven months or more, when they are planted in combination with vineyards.

If the benefits of insectary plants can be validated, then their planting could become a common component of biological conservation control and be adopted widely, as the underpinning component of an IPM strategy by wine grape growers throughout Australia. This study provides empirical evidence of the biodiversity benefits of incorporating the three locally-adapted native plants assessed, which have the potential to enhance conservation biological control.

The costs and benefits of insectary plants need to be evaluated for different plant combinations. This would require an updated estimation of the economic costs of damage and control measures directed against arthropod pests and pathogenic diseases on Australian wine grapes. Progress is also needed to capture the true value of natural capital improvements via environmental-economic accounting, when insectary plants are incorporated in and around production landscapes and the associated biodiversity enhancement benefits.

Environmental stewardship

Australia is the fifth largest wine producer in the world and the largest in the southern hemisphere (Wine Australia, 2018c). Vineyards comprising an estimated 135,177 of planted hectares are located within 65 discrete wine regions (Bailey, 2016). Wine grapes are grown in every Australian state and territory, and South Australia is the largest wine grape producer (AgEconPlus, 2015). The proposal to incorporate native insectary habitat provides an opportunity for wine grape growers to demonstrate their environmental credentials, and improve their individual biodiversity scores, via national environmental stewardship programs such as Entwine Australia (Hoffmann and Thomson, 2011; AWRI, 2018b). There is also the capacity to create change at the landscape scale (Harrison and Bruna, 1999; Thomson and Hoffmann, 2013; Karp et al. 2018) to harness a broad suite of ecosystem services benefits (Close et al. 2009; Mace et al. 2012; Schellhorn et al. 2015). It is reported that consumers in mainland China, South Korea and Singapore strongly associate Australia with wines produced in a sustainable way (Wine Australia, 2018b). Consumer research by Tourism Australia by AgEconPlus (2015) showed that 'world class beauty and natural environments' rated as the fourth major factor influencing international holiday destination selection. The use of native insectary plants, the enhancement of biodiversity and the telling of these stories can potentially add to Australia's capacity to demonstrate this market perception of being 'clean and green'. The incorporation of naturally adapted, native vegetation is a tangible way that vineyards and wineries can potentially convey their unique Australian offerings and stand out in a crowded international market place.

Conclusion

These studies support a truly integrated approach to pest management, which incorporates natural biological control and the use of native insectary plants to potentially provide long-term and sustainable solutions. The study has revealed for the first time that larval *A. rudisana*, lucerne leafroller, *Merophyas divulsana,* and cotton tipworm, *Crocidosema plebejana* can be found on the grapevine canopy in South Australian vineyards at low densities. These lepidopteran species may provide a valuable source of alternative hosts for parasitoids and alternative prey for predators, if they are located in and around vineyards.

The incorporation of native insectary plants *B. spinosa*, *L. continentale* and *Rytidosperma* ssp. has the potential to enhance biodiversity, and CBC efforts by providing a suitable habitat to support diverse and functional populations of predatory arthropods. The opportunity to plant selected native insectary species could help wine grape growers save time and resources by producing fruit with lower pest incidence, while enhancing biodiversity of their vineyards.

These insectary plants are naturally adapted to all of the major wine growing regions within Australia and could be potentially planted virtually wherever wine grapes are grown. In addition, the results of this study may be applicable to a range of Australian production systems including, but not limited to, apple and citrus production.

Appendices

Appendix 1: Desktop review of candidate insectary plants

Habit	Family	Genus	Species	Common name	Regions of SA where the plant occurs naturally				Location	Height (m)	Spread (m)	Form	Rain (mm)	Soil texture	Soil pH	Frost	Flower colour	Flowering time		Food
					Northern Lofty	Murray	Southern Lofty	South Eastern											Pollen	Nectar
Trees and shrubs	Fabaceae	*Acacia*	*acinacea*	round-leaved wattle	Rare	Rare	Indigenous	Indigenous	Shelter belt	1 to 2	1 to 2	Small shrub wide	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Yellow/Gold	Winter to Spring		Extrafloral Nectaries (EFN) present?
Trees and shrubs	Fabaceae	*Acacia*	*myrtifolia*	myrtle wattle	Endangered	Vulnerable	Indigenous	Indigenous	Shelter belt	1 to 2	1 to 2	Small shrub wide	250 to 500	Clay, Loam	Calcareous soils with pH > 7, Soils with pH less < 8	Moderately sensitive	Yellow/Gold	Spring		Extrafloral Nectaries (EFN) present?
Trees and shrubs	Fabaceae	*Acacia*	*pycnantha*	golden wattle	Indigenous	Indigenous	Indigenous	Indigenous	Shelter belt	2 to 5	2 to 5	Medium tree	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Yellow/Gold	Winter to Spring	Yes	Extrafloral Nectaries (EFN) present?
Ground cover	Rosaceae	*Acaena*	*novae-zelandiae*	biddy-widdy					Mid row								Pink			Extrafloral Nectaries (EFN) present?
Ground cover	Lamiaceae	*Ajuga*	*australis*	austral bugle	Rare	Rare	Indigenous	Indigenous	Shelter belt	< 0.5	0.5 to 1	Understory	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Pink	Spring to Summer	Yes	
Grasses	Poaceae	*Rytidosperma*	*geniculatum*	kneed wallaby-grass	Rare	Threatened	Indigenous	Indigenous	Mid row	< 0.5	< 0.5	Grass	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Cream	Spring to Autumn	Yes	
Trees and shrubs	Pittosporaceae	*Bursaria*	*spinosa ssp. Lasiophylla*	Christmas bush		Rare	Rare		Headland	1 to 2	0.5 to 1	Small shrub erect	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	White	Spring to Summer	Yes	Yes
Trees and shrubs	Pittosporaceae		*spinosa ssp. spinosa*	Christmas bush	Indigenous	Indigenous	Indigenous	Indigenous	Headland	2 to 5	1 to 2	Small shrub erect	250 to 500	Clay, Loam, Limestone	Acid pH < 7 through to calcarious soils pH > 7	Resistant	White	Summer	Yes	Yes

Ground cover	Goodeniaceae	*Dampiera*	*rosmarinifolia*	wild rosemary	Vulnerable	Indigenous	Rare	Indigenous	Mid row	< 0.5	0.5 to 1	Understory	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Purple	All year		
Trees and shrubs	Myrtaceae	*Melaleuca*	*lanceolata*	dryland tea tree 'Moonah'	Threatened	Indigenous	Indigenous	Indigenous	Shelter belt	5 to 10	2 to 5	Medium tree	< 250	Sand, Clay, Loam, Limestone	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Cream	Spring to Summer		
Trees and shrubs	Myrtaceae	*Eucalyptus*	*leucoxylon ssp. Leucoxylon*	S.A. blue gum	Indigenous	Rare	Indigenous	Indigenous	Shelter belt	> 10	5 to 10	Large tree	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	White	Summer		Yes
Trees and shrubs	Myrtaceae	*Eucalyptus*	*odorata*	peppermint box	Indigenous	Rare	Indigenous	Rare	Shelter belt	> 10	> 10	Large tree	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Yellow/Gold	All year		Yes
Trees and shrubs	Goodeniaceae	*Goodenia*	*amplexans*	clasping goodenia			Indigenous		Headland	0.5 to 1	0.5 to 1	Small shrub wide	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Yellow/Gold	Spring to Summer	Yes	Yes
Trees and shrubs	Goodeniaceae		*ovata*	hop goodenia		Vulnerable	Indigenous	Indigenous	Headland	1 to 2	1 to 2	Small shrub wide	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Yellow/Gold	Spring to Summer	Yes	Yes
Ground cover	Goodeniaceae		*pinnatifida*	cut-leaf goodenia					Mid row								Yellow/Gold	Spring		
Trees and shrubs	Myrtaceae	*Leptospermum*	*continentale*	prickly tea-tree		Rare	Indigenous	Indigenous	Shelter belt	2 to 5	2 to 5	Medium tree	250 to 500	Sand, Clay, Loam	Acid with pH < 7, Neutral soils with pH 7	Resistant	Cream	Spring to Summer	Yes	Yes
Strap leaved plants	Asparagaceae	*Lomandra*	*densiflora*	pointed mat-rush	Indigenous	Rare	Indigenous		Mid row	< 0.5	< 0.5	Grass	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Green	Winter to Spring	Yes	Yes
Strap leaved plants	Asparagaceae		*effusa*	scented mat-rush	Indigenous	Indigenous	Rare	Indigenous	Mid row	< 0.5	< 0.5	Grass	250 to 500	Sand, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Cream	Winter to Spring	Yes	Yes
Strap leaved plants	Asparagaceae		*micrantha ssp. Micrantha*	small-flowered mat-rush	Indigenous	Indigenous	Indigenous	Indigenous	Mid row	0.5 to 1	0.5 to 1	Grass	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	White	Autumn to Spring	Yes	Yes

Strap leaved plants	Asparagaceae		*multiflora ssp. Dura*	many-flowered mat-rush	Indigenous	Rare	Indigenous		Mid row	0.5 to 1	< 0.5	Grass	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Cream	Winter to Summer	Yes	Yes
Trees and shrubs	Scrophulariaceae	*Myoporum*	*insulare*	boobialla		Rare	Indigenous	Indigenous	Shelter belt	2 to 5	2 to 5	Small shrub wide	250 to 500	Sandy, Loam, Limestone	Acid pH < 7 through to calcarious soils pH > 7	Sensitive	White	Spring	?	Yes
Ground cover	Myoporaceae		*parvifolium*			Rare	Vulnerable	Rare	Mid row	< 0.5	1 to 2	Understory	250 to 500	Sand, Clay, Loam, Limestone	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	White	Spring to Summer	Yes?	Yes
Trees and shrubs	Fabaceae	*Pultenaea*	*daphnoides*	long-leaved bush-pea			Indigenous		Headland	1 to 2	0.5 to 1	Small shrub wide	500 to 750	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Yellow/Gold	Spring	Yes	Extrafloral Nectaries (EFN) present? Nectaries protected?
Trees and shrubs	Fabaceae	*Pultenaea*	*largiflorens*	twiggy bush-pea	Indigenous	Rare	Indigenous	Indigenous	Headland	0.5 to 1	0.5 to 1	Small shrub wide	500 to 750	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Yellow/Gold	Winter to Spring		Extrafloral Nectaries (EFN) present? Nectaries protected?
Trees and shrubs	Plantaginaceae	*Stemodia*	*florulenta*	blue-rod	Rare	Indigenous	Indigenous		Shelter belt	< 0.5	< 0.5	Understory	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Resistant	Blue	Spring	Yes	Yes
Trees and shrubs	Goodeniaceae	*Scaevola*	*crassifolia*				Rare	Indigenous	Shelter belt	0.5 to 1	0.5 to 1	Small shrub wide	250 to 500	Sand, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Blue	Spring to Summer	Yes	Yes
Ground cover	Goodeniaceae		*aemula*	fairy fan flower		Rare	Endangered	Indigenous	Mid row	< 0.5	0.5 to 1	Understory	250 to 500	Sand, Clay, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Pink	All year	Yes	
Ground cover	Goodeniaceae		*calendulacea*	dune fan flower			Endangered	Endangered	Mid row	< 0.5	0.5 to 1	Understory	250 to 500	Sand, Loam	Acid pH < 7 through to calcarious soils pH > 7	Moderately sensitive	Blue	All year		

Appendix 2: Summary of predatory arthropods

Predator taxa	Genus and species	Common name
INSECTA		
ODONATA		dragonfly
DERMAPTERA		
Forficulidae	*Forficula auricularia*	European earwig
MANTODEA		
HEMIPTERA		
Anthocoridae	*Orius* ssp.	minute pirate bug
Nabidae	*Nabis kinbergii*	Pacific damsel bug
Pentatomidae	*Cermatulus nasalis*	glossy shield bug
	Oechalia schellenbergii	predatory shield bug
Reduviidae	*Coranus* ssp.	brown assassin bug
	Coranus granosus	
	Emesinae ssp.	thread-legged bug
	Gminatus australis	orange assassin bug
	Peirates ssp.	black ground assassin bug
	Pnirsus cinctipes	
HYMENOPTERA		parasitoid wasp
NEUROPTERA		
Chrysopidae	*Mallada signata*	green lacewing
Hemerobiidae	*Micromus tasmaniae*	brown lacewing
Mantispidae		mantid lacewing
COLEOPTERA		
Anthicidae		ant-like flower beetle
Cantharidae		soldier beetle
Carabidae	*Geosccapitus* ssp.	
Cleridae		
Coccinellidae	*Coccinella transversalis*	transverse ladybird beetle
	Cryptolaemus montrouzieri	mealybug destroyer ladybird
	Diomus notescens	minute two-spotted ladybird
	Eleale ssp.	checkered beetle
	Harmonia conformis	common spotted ladybird
	Scymnus ssp.	
Melyridae	*Dicranolaius bellulus*	red and blue beetle
Staphylinidae		rove beetle
DIPTERA		
Syrphidae		hoverfly
Asilidae		predatory robber fly
ARACHNIDA		
ACARI	*Phytoseiulus* ssp.	predatory mite
ARANEAE		
Araneidae	*Arkys* ssp.	triangular spider
	Celaenia ssp.	bird-dropping spider
	Eriophora ssp.	orb weaving spider
Deinopidae		net-casting spider
Dysderidae		woodlouse or slater hunters
Gnaphosidae		ground spider
Linyphiidae	*Erigone* ssp.	money spider
Lycosidae		wolf spider
Oxyopidae		lynx spider
Philodromidae		philodromid crab spider
Pholcidae		cellar spider
Salticidae		jumping spider
Sparassidae		huntsman spider
Tetragnathidae		long-jawed spider
Theridiidae	*Latrodectus hasselti*	redback spider
Thomisidae		crab spider
Zodariidae		ant spider
PSEUDOSCORPIONES		pseudoscorpion

Appendix 3: Sampling methods

Modified beat net

The beat net was constructed using a metal ‘card table’ frame with retractable legs measuring 700 x 700 mm, with calico and insect net inserts. The funnel (Multipurpose Funnel 28 cm diameter; Lion Aust. Consolidated) was modified to hold a 250 mL (techno-plas; Rowe Scientific) collection container, secured with velcro.

Modified sweep net

An insect sweep net (370 mm in diameter), was modified to hold a funnel (Multipurpose Funnel 16 cm diameter; Lion Aust. Consolidated) and a 250 mL (techno-plas; Rowe Scientific) collection container, secured with velcro.

Pitfall trap

Round plastic 850 mL containers with a diameter of 120 mm were placed in a PVC plastic sleeve, flush with the soil surface. Wire covers, 1 mm thick with 25 mm hexagonal gaps were used to limit non-target catch. Plastic shields, 250 x 250 mm were placed above the traps to exclude rainfall.

Appendix 4: Photos taken at each survey site during 2013/14

Site photos are presented to show the appearance of plants throughout the sampling period and site characteristics to provide a clear impression of what was done and where.

Site 1a: Christmas bush, *Bursaria spinosa*

Photo taken: 12 September 2013

Photo taken: 24 September 2013

Photo taken: 15 October 2013

Photo taken:18 October 2013

Photo taken: 1 November 2013

Photo taken: 15 November 2013

Photo taken: 28 November 2013

Photo taken: 13 December 2013

Photo taken: 16 December 2013

Photo taken: 10 January 2014

Photo taken: 25 January 2014

Photo taken: 20 February 2014

Photo taken: 28 March 2014

Photo taken: 20 May 2014

Site 1b: Prickly tea-tree, *Leptospermum continentale*

Photo taken: 12 September 2013

Photo taken: 5 October 2013

Photo taken:18 October 2013

Photo taken: 1 November 2013

Photo taken: 28 November 2013

Photo taken: 13 December 2013

Photo taken: 16 December 2013

Photo taken: 10 January 2014

Photo taken: 25 January 2014

Photo taken: 20 February 2014

Photo taken: 20 May 2014

Photo taken: 4 August 2014

Site 1c: *Vitis vinifera* cv. Chardonnay

Photo taken: 12 September 2013

Photo taken: 24 September 2013

Photo taken: 5 October 2013

Photo taken: 18 October 2013

Photo taken: 1 November 2013

Photo taken: 28 November 2013

Photo taken: 13 December 2013

Photo taken: 27 December 2013

Photo taken: 10 January 2014

Photo taken: 25 January 2014

Photo taken: 20 February 2014

Photo taken: 28 March 2014

Photo taken: 20 May 2014

Photo taken: 4 August 2014

Site 5b: Wallaby grass, *Rytidosperma* ssp.

Photo taken: 30 September 2013

Photo taken: 7 October 2013

Photo taken: 21 October 2013

Photo taken: 4 November 2013

Photo taken: 18 November 2013

Photo taken: 2 December 2013

Photo taken: 16 December 2013

Photo taken: 30 December 2013

Photo taken: 10 January 2014

Photo taken: 10 March 2014

Photo taken: 21 May 2014

Photo taken: 5 August 2014

Site 5b: *Vitis vinifera* cv. Shiraz

Photo taken: 30 September 2013

Photo taken: 7 October 2013

Photo taken: 21 October 2013

Photo taken: 4 November 2013

Photo taken: 18 November 2013

Photo taken: 2 December 2013

Photo taken: 16 December 2013

Photo taken: 30 December 2013

Photo taken: 10 January 2014

Photo taken: 25 January 2014

Photo taken: 24th February 2014

Photo taken: 28th March 2014

Photo taken: 21 May 2014

Photo taken: 5 August 2014

Site 6: Wallaby grass, *Rytidosperma* ssp.

Photo taken: 12 September 2013

Photo taken: 30 September 2013

Photo taken: 7 October 2013

Photo taken: 18 October 2013

Photo taken: 4 November 2013

Photo taken: 18 November 2013

Photo taken: 2 December 2013

Photo taken: 16 December 2013

Photo taken: 30 December 2013

Photo taken: 25 January 2014

Photo taken: 10 March 2014

Photo taken: 28 March 2014

Photo taken: 21 May 2014

Photo taken: 5 August 2014

Site 6: *Vitis vinifera* cv. Shiraz

Photo taken: 12 September 2013

Photo taken: 30 September 2013

Photo taken: 7 October 2013

Photo taken: 18 October 2013

Photo taken: 4 November 2013

Photo taken: 18 November 2013

Photo taken: 2 December 2013

Photo taken: 16 December 2013

Photo taken: 27 December 2013

Photo taken: 25 January 2014

Photo taken: 24 February 2014

Photo taken: 28 March 2014

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Photo taken: 5 August 2014

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