



Libraries and Learning Services

University of Auckland Research Repository, ResearchSpace

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognize the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the [Library Thesis Consent Form](#) and [Deposit Licence](#).

Biocontrol of invasive tomato/potato psyllid

***Bactericera cockerelli*:**

Effects of predatory mite, entomopathogenic fungi, diets and ants

Jianfeng Liu

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy in the Biological Sciences, the University of Auckland, 2019.

Abstract

Global food production increasingly faces the problem of reducing the impact of a few widespread pests and diseases, without causing environmental harm from the overuse of pesticides. Biological control using predators and pathogens to control the pest offers one solution, but identifying suitable biological control agents and strategies to apply them is challenging. One such pest, the tomato/potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an important, difficult-to-control, agricultural insect pest of solanaceous crops in United States, Mexico, Central America and New Zealand. Although long-term use of chemical pesticides has resulted in insecticidal resistance, no alternative control strategies are available against TPP. The main purpose of this thesis was to develop a more effective control tool for TPP using biological control. I explored the effects of diet and habitat shelter on the performance of a generalist predatory mite *Amblydromalus limonicus* (Garman & McGregor) (Acari: Phytoseiidae), assessed interactions between TPP, entomopathogenic fungi (EPF), and predatory mites, and evaluated the effect of ants on the life table parameters and predation rates of *A. limonicus* on TPP.

To find an alternative diet for the mass-rearing and preventative release of a predatory mite against TPP, I evaluated factitious and artificial diets on the performance of *A. limonicus*. Different diets significantly influenced survival rate, developmental time and reproduction of *A. limonicus*. *A. limonicus* had a faster developmental time and higher survival rate and fecundity when fed with *Typha orientalis* pollen than on punctured *Ephestia kuehniella* eggs and an artificial diet. Dispersed patches of pollen strongly increased the population growth of immature and female *A. limonicus* compared with aggregated patches when the mites fed on *T. orientalis* pollen under laboratory conditions. *T. orientalis* pollen resulted in a higher total number of *A. limonicus* in 7 days than *E. kuehniella* eggs. Greenhouse experiments demonstrated that the application of *A. limonicus* significantly reduced the total number of TPP on bell peppers after seven weeks compared with the control. Moreover, *A. limonicus* supplemented with *T. orientalis* pollen significantly maintained a higher number of predatory mites and reduced the mean number of total, eggs, and nymphs TPP on plants over 7 weeks. So providing supplementary *T. orientalis* pollen in a crop system could enhance the control efficiency of *A. limonicus* on TPP.

To identify other suitable candidate organisms that could form the basis of an integrated pest management strategy for the control of TPP in New Zealand, I screened 16 native strains of EPF against TPP adults and nymphs. The most successful fungus was *Beauveria bassiana* ICMP 8701, which induced high mortality and short median lethal times (LT₅₀) values in TPP at 1×10^7 mL⁻¹ in 10 days. In the parental generation, the reproduction parameters of TPP adults were significantly reduced by two sublethal concentrations of *B. bassiana*. Physiological effects from the parental generation infected by *B. bassiana* (LC₃₀) could be carried over to their offspring, significantly reducing developmental rates, and life table parameters of TPP. The integrated strategy of combining the release of predatory mites and EPF may further promote biocontrol of pests. To examine the compatibility of *A. limonicus* and *B. bassiana*, I tested the lethal and sublethal effects of *B. bassiana* on *A. limonicus* on TPP. *A. limonicus* females were susceptible to *B. bassiana*. Sublethal concentrations of *B. bassiana* (LC₃₀) significantly reduced reproduction parameters and predation rates of F₀ *A. limonicus* females. However, life table parameters, predation rates and population parameters of F₁ *A. limonicus* were not influenced by sublethal concentrations (LC₃₀). This result suggests that transgenerational effects of *B. bassiana* cannot be carried over to the F₁ generation. Application of *B. bassiana* suspensions did not affect *A. limonicus* densities when they were combined in greenhouse conditions. The combined use of *A. limonicus* with *B. bassiana* suspensions resulted in significantly decreased TPP populations and greater plant biomass and fruit growth. Therefore *A. limonicus* and *B. bassiana* were compatible and could further improve the control of TPP.

Predator cues can induce predation stress in prey that negatively influence survival, development, reproduction, and feeding behaviour of the prey. Hemipteran-tending ants such as *Technomyrmex albipes* can negatively influence the predation behavior of mites. This thesis also evaluated the effects of ant odour on life table parameters and predation rates of *A. limonicus* when feeding on TPP. Exposure to ant odour did not alter the development, survival rate and fecundity of *A. limonicus*. Consumption rates of TPP also showed a dimorphic response—predation rates increased in female *A. limonicus*, but not in males. To my knowledge, this is the first report indicating increased consumption rates by natural enemies exposed to the odour of a mutualist (ant) of the pest (TPP).

My findings will fill some knowledge gaps regarding the development of commercially available biological control agents in New Zealand. *T. orientalis* pollen would be an effective alternative in solving the problem of mass-rearing of predatory mites and provided supplemental foods to maintain their populations in protected crops after release in the greenhouse in New Zealand. Based on the tested result, *T. orientalis* pollen might be registered as a commercial pollen product for predatory mites. Local strains of *B. bassiana* ICMP 8701 has the potential to develop as biopesticides against TPP for New Zealand. The simultaneous application of two agents provides a useful foundation for developing an IPM program for the control of TPP in particular and invasive alien pests in general. *B. bassiana* ICMP 8701 is compatible with *A. limonicus*. The combined application of two agents could overcome the shortcoming of individual agent and make crop protection more sustainable. The presence of hemipteran-tending ant odour did not influence the life table parameters of *A. limonicus*, but further improved its control efficiency. This finding may provide new insights into understanding tri-partite interactions involving a pest, its predator and a mutualist of the pest.

Acknowledgements

I could not have completed this PhD thesis at all without all of people who had helped, supported and accompanied me in these three years.

First, I would like to thank my supervisors Professor Zhi-Qiang Zhang and Professor Jacqueline Beggs for their assistance and continuous encouragement throughout my PhD. I thank Zhi-Qiang for guiding me into the world of Acarology. Your passion for Acari research really inspired and encouraged me to further conduct my studies. Thank you for forgiving me for asking silly questions or doing silly things, but these things inspired and expanded my horizons into thinking deeply before I acted. Jacqueline, you have taught me how to think and organise a paper, and communicate with readers through English writing. Thank my two supervisors for still supporting me when you suffered from great pains in your family.

I am especially grateful to Xiaoying Wei (The University of Auckland) for providing assistance for laboratory experiments. I also thank Guangyun Li, Jieyun Wu, Chen Wu, Wendy Lam, Ming Hui Lee, Kajal Patel, and Jiawei Shen at The University of Auckland, who helped maintaining insects and predatory mites and providing guidance. I thank Adrian Turner from The University of Auckland for his guidance and advice on SEM and TEM observation.

I am grateful to Chris Winks, Elsa Paderes, Stanley Bellgard, and Zane McGrath (Landcare Research, Auckland) for help in preparing the experiment and technical support. I am also thankful to Bevan Weir and Duckchul Park (Landcare Research, Auckland) for identifying entomopathogenic fungi strains tested and Darren Ward (Landcare Research, Auckland) for the identification of the ant *Technomyrmex albipes*. I am grateful to Hugh Gourlay (Landcare Research, Christchurch) for preparing the artificial diet used in this thesis. I also thank Anne Austin (Landcare Research, Palmerston North) and Ray Prebble (Landcare Research, Christchurch) for reading and commenting on this thesis. I wish to thank Dr Qing-Hai Fan, Dr Donemei Li, Joe Tang (Ministry for Primary Industries, New Zealand) and Dr Zengqi Zhao (Landcare Research, Auckland) for offering me wise guidance during my PhD.

Thanks also to Professor Xiao Zou (Guizhou University, Guiyang, China) for sharing his knowledge of entomopathogenic fungi and advice on experimental design. I would like to thank Professor Hsin Chi (Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, Niğde, Turkey) for providing the software and instructions for the analysis of life table data. I thank Chengxu Wu (Chinese Academy of Forestry, Beijing, China) who provided advice for statistics analysis.

To Lixia Xie, Min Ma, Peiying Peng, Suqin Shang, Tao Jiang, Xiao Han, and Yumei Xu at Ecology laboratory, thank you for providing me a warm and friendly working environment. Thanks also to my landlords Yang Li and Xiaomei Cao for providing bell pepper plants for the experiments and friendly living condition.

I would like to express my gratitude to the Systematic & Applied Acarology Society and to the University of Auckland for financial support. Many thanks Chief editor Zhi-Qiang for giving me the opportunity to be an assistant editor for Systematic & Applied Acarology; this experience greatly enhanced my English writing skills. In addition, this thesis was supported in part by a subcontract on *Amblydromalus limonicus* rearing from Plant & Food Research (Christchurch).

Finally, thanks to my lovely wife Jifeng Hu for her ongoing encouragement and support during my PhD. Without her encouragement, I believe that I could not have finished my thesis. I would like to thank my parents and parents in-law, grandmother, sisters, and brothers for their encouragement and selfless love.

Table of Contents

Abstract	i
Acknowledgements	iv
Table of Contents	vi
List of Figures	xii
List of Tables	xv
Chapter 1	1
General introduction	1
1.1 Invasive alien insects	2
1.1.1 Ecological effects of invasive alien insects.....	2
1.1.2 Management of invasive alien insects.....	3
1.1.3 Biological control of invasive alien insects	4
1.2 Invasive species of Hemiptera	5
1.2.1 Invasive Hemiptera of agricultural and forest importance.....	5
1.2.2 Cypress aphid.....	6
1.2.3 Sweet potato whitefly.....	7
1.2.4 Asian citrus psyllid.....	8
1.3 Tomato/potato psyllid	9
1.3.1 Spread of tomato/potato psyllid	9
1.3.2 Damage of tomato/potato psyllid.....	9
1.3.3 Management of tomato/potato psyllid	10
1.4 Biological control of tomato/potato psyllid	11
1.4.1 Parasitoids	13
1.4.2 Predatory bugs.....	14
1.4.3 Predatory ladybirds	15
1.4.4 Predatory lacewing and hoverfly	16
1.4.5 Predatory mites	17
1.4.6 Entomopathogenic fungi.....	17
1.5 Interactions between entomopathogenic fungi and predatory mites.....	19
1.5.1 Diversity of EPF and predatory mite biocontrol agents.....	19
1.5.2 Compatibility	24
1.5.3 Behavior and predation	24
1.5.4 Life table parameters.....	25
1.5.5 Control efficiency	26

1.6 Rearing of phytoseiid mites	26
1.6.1 Artificial diets	27
1.6.2 Pollen	28
1.6.3 Factitious food	30
1.6.4 Supplemental food for predatory mites.....	33
1.6.5 Application method of supplemental food.....	35
1.7 Tri-partite complexity among hemipterans, ants and natural enemies.....	35
1.8 Thesis objectives	37
Chapter 2	40
Development, survival and reproduction of a New Zealand strain of <i>Amblydromalus limonicus</i> (Acari: Phytoseiidae) on <i>Typha orientalis</i> pollen, <i>Ephestia kuehniella</i> eggs and an artificial diet	40
2.1 Introduction.....	41
2.2 Material and methods.....	43
2.2.1 Mite cultures and diets	43
2.2.2 Preparation of artificial diet	44
2.2.3 Experimental arenas.....	44
2.2.4 Experiment 1: development and reproduction of <i>A. limonicus</i>	45
2.2.5 Experiment 2: influence of ambient relative humidity on the developmental time and immature survival of <i>A. limonicus</i> on <i>E. kuehniella</i> eggs.....	46
2.2.6 Statistical analysis	46
2.3 Results.....	47
2.3.1 Experiment 1: development and reproduction of <i>A. limonicus</i> on different diets.....	47
2.3.2 Experiment 2: influence of ambient relative humidity on the development and immature survival of <i>A. limonicus</i> on <i>E. kuehniella</i> eggs at 25 °C.....	49
2.4 Discussion	50
2.4.1 Water availability for <i>A. limonicus</i> feeding on <i>E. kuehniella</i> eggs	50
2.4.2 The performances of <i>A. limonicus</i> on different diets	51
2.5 Conclusion	53
Chapter 3	55
Population development of the predatory mite <i>Amblydromalus limonicus</i> is modulated by habitat dispersion, diet and density of conspecifics	55
3.1 Introduction.....	56
3.2 Methods.....	58
3.2.1 Mite cultures and diets	58
3.2.2 Experimental set-up	58
3.2.3 Experimental procedure	59

3.2.4 Statistical analysis	60
3.3 Results	61
3.4 Discussion	63
3.4.1 Habitat dispersion	63
3.4.2 Diet-dependent development	65
3.4.3 Density-dependent development	66
3.5 Concluding remarks	66
Chapter 4	67
Lethal and sublethal effects of entomopathogenic fungi on tomato/potato psyllid, <i>Bactericera</i>	
<i>cockerelli</i> (Šulc) (Hemiptera: Triozidae) in capsicum.....	67
4.1 Introduction.....	68
4.2 Materials and Methods.....	71
4.2.1 Host plant	71
4.2.2 Tomato/potato psyllid colony	71
4.2.3 Entomopathogenic fungi strains.....	72
4.2.4 Pathogenicity of fungal stains against psyllid first instar nymphs and adults.....	72
4.2.5 Effects of different concentrations on susceptibility of adults.....	74
4.2.6 Evaluation of sublethal effects of <i>B. bassiana</i> on reproduction of <i>B. cockerelli</i> adults	74
4.2.7 Evaluation of sublethal effects of <i>B. bassiana</i> on offspring of the treated <i>B. cockerelli</i>	74
4.2.8 Data analyses.....	75
4.3 Results.....	76
4.3.1 Pathogenicity of fungal isolates	76
4.3.2 Toxicity effect of <i>B. bassiana</i> ICMP 8701 on <i>B. cockerelli</i> adults.....	77
4.3.3 Sublethal effects of <i>B. bassiana</i> ICMP 8701 on longevity and fecundity of <i>B.</i>	
<i>cockerelli</i> adults	77
4.3.4 Sublethal effects of <i>B. bassiana</i> ICMP 8701	79
4.3.5 Population growth parameters	83
4.4 Discussion	83
4.5 Conclusion	87
Chapter 5	88
Influence of pathogenic fungi on the life history and predation rate of mites attacking a psyllid pest	
.....	88
5.1. Introduction.....	89
5.2. Material and methods.....	91
5.2.1 Predatory mite and psyllid colonies	91

5.2.2 Fungal preparation	91
5.2.3 Experimental set-up	92
5.2.4 Lethal effect of <i>B. bassiana</i> on <i>A. limonicus</i> females	92
5.2.5 Sublethal effect of <i>B. bassiana</i> on F ₀	93
5.2.6 Sublethal effect of <i>B. bassiana</i> on F ₁	93
5.2.7 Data analysis	93
5.3. Results.....	94
5.3.1 Toxicity of <i>B.bassiana</i> on <i>A. limonicus</i> females.....	94
5.3.2 Sublethal effects of <i>B. bassiana</i> on F ₀	94
5.3.3 Sublethal effects of <i>B. bassiana</i> on the survival rates of F ₁	95
5.3.4 Sublethal effects of <i>B. bassiana</i> on developmental time, adult longevity and fecundity of F ₁	96
5.3.5 Population parameters of F ₁ <i>A. limonicus</i> of the treated females exposed to LC ₁₀ and LC ₃₀ sublethal concentrations of <i>B. bassiana</i>	99
5.3.6 Predation rate	100
5.3.7 Population and predation projection	102
5.4. Discussion	105
5.4.1 Compatibility	105
5.4.2 Sublethal effect of <i>B. bassiana</i> on F ₀	106
5.4.3 Sublethal effect of <i>B. bassiana</i> on F ₁	107
5.4.4 Sublethal effect of <i>B. bassiana</i> on predation performance of <i>A. limonicus</i>	108
Chapter 6	110
Provisioning predatory mites with entomopathogenic fungi or pollen improves biological control of a greenhouse psyllid pest.....	110
6.1 Introduction.....	111
6.2 Materials and methods	114
6.2.1 Plants, insects and biological control agents.....	114
6.2.2 Greenhouse experiments	115
6.2.3 Sampling of predatory mites and TPP	116
6.2.4 Plant parameter assessments	117
6.2.5 Data analysis	117
6.3 Results.....	118
6.3.1 Effects of predatory mites on TPP	118
6.3.2 Application of <i>B. bassiana</i> suspension and predatory mites on TPP.....	118
6.3.3 Combined use of <i>T. orientalis</i> pollen and predatory mites on TPP	119
6.3.4 Combined use of dry conidia and predatory mites on TPP.....	121

6.3.5 Efficacy of predatory mites, <i>B. bassiana</i> suspension, <i>T. orientalis</i> pollen, and <i>B. bassiana</i> dry conidia for plant growth under greenhouse conditions.....	121
6.4. Discussion	122
6.4.1 Effect of entomopathogenic fungi.....	123
6.4.2 Effect of predatory mites.....	123
6.4.3 Effect of <i>T. orientalis</i> pollen	124
6.4.4 Combination effect of predatory mites with entomopathogenic fungi and <i>T. orientalis</i> pollen.....	126
6.5. Conclusion	127
Chapter 7	128
Tri-partite complexity: Odour from a psyllid’s mutualist ant increased predation by a predatory mite on the psyllid	128
7.1 Introduction.....	129
7.2 Material and Method.....	131
7.2.1 Mites and insect colonies	131
7.2.2 Experimental set-up	132
7.2.3 Life table analyses.....	132
7.2.4 Predation rate calculation and analysis	135
7.2.5 Population dynamics	136
7.2.6 Statistical analysis	137
7.3 Results.....	137
7.3.1 Immature development	137
7.3.2 Survival, life expectancy, and longevity	137
7.3.3 Reproduction.....	138
7.3.4 Life table parameters.....	139
7.3.5 Consumption rate	141
7.3.6 Population projection	144
7.4 Discussion	144
Chapter 8	148
General discussion	148
8.1 Effect of diets and habitat dispersion on the performance of <i>A. limonicus</i>	149
8.2 Effects of entomopathogenic fungi on <i>B. cockerelli</i> and <i>A. limonicus</i>	151
8.3 How hemipteran-tending ant odour influenced the performance of <i>A. limonicus</i>	152
8.4 Future research priorities	153
8.4.1 Effects of diet and habitat dispersion on predatory mites	153
8.4.2 The interactions among <i>B. cockerelli</i> , <i>B. bassiana</i> and <i>A. limonicus</i>	154

8.4.3 Non-consumptive effects of ant odour on <i>A. limonicus</i>	155
8.5 Concluding remarks	155
Appendix A.....	157
Provisioning predatory mites with entomopathogenic fungi or pollen improves biological control of a greenhouse psyllid pest.....	157
References.....	160

List of Figures

Figure 3.1. Three different arrangements of 8 pieces of threads in a Munger cell..... 60

Figure 3.2. Effects of three different habitat dispersions on population density of *A. limonicus* over 7 days.. 62

Figure 4.1. Sublethal effects of *B. bassiana* ICMP 8701 on longevity and fecundity of *B. cockerelli* adults. Means followed by the same letter in each bar are not significantly different. 78

Figure 4.2. Age-stage specific survival rate (S_{xj}) of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)... 79

Figure 4.3. Age-specific survival rate (l_x), female age-specific fecundity (fx_7), age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)..... 80

Figure 4.4. Age-stage specific reproductive rates (v_{xj}) of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)..... 81

Figure 4.5. Life expectancy (e_{xj}) values of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)... 82

Figure 5.1. Sublethal effects of *B. bassiana* ICMP 8701 on fecundity, longevity, oviposition period and predation rates of *A. limonicus* females. Means followed by the same letter in each bar are not significantly different 95

Figure 5.2. Age-stage specific survival rate (s_{xj}) of F₁ immatures and adults from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)..... 96

Figure 5.3. Age-specific survival rate (l_x), female age-specific fecundity (f_{xj}), age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) of F₁ from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)..... 98

Figure 5.4. Age-specific life expectancy (e_{xy}) of F₁ immatures and adults from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)..... 99

Figure 5.5. Age-stage specific reproductive value (v_{xj}) of F₁ immatures and female from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80)..... 100

Figure 5.6. Age-stage, two-sex predation rate (c_{xj}) of F ₁ immatures and adults from <i>A. limonicus</i> females exposed to sublethal concentrations (LC ₁₀ and LC ₃₀) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80).....	101
Figure 5.7. Age-specific predation rate (k_x) and age-specific net predation rate (q_x) of F ₁ from <i>A. limonicus</i> females exposed to sublethal concentrations (LC ₁₀ and LC ₃₀) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80)	101
Figure 5.8. Computer simulation of population growth of F ₁ from <i>A. limonicus</i> females exposed to sublethal concentrations (LC ₁₀ and LC ₃₀) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80) .	103
Figure 5.9. Computer simulation of stage growth rate of F ₁ from <i>A. limonicus</i> females exposed to sublethal concentrations (LC ₁₀ and LC ₃₀) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80) .	104
Figure 5.10. Computer simulation of total population and consumption of F ₁ from <i>A. limonicus</i> females exposed to sublethal concentrations (LC ₁₀ and LC ₃₀) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80).....	104
Figure 6.1. Temperature (°C) and relative humidity (%) under the greenhouse at daily interval were recorded during the experiment from early December 2017 to late January 2018	116
Figure 6.2. The effects of predatory mite species, <i>B. bassiana</i> suspension, <i>T. orientalis</i> pollen, and <i>B. bassiana</i> dry conidia on the mean number (\pm SE) of <i>B. cockerelli</i> (total, eggs, nymphs, and adults) per bell pepper leaf over a period of 7 weeks in the greenhouse.	119
Figure 6.3. The effects of <i>B. bassiana</i> suspension, <i>T. orientalis</i> pollen, and <i>B. bassiana</i> dry conidia on mean number (\pm SE) of mobile mites and mite eggs per bell pepper leaf over a period of 7 weeks in the greenhouse. Initially 10 TPP females and 40 predatory mite females were released per bell pepper plant.....	120
Figure 7.1. Age-stage specific survival rate (s_{xj}) of the predatory mite <i>A. limonicus</i> immatures and adults fed on TPP first instar nymph without or with ant odour	139
Figure 7.2. Age-specific survival rate (l_x), female age-specific fecundity (f_{x5}), and age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) of the predatory mite <i>A. limonicus</i> fed on TPP first instar nymph without or with ant odour	140
Figure 7.3. Age-specific life expectancy (e_{xy}) of immatures and adults <i>A. limonicus</i> fed on TPP first instar nymph without or with ant odour	140
Figure 7.4. Age-stage specific reproductive value (v_{xj}) of <i>A. limonicus</i> immatures and female fed on <i>B. cockerelli</i> first instar nymph without or with ant odour	140
Figure 7.5. Age-stage, two-sex predation rate (c_{xj}) of <i>A. limonicus</i> immatures and adults fed on TPP first instar nymph without or with ant odour.....	141
Figure 7.6. Age-specific predation rate (k_x , a) and age-specific net predation rate (q_x , b) of <i>A. limonicus</i> fed on <i>B. cockerelli</i> first instar nymph without or with ant odour.....	142

Figure 7.7. Computer simulation of population growth of *A. limonicus* fed on *B. cockerelli* first instar nymph without or with ant odour 142

Figure 7.8. Computer simulation of stage growth rate of *A. limonicus* fed on *B. cockerelli* first instar nymph without or with ant odour 142

Figure 7.9. Computer simulation of a) total population and b) consumption of *A. limonicus* with or without ant odour 143

List of Tables

Table 1.1. Reports of parasitoids and predators evaluated for the control of tomato/potato psyllids in the United States, Mexico, and New Zealand.	12
Table 1.2. Reports of entomopathogenic fungi strains evaluated for the control of tomato/potato psyllids in the United States, Mexico, and New Zealand.....	20
Table 1.3. Reports in the interactions between entomopathogenic fungi strains and predatory mite .	22
Table 2.1. Immature survival of <i>A. limonicus</i> reared on 6 diets at 25 °C.	48
Table 2.2. Developmental time of <i>A. limonicus</i> fed on four diets at 25 °C.	48
Table 2.3. Reproductive parameters of <i>A. limonicus</i> fed on three diets at 25 °C.	48
Table 2.4. Immature survival of <i>A. limonicus</i> on <i>E. kuehniella</i> eggs under two different ambient relative humidities with punctured and intact leaf discs at 25 °C.	49
Table 2.5. Developmental time of <i>A. limonicus</i> on <i>E. kuehniella</i> eggs with different ambient relative humidities 25 °C.....	49
Table 3.1. ANOVA results for the influence of habitat dispersion (aggregated, general and dispersed patches), diet (<i>T. orientalis</i> pollen, <i>E. kuehniella</i> eggs), and density (1 or 2 founder females) on population growth of <i>A. limonicus</i>	61
Table 4.1. List and origin of entomopathogenic fungal strains against <i>Bactericera cockerelli</i>	73
Table 4.2. Mortalities of 16 entomopathogenic fungal isolates against <i>Bactericera cockerelli</i> first instar nymphs and adults at $1 \times 10^7 \text{ mL}^{-1}$ after 10 days. The isolate which caused the highest mortality (in bold) was selected for further study.....	77
Table 4.3. Acute toxicity of <i>B. bassiana</i> ICMP 8701 against <i>B. cockerelli</i> adults at 10 days post-treatment.....	78
Table 4.4. Means (\pm SE) developmental time (days) of offspring from females of <i>B. cockerelli</i> exposed to sublethal concentrations (LC_{10} and LC_{30}) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80).....	81
Table 4.5. Means (\pm SE) life table parameters of offspring from females of <i>B.cockerelli</i> exposed to sublethal concentrations (LC_{10} and LC_{30}) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80)...	83
Table 5.1. Acute toxicity of <i>B. bassiana</i> ICMP 8701 against <i>A. limonicus</i> females at 10 days post-treatment.....	95
Table 5.2. Mean (\pm SE) of egg hatching rate, larva, protonymph, deutonymph, preadult survival rate and proportion of female adult Nf/N (%) of F_1 from females of <i>A. limonicus</i> exposed to sublethal concentrations (LC_{10} and LC_{30}) of <i>B. bassiana</i> ICMP 8701	96
Table 5.3. Means (\pm SE) developmental time (days) of F_1 from <i>A. limonicus</i> females exposed to sublethal concentrations (LC_{10} and LC_{30}) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80)...	97

Table 5.4. Means (\pm SE) population parameters of F1 from <i>A. limonicus</i> females exposed to sublethal concentrations (LC ₁₀ and LC ₃₀) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80)	100
Table 5.5. Predation rates of F ₁ from <i>A. limonicus</i> females exposed to sublethal concentrations (LC ₁₀ and LC ₃₀) of <i>B. bassiana</i> ICMP 8701 or water (0.05% Tween 80) fed on <i>B. cockerelli</i>	102
Table 6.1. Description of treatments for predatory mites, <i>B. bassiana</i> suspension and dry conidia, and <i>T. orientalis</i> pollen in greenhouse experiments.....	118
Table 6.2. Effects of different mite treatments on bell pepper growth parameters as assessed after 7 weeks under greenhouse conditions	121
Table 7.1. Developmental time, longevity, and fecundity (eggs) (\pm SE) of <i>A. limonicus</i> fed on TPP without or with ant odour	138
Table 7.2. The mean (\pm SE) two-sex life table parameters of <i>A. limonicus</i> fed on TPP without or with ant odour.....	139
Table 7.3. Predation rates of <i>A. limonicus</i> fed on <i>B. cockerelli</i> without or with ant odour.....	141
Table A1. The effects of predatory mite species, <i>B. bassiana</i> suspension, <i>T. orientalis</i> pollen, and <i>B. bassiana</i> dry conidia on the mean total, eggs, nymphs, adults numbers (\pm SE) of <i>B. cockerelli</i> and mean mobile mites and mite eggs (\pm SE) of predatory mites per bell pepper leaf were analysed using Friedman's test over a period of 7 weeks in the greenhouse.....	158
Table A2. Effects of different mite treatments on bell pepper growth parameters (Means \pm SE) as assessed after 7 weeks under greenhouse conditions	159

Chapter 1

General introduction

1.1 Invasive alien insects

Globalization increases the spread of invasive alien species (IAS) particularly as international commerce discovers new markets and commodities (Meyerson & Mooney 2007). IAS are a major element of global change and often have dramatic impacts on the loss of biodiversity and ecosystem functions for a new location (Early *et al.* 2016; Pysek & Richardson 2010; Ricciardi *et al.* 2017). In the twenty-first century, Early *et al.* (2016) claim that 17 % of the global land surface is vulnerable to IAS, and these IAS threats occur in biodiversity hotspots and developing economies as well. Understanding the mechanisms of the ecological effects of IAS in the invaded region is a key step for mitigating their impacts on biodiversity, economies, food security, and human livelihoods (Kenis *et al.* 2009). Compared with invasive alien plants, vertebrates, or aquatic organisms, significantly less attention has been given to invasive alien insects regarding their ecological effects on the environment (Kenis *et al.* 2009; Parker *et al.* 1999).

1.1.1 Ecological effects of invasive alien insects

Invasive alien insects can cause biodiversity losses in a direct fashion through herbivory, predation or parasitism on native species and communities. They also indirectly influence native species and ecosystems through a range of ecological mechanisms, including transmitting diseases and competing for the same resource (e.g., space or food) with native species (Kenis *et al.* 2009; Wagner & Van Driesche 2010; Wyckhuys *et al.* 2013). Alien herbivores pose a major threat to native plant populations in the invaded region. The fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) is native to North America, but was accidentally introduced to Asia in the early 1940s (Umeya 1977) and is now found in more than 30 countries. Outbreaks of this species may be due to their abundant and diverse (>400 species) host plants/range, higher fertility and adaptability, and few natural enemies (Ge *et al.* 2018; Schowalter & Ring 2017). Although this invasive species is of minor importance to forest trees, there is substantial direct effect via herbivory of larvae on the foliage of a range of host plants, especially fruit trees, and urban shade and ornamental trees (Schowalter & Ring 2017). In China, the invasion of *H. cunea* occurred in 2004, yet caused in total economic losses of about 107.5 billion RMB (Zhao 2005). The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae),

is listed by the International Union for the Conservation of Nature (IUCN) as one of the world's 100 worst invasive alien species (Lowe *et al.* 2000). The rapid global spread of this invasive mosquito and its capacity to vector various arboviruses significantly influences public health worldwide (Bonizzoni *et al.* 2013). *Aedes albopictus* is an epidemiologically important vector for many pathogens and arboviruses, such as dengue fever, yellow fever virus, and Chikungunya fever (Gratz 2004; Reiter 2010). This mosquito is originally indigenous to Southeast Asia, but has spread to Africa, the Middle East, Europe, North and South America (Gratz 2004). *A. albopictus* was mainly transported by dormant eggs in used tyres (Grist 1993). This invasive pest is very difficult to control due to its great adaptive ability in various environments.

1.1.2 Management of invasive alien insects

Reducing the rapid and widespread invasion of alien insects into new locations is the first step during management operations (Beggs *et al.* 2011). In non-invaded regions, establishing reactive national policies and proactive responses are necessary to manage alien insects. For example, governments issue import quarantine order for material potentially carrying invasive alien insects when there is a need to import such materials from invaded regions (Early *et al.* 2016). Also, a series of surveillance protocols would be established, including early identification, real-time monitoring, eradication schemes, and control methods (Biondi *et al.* 2018). Increasing globalization of trade facilitates the risk for invasion. Most countries have a limited national response capacity against invasions, because they lack the financial resources, relevant research, management coordination, and early-warning of known invasive insects (Early *et al.* 2016). For example, *Drosophila suzukii* spread rapidly in Europe from 2008 to 2011 due to the initial lack of integrated, precise and area-wide monitoring network of any *Drosophila* (Cini *et al.* 2012). Besides, standard protocols also have a low probability of detection for some alien insects, such as thrips, whiteflies, and mites (Kenis *et al.* 2009). Normally, invasive alien insects have some common abilities regarding the invasion processes, such as fewer natural enemies, short generation time, wide host plant range, extreme fecundity, and high dispersal potential (Cini *et al.* 2012; Sakai *et al.* 2001). When alien insects newly discover and establish populations in non-invaded regions, management of invasive species in the

early phases is heavily reliant on chemical insecticides to limit the initial establishment (Grafton-Cardwell *et al.* 2013). However, pesticide regulation of alien insects usually only has a short-term effect on invasion. Intensive insecticide use in crops may cause the spread of invasive species. For example, the repeated application of pesticides is known to commonly increase the spread of invasive *Bactericera cockerelli* population in western North America possibly due to the pesticide resistance (Liu & Trumble 2007). A similar result was found for western flower thrips *Frankliniella occidentalis* (Pergande). The start of the spread of thrips was probably caused by intensive insecticide use during 1970 and 1980 which selected an insecticide resistant strain or strains of thrips which then proliferated (Kirk & Terry 2003). The application for biological control (using the natural enemies of the pest) is that there needs to be an effective and sustainable solution for invasive pests over the longer-term (Cini *et al.* 2012), as it does not drive the selection of resistant strains, and biological control agents are highly specific and are usually found in the native home-range of the invading species.

1.1.3 Biological control of invasive alien insects

Due to the absence of effective coevolved natural enemies, invasive alien insects could relax selection for defense and focus on explosive population increase and spread in geographical distribution to a non-invaded region (Hänfling & Kollmann 2002; Wyckhuys *et al.* 2013). As alien insects are capable of establishing and spreading readily in “enemy-free space” in the invaded region, releasing biological control agents could be considered a key factor in sensitive receiving habitats (Hoddle 2004). In classical biological control, exotic natural enemies are usually introduced to control invasive alien insects. There is debate about the efficacy of biological control as only about one-third of introduced biological control agents are capable of establishing populations, and only about one third of those that establish are able to suppress alien insects (Babendreier 2007). In addition, the introduction of exotic natural enemies might pose a threat to human health, economics, and the environment (Babendreier 2007). For example, the multicolored Asian lady beetle, *Harmonia axyridis*, is an effective generalist predator in controlling some hemipteran pests (Pell *et al.* 2008). In 1916 it was first released as a classical biocontrol agent in the United States. But by the 1980’s it had instead become regarded as an invasive alien insect

(Koch 2003). *H. axyridis* poses significant threats to the diversity of other natural enemies and ecosystem services predominantly through intraguild predation (Pell *et al.* 2008; Roy & Wajnberg 2008). Compared with the introduction of exotic natural enemies, releasing native natural enemies may be safer (Babendreier 2007; Collier & Van Steenwyk 2004). However, van Lenteren (2012) found that in Africa more than 90% of the permitted commercially-available natural enemies were mass-reared on other continents or original material/entities collected from other continents/countries. A similar phenomenon was also found in some countries, such as Japan, Mexico, Canada and South Korea (Van Lenteren 2012). More attention should be paid to investigate the biological control values of native natural enemies to avoid the risk of importing exotic species, especially against small and highly mobile Hemiptera (De Clercq 2002).

1.2 Invasive species of Hemiptera

1.2.1 Invasive Hemiptera of agricultural and forest importance

Among animal taxa, invasive insect species form the majority of non-native animal species established outside of their native range in different parts of the world. This includes 3,540 species in North America (Yamanaka *et al.* 2015), 2651 species in Hawaii (Yamanaka *et al.* 2015), 1,306 species in the European Union (Roques 2009), 1477 species in New Zealand (Edney-Browne *et al.* 2018), and 471 species in mainland Japan (Yamanaka *et al.* 2015). The taxonomic composition of non-native insect species shows that Hemiptera contributed 27.3%, 16.4%, 26%, 20%, and 22.5% of all established insects in North America, Hawaii, European Union, New Zealand, and Japan, respectively (New 2017). In these areas, the taxonomic composition of non-native species of Hemiptera was higher than those of native species (Yamanaka *et al.* 2015). Non-native Hemiptera herbivores pose a threat to native plant populations. For example, in New Zealand 47.7% of non-native insect feeding guild were herbivores, and principle, non-native species belonged to the family Aphididae (Hemiptera) (Edney-Browne *et al.* 2018). Sap-sucking hemipterans have become major agricultural pests. Most hemipterans are phytophagous and damage multiple economically important crops and forest trees in the world (Chougule & Bonning 2012). These notable phytophagous hemipterans include aphids, plant bugs, psyllids, stink bugs and whiteflies. Due to the international trade and changes in agricultural

practices, some insects widespread and emerged to primary pests from miner or secondary pests (Chougule & Bonning 2012).

1.2.2 Cypress aphid

The family Aphididae are one of the most successful families of insects, with about 4,700 species in the world and many species are agricultural and forest important pests (Montalva *et al.* 2017; Remaudiere & Remaudiere 1997). The cypress aphid, *Cinara cupressi*, was listed in the IUCN's 100 of the world's worst invasive alien species (Lowe *et al.* 2000). *C. cupressi* was a native to Europe and the Near East (Montalva *et al.* 2017). This invasive species has spread in North America, South America, Southwest Asia, and Africa (Blackman & Eastop 1994). *C. cupressi* preferentially attacks taxa of the cypress family Cupressaceae, including *Cupressus lusitanica*, *Juniperus procera*, *Widdringtonia nodiflora*, and it has caused millions of US dollars damage to *C. lusitanica* in Africa (Ciesla 1991; Murphy *et al.* 1994). Besides, this invasive species had been recorded on 23 different tree species in Chile, especially to some vulnerable and endangered tree species (*Austrocedrus chilensis* and *Fitzroya cupressoides*) (Montalva *et al.* 2017). *C. cupressi* infestations resulted in widespread damage or even death of host trees (Chilima 1995). Cypress aphid causes initial damage through injecting aphid saliva into the tree's stem which leads to brown foliage of the attacked tree, the death of branches, and finally whole-tree mortality (Orondo & Day 1994; Peña & Altmann 2009). Moreover, phloem-feeding aphids can excrete large amounts of honeydew which induce growth and development of sooty moulds and influence the photosynthesis and gas exchange of plants (Ciesla 1991). In Chile, the insecticide dimetoate had been injected into attacked tree trunks, significantly reducing the symptoms of aphid damage (Baldini 2008). The most used control agents associated with *Cinara* sp. are parasitoids, especially, *Pauesia* sp. (Montalva *et al.* 2010). The solitary endoparasitoid, *Pauesia juniperorum* (Stary), was introduced from UK and France into Africa and Chile for the biological control of *C. cupressi*, and the severity of damage by *C. cupressi* had been declined in Malawi and Kenya possible due to the action of *P. juniperorum* (Chilima 1995; Day *et al.* 2003; Kairo & Murphy 2005; Montalva *et al.* 2010). Montalva *et al.* (2017) reported that native Chilean *Lecanicillium attenuatum* strains, isolated from naturally infected *C. cupressi*, had considerable potential to become the natural enemies of cypress aphid.

1.2.3 Sweet potato whitefly

Sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), was also listed in the IUCN's 100 of the world's worst invasive alien species (Lowe *et al.* 2000). *B. tabaci* has been considered as a complex species and has more than 24 biotypes (De Barro *et al.* 2011; Perring 2001). *B. tabaci* causes serious damage not only through directly extracting large quantities of phloem sap and excreting honeydew, but also indirectly transmitting numerous plant pathogenic viruses (111 species) (Byrne & Bellows 1991; Jiu *et al.* 2007; Jones 2005). Due to the occurrence of B biotype, *B. tabaci* rose to international prominence and had spread around the world rapidly, becoming a major pest in many cultivated and non-crop plants in the past 30 years, including, vegetable, grain legume, ornamental, and cotton plants (De Barro *et al.* 2011; Jiu *et al.* 2007; Liu *et al.* 2010). Moreover, two widespread biotypes (biotype B and Q) can result in the displacement of indigenous biotypes of the whitefly during the invasion process (Liu *et al.* 2007, 2010). As a consequence of extensive exposure to chemical insecticides, *B. tabaci* has developed resistance to almost 40 insecticides, such as bifenthrin, endosulfan, α -cypermethrin, pirimiphos-methyl, pyrethroids, imidacloprid, and neonicotinoids (Bass *et al.* 2015; Ma *et al.* 2007; Roditakis *et al.* 2005). The development of resistance had resulted in the failure of insecticides to control this whitefly (Roditakis *et al.* 2005). The development of insecticide resistance of a widespread invasive species leads to major worldwide apprehension, which has stimulated numerous studies on IPM strategies through biological control (Roush & Daly 1990).

Some previous studies discuss the importance of predators, parasitoids and entomopathogenic fungi (Faria & Wraight 2001; Gerling *et al.* 2001). Predators and parasitoids were mainly studied and applied to severe outbreaks of *B. tabaci* in the last 30 years (Gerling *et al.* 2001). The arthropods identified as predators in whitefly control consist of nine orders and 31 families, such as predatory beetles, true bugs, mites, and spiders. Also, 34 parasitoid species of *Encarsia* and 11 species of *Eretmocerus* were recognized as natural enemies of *B. tabaci* (Gerling *et al.* 2001). Whiteflies are phloem feeders, piercing the tissues of hosts and directly sucking sap from the vascular bundles (Faria & Wraight 2001). This style of feeding makes entomopathogenic fungi ideal biocontrol agents of whiteflies although sap sucking

whiteflies are not susceptible to some insect pathogenic bacteria and viruses which are transmitted through host feeding on contaminated foliage (Faria & Wraight 2001). In order to successfully control *B. tabaci* using natural enemies, further investigation is needed on the interactions among *B. tabaci*, host plants and natural enemies (Gerling *et al.* 2001).

1.2.4 Asian citrus psyllid

Psyllids (Hemiptera: Psylloidea) are important pests of agricultural crops worldwide (Burckhardt 1986, 1994; Munyaneza 2010). These insects caused serious damage through direct feeding by nymphs and adults. At least four psyllids can serve as a vector of bacterium “*Candidatus Liberibacter*” to *Citrus* spp. and solanaceous crops (Munyaneza 2010). The Asian citrus psyllid, *Diaphorina citri* Kumayama and the African citrus psyllid, *Trioza erythrae* Del Guercio can both vector Huanglongbing (HLB, citrus greening disease) “*Candidatus Liberibacter asiaticus*” (Las) which is a highly destructive disease of citrus (Bové 2006). Similarly, the potato/tomato psyllid, *Bactericera cockerelli* Sulc, carrot psyllid *Trioza apicalis* Förster, and psyllid *Bactericera trigonica* Hodkinson are associated with the pathogen “*Candidatus Liberibacter solanacearum*” (four haplotypes: LsoA, LsoB, LsoC and LsoD) in the Americas, New Zealand and Europe (Liefting *et al.* 2008; Munyaneza 2010; Munyaneza *et al.* 2010a; b; Nelson *et al.* 2013). This pathogen seriously affects the yield of potato, tomato, bell pepper, eggplant, tamarillo, and carrot crops (Liefting *et al.* 2009a; Nelson *et al.* 2013).

D. citri and its transmission of HLB pose a major threat to the world citrus industry. The infectious nature of HLB associated with *D. citri* was first reported in South China (Lin 1956). The psyllid has spread to 40 different Asian, Oceanian, and American countries (Bové 2006). The spread of *D. citri* and HLB was primarily via infected psyllids and infected plant material by natural and human-assisted transport (Halbert *et al.* 2010; Halbert & Manjunath 2004). The management of *D. citri* and HLB is difficult and should involve all aspects of an IPM program. Previous literature suggests that applying pesticides was necessary to control psyllids and HLB (Tolley 1990). Susceptibility of *D. citri* to insecticides showed that it had resistance to various pesticides (Grafton-Cardwell *et al.* 2013).

There are a range of parasitoids, predators and pathogens that could be used in an IPM program. There were two primary ectoparasitoids of *D. citri*, *Tamarixia radiata* (Waterston) and *Diaphorencyrtus aligarhensis* (Shafee, Alam and Argarwal) (Étienne *et al.* 2001; McFarland & Hoy 2001). The major predators of *D. citri* nymphs are *Exochomus childreni* Mulsant, *Harmonia axyridis* (Pallas), and *Olla v-nigrum* (Mulsant) (Michaud 2004). Additionally, a number of entomopathogenic fungi have been evaluated for control of *D. citri* nymphs and adults (Grafton-Cardwell *et al.* 2013). In this thesis, I focused on another invasive psyllid species, tomato/potato psyllid.

1.3 Tomato/potato psyllid

1.3.1 Spread of tomato/potato psyllid

The tomato/potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), has been a pest species of Solanaceae crops for nearly a century (Butler & Trumble 2012b; Prager & Trumble 2017). In the early 1900s TPP was first recognized as an injurious insect with explosive population growth (Compere 1915, 1916; Essig 1917; Sulc 1909). In the early 1920s and 1930s, its pest status increased to a serious and destructive pest due to psyllid's association with a new disease that was known as "psyllid yellows" (Binkley 1929; List & Daniels 1934; Richards 1928, 1931). This new disease was given the name "Zebra Chip" (ZC), and it caused substantial losses to potato growers in the United States and Mexico in the middle to late 2000s (Munyaneza *et al.* 2007a; b). TPP is native to North America and Central America dating to early 1990s (Prager & Trumble 2017), and it was accidentally introduced into New Zealand in May 2006 (Gill 2006). Teulon *et al.* (2009) found that TPP had spread throughout both in the North and South Island in April 2009, possibly dispersed by the combination of natural and human-vectors, especially through the transport of infected host plants.

1.3.2 Damage of tomato/potato psyllid

TPP causes both direct and indirect damage to Solanaceae crops. Direct damage is caused by adults and nymphs heavy feeding upon the phloem by using piercing-sucking mouthparts, and indirect damage results from the ability of *TPP* to transmit *Candidatus Liberibacter solanacearum* (Munyaneza 2012). Although both TPP adults

and nymphs are able to transmit Lso, adults are more efficient vectors of Lso because they have better flying and jumping skill than those of sedentary nymphs (Buchman *et al.* 2011; Munyaneza 2012). The primary means of ZC transmission is through feeding on infected plants. Even a single infective TPP per plant can effectively transmit Lso to potatoes, resulting in substantial spread of ZC (Munyaneza 2012, 2015). The putative causal agent of ZC was unknown until 2008; researchers subsequently showed that ZC was detected as “*Candidatus Liberibacter solanacearum*” (Lso) in potatoes (*Solanum tuberosum*), tomatoes (*S. lycopersicum*), bell pepper (*Capsicum annuum*), cape gooseberry (*Physalis peruviana*), eggplant (*S. melongena*), tamarillo (*Solanum betaceum*) in the United States, Mexico and New Zealand (Butler & Trumble 2012a; Liefting *et al.* 2009a; Munyaneza 2012, 2015, Munyaneza *et al.* 2007a; b, 2008, 2009; Prager & Trumble 2017; Teulon *et al.* 2009). Psyllids and ZC posed a major threat to the United States potato production and resulted in millions of dollars of losses (Munyaneza 2012; Rosson *et al.* 2009). In some potato growing regions of the United States, growers had to abandon entire fields due to the psyllids and ZC (Munyaneza 2012; Munyaneza *et al.* 2007a). In New Zealand, the economic impact of psyllids and ZC has resulted in millions of dollars of losses since TPP arrived, including crop losses, increased control costs, and the reduction of export trade (Teulon *et al.* 2009). In 2008 export receipts of capsicums were reduced around NZ \$5.22 million because export markets were closed due to the presence of TPP and the time to regain access to overseas markets (Teulon *et al.* 2009). The cost of TPP to the potato industry in New Zealand was over NZ \$28 million in 2010–2011 alone (Kale 2011). Tamarillo production volumes were reduced from 800 tonnes worth NZ \$3.5 million to 250 tonnes valued at NZ \$1.3 million (Teulon *et al.* 2009). Therefore, tomato/potato psyllid and ZC could pose a global threat to Solanaceae crop industry if ZC and its spread by TPP were not controlled (Munyaneza 2015).

1.3.3 Management of tomato/potato psyllid

Currently, it is difficult to manage ZC once the disease has established in one area because of the short life cycle, high reproductive potential and strong dispersal ability of TPP adults (Ramírez-Ahuja *et al.* 2017). The primary control tactics for ZC are targeted against TPP (Munyaneza 2015). The suppression of TPP populations is dominated by repeated applications of comparatively broad-spectrum insecticides

(Butler 2011; Gharalari *et al.* 2009; Guenthner *et al.* 2012). Insecticide applications associated with psyllid and ZC control is costly in both labor and materials. In Texas 23 different insecticides were used by growers for the 2009–2013 crops with an average of US\$ 285 per acre for an average number of 8.9 applications of insecticides, and during the same timeframe in Kansas and Nebraska growers used a total of 27 insecticides with an average of US\$ 283 and 228 per acre for an average number of 9.4 applications of insecticides (Guenthner & Greenway 2013). In Mexico, Coahuila and Nuevo Leon growers delivered up to 30 applications of insecticides for controlling TPP and ZC during crop seasons (Almeyda-León *et al.* 2008). The average number of insecticides applied in Pukekohe potato regions (New Zealand) increased to 16 for TPP control (Wright *et al.* 2017). In addition, increased applications of insecticides have resulted in the development of resistance by TPP to pesticides such as imidacloprid and spinosad¹ (Almeyda-León *et al.* 2008; Liu & Trumble 2007). The application of a highly toxic broad-spectrum insecticide, methomyl, had led to increased TPP populations in potato and pepper crops due to the pesticide resistance development (Prager *et al.* 2016). Biological control has been suggested as a sustainable pest control strategy to control the psyllid. It is critical to integrate multiple biological control agents in an IPM strategy for the control of TPP in order to use less toxic and smaller quantities of insecticides.

1.4 Biological control of tomato/potato psyllid

Biological control is an important component of TPP IPM programmes to reduce the reliance on chemical pesticides. Numerous previous laboratory assessments and surveys of natural enemies of TPP have been investigated in the United States, Mexico and New Zealand (Castillo Carrillo *et al.* 2016; Prager & Trumble 2017; Walker *et al.* 2011). These natural enemies include parasitoids, predatory bugs, predatory ladybirds, predator lacewing, predator hoverfly, predatory mites and entomopathogenic fungi (Table 1.1).

¹ Spinosad is an insecticide based on chemical compounds found in the bacterial species *Saccharopolyspora spinosa*.

Table 1.1. Reports of parasitoids and predators evaluated for the control of tomato/potato psyllids in the United States, Mexico, and New Zealand.

Parasitoids and predators	Host plants of TPP	Tested TPP stages	Condition and location	References
Parasitoids				
<i>Tamarixia triozae</i>	<i>S. tuberosum</i> , <i>S. lycopersicum</i>	Nymphs	Field investigation, NZ Lab, field investigation, USA, Mexico	(Bravo & López 2007; Butler 2011; Jensen 1957; Johnson 1971; Martinez <i>et al.</i> 2015; Morales <i>et al.</i> 2018; Pletsch 1947; Rojas <i>et al.</i> 2015)
<i>Metaphycus psyllidis</i>	<i>Capsicum</i> sp.	nymphs	Field investigation, USA	(Compere 1943)
Predatory bugs				
<i>Engytatus varians</i>	<i>S. tuberosum</i>	3 rd nymphs	Mexico	(Mart ínez <i>et al.</i> 2014)
<i>Dicyphus hesperus</i>	<i>S. lycopersicum</i>	-	Greenhouse, Mexico	(Calvo <i>et al.</i> 2016, 2018a; b)
<i>Nabis kinbergii</i>	<i>S. tuberosum</i>	Eggs, all nymphs	Lab, field investigation, NZ	(MacDonald <i>et al.</i> 2016; Walker <i>et al.</i> 2011)
<i>Oechalia schellenbergii</i>	<i>S. tuberosum</i>	-	Field investigation, NZ	(Walker <i>et al.</i> 2011)
<i>Orius tristicolor</i>	<i>Solanum dulcamara</i>	-	Field investigation, USA	(Castillo Carrillo <i>et al.</i> 2016)
<i>Orius vicinus</i>	<i>S. tuberosum</i>	Eggs, all nymphs	Lab, NZ	(Tran 2012)
Ladybirds				
<i>Cleobora mellyi</i>	<i>S. lycopersicum</i> , <i>S. tuberosum</i>	3 rd , 4 th , 5 th nymphs	Lab, greenhouse	(O'Connell <i>et al.</i> 2012; Pugh <i>et al.</i> 2015)
<i>Coccinella undecimpunctata</i>	<i>C. annuum</i> , <i>S. tuberosum</i>	Eggs, all nymphs	Lab, field investigation, NZ	(MacDonald <i>et al.</i> 2016; Walker <i>et al.</i> 2011)
<i>Cryptolaemus montrouzieri</i>	<i>S. lycopersicum</i> , <i>S. tuberosum</i>	1 st -5 th nymphs	Lab, NZ	(O'Connell <i>et al.</i> 2012)
<i>Harmonia conformis</i>	<i>C. annuum</i> , <i>S. tuberosum</i>	Eggs, all nymphs	Lab, field investigation, NZ	(MacDonald <i>et al.</i> 2016; Walker <i>et al.</i> 2011)
<i>Hippodamia convergens</i>	-	5 th nymphs	Lab, NZ	(Knowlton 1933)
<i>Scymnus loewii</i>	<i>S. lycopersicum</i> , <i>S. tuberosum</i>	1 st -5 th nymphs	Lab, NZ	(O'Connell <i>et al.</i> 2012)
Hoverfly				
<i>Melanostoma fasciatum</i>	<i>C. annuum</i> , <i>S. tuberosum</i>	Eggs, all nymphs	Lab, field investigation, NZ	(MacDonald <i>et al.</i> 2016; MacDonald & Walker 2012; Walker <i>et al.</i> 2011)
Lacewing				
<i>Micromus tasmaniae</i>	<i>C. annuum</i> , <i>S. tuberosum</i>	Eggs, all nymphs	Lab, field investigation, NZ	(MacDonald <i>et al.</i> 2016; MacDonald & Walker 2012; Walker <i>et al.</i> 2011)
Predatory mites				
<i>Amblydromalus limonicus</i>	<i>C. annuum</i>	Eggs, 1 st -3 rd nymphs, sugar droplets	Lab, NZ	(Davidson <i>et al.</i> 2016; Patel & Zhang 2017a; b; Xu & Zhang 2015)
<i>Anystis baccharum</i>	<i>Lycium ferocissimum</i>	Egg and younger nymphs	Lab, NZ	(Geary <i>et al.</i> 2016)
<i>Neoseiulus cucumeris</i>	<i>C. annuum</i>	Eggs, 1 st nymph	Lab, NZ	(Patel & Zhang 2017a; b)

“-” indicated no record in the reference.

1.4.1 Parasitoids

There are a number of parasitoids which could be used in an IPM program to control TPP. Two primary parasitoids, *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) and *Metaphycus psyllidis* Compere (Hymenoptera: Encyrtidae) (Table 1.1), have been reported on TPP nymphs (Bravo & López 2007; Compere 1943; Jensen 1957; Lomeli-Flores & Bueno 2002; Rojas *et al.* 2015). *T. triozae* is an important native ectoparasitoid of TPP in the United States and Mexico (Jensen 1957; Lomeli-Flores & Bueno 2002). High levels of parasitism (70–80%) by *T. triozae* were also observed without insecticides in southern Mexico (Bravo & López 2007). Besides, *T. triozae* females, a synovigenic parasitoid, could consume one TPP 4th instar nymph during 6-h period acquiring protein for egg production and increasing their efficiency to reduce host populations (Martinez *et al.* 2015; Morales *et al.* 2018; Rojas *et al.* 2015). Rojas *et al.* (2015) studied the detailed biology and life table parameters of *T. triozae* on TPP nymphs, finding that *T. triozae* might have great potential to control TPP based on the life table comparison between *T. triozae* on tomato and on eggplant and pepper in laboratory conditions. However, Johnson (1971) investigated numerous fields in north central Colorado and found that *T. triozae* populations were poorly synchronized with TPP populations and the pupae of this species suffered from high mortalities (38 to 100%). The parasitism levels reported for *T. triozae* were quite low under some circumstances. Twenty-three percent of TPP were recorded as attacked by *T. triozae* in a tomato field in Montana (Pletsch 1947). Similar studies also reported low parasitism level of *T. triozae* in agricultural fields; less than 20% in southern California and only 5–20% in south Texas (Butler 2011; Liu *et al.* 2012). Although *M. psyllidis* was described as a new primary parasitoid on TPP nymphs on chili pepper, there was no further research of this parasitoid as a control agent of TPP after that (Compere 1943).

The successful parasitism of two parasitoid developmental stages (adults and host parasitizing nymphs) could be influenced by multiple factors, such as host plants, lack of alternative hosts, and the widespread use of various insecticides. In the model Solanaceous plants-TPP-*T. triozae*, host plants influenced the foraging behavior, parasitism and reproduction parameters of *T. triozae* on psyllids (Hernández-moreno *et al.* 2017). *T. triozae* only parasitized on TPP 4th and 5th instar nymphs and could not

rapidly prevent plant disease transmission by the first and second instar psyllids on potato and tomato (Rojas *et al.* 2015).

Pesticide treatments can significantly reduce the survival and performance of *T. triozae* due to the developmental stage of this parasitoid inside TPP nymphs. The maximum field recommended concentration (1L ha⁻¹) of Imidacloprid inhibits 100% parasitoid adult emergence and abamectin inhibits 42% pupae parasitized in TPP 4th instar nymphs (Luna-Cruz 2011). *T. triozae* adults were highly sensitive to pesticides, such as abamectin, imidacloprid-cyfluthrin, spinetoram, and spinosad (Liu *et al.* 2012; Luna-Cruz 2011; Martinez *et al.* 2015). Abamectin and spinosad are placed in toxicity categories 3 (moderately persistent) and 4 (persistent) to *T. triozae* according to the classification of the International Organization of Biological Control (Luna-Cruz 2011; Luna-Cruz *et al.* 2015). Soybean oil insecticide EPA 90, imidacloprid and abamectin could result in between 67 and 100 *T. triozae* adult mortalities after 48h post treatment (Martinez *et al.* 2015). A sublethal concentration (LC₅₀) of these three insecticides also caused higher mortality in eggs and larvae of *T. triozae*, but not in their pupae stage (Morales *et al.* 2018).

The high cost along with the requirement of relatively higher numbers of *T. triozae* in the greenhouse might also influence the enthusiasm of growers for IPM to achieve adequate control of TPP. In New Zealand, Plant & Food Research imported *T. triozae* into quarantine facilities to assess its host specificity on psyllids in 2009. It was first released in Hawke's Bay and Canterbury in August 2017 (Barnes 2017). However, it could be a little bit more difficult to conduct the mass-rearing of the parasitoid in the factory condition due to the high cost in a supply of living TPP nymphs and host plants.

1.4.2 Predatory bugs

Predatory bugs are efficient biological control agents against TPP (Calvo *et al.* 2016, 2018a; b; Martínez *et al.* 2014). *Dicyphus hesperus* Knight (Heteroptera: Miridae) is native to North America but is now a widely distributed predator used for the control of tomato pests (Gillespie *et al.* 2007; Henry & Wheeler Jr 1988). Calvo *et al.* (2016) reported that *D. hesperus* populations could establish well and suppress TPP

populations in 10 weeks. Furthermore, this biocontrol agent did not cause damage on tomato leaves or fruit in the greenhouse. They also showed that the combined application of *D. hesperus* and *T. triozae* resulted in better TPP control efficiency on greenhouse tomato crops than that of a single application of either agent (Calvo *et al.* 2018a; b). Therefore, *D. hesperus* might have great potential in controlling TPP.

In Mexico, *Engytatus varians* (Distant) (Heteroptera: Miridae) were reported to predate on TPP 3rd nymphs in greenhouses (Martínez *et al.* 2014). Based on field studies, *Orius tristicolor* (White) (Hemiptera: Anthocoridae) was identified as the key biological control agents of TPP in potato, tomato, and pepper crops in southern California (2009–2010), and *O. tristicolor* made up 5% of over generalist predators in bittersweet nightshade patches in northwestern United States (2012–2013) (Butler & Trumble 2012a; Castillo Carrillo *et al.* 2016). In New Zealand, Walker *et al.* (2011) monitored unsprayed potatoes at Pukekohe weekly from July 2009 to March 2010 and found that *Nabis kinbergii* Reuter (Hemiptera: Nabidae) and *Oechalia schellenbergii* (Guerin) (Hemiptera: Pentatomidae) were foliage-dwelling insect predators. Observation from laboratory assays showed that *N. kinbergii* ate all life stages of TPP, particularly TPP eggs (MacDonald *et al.* 2016). *O. vicinus* were also able to attack eggs and younger nymphs of TPP in laboratory conditions (Tran 2012). These observations indicated that and these predatory bugs may be promising biocontrol agents against TPP.

1.4.3 Predatory ladybirds

Predatory ladybirds are one of important natural enemies of a variety of psyllid species (Hodek & Honěk 2009). In the early 1930s, Knowlton (1933) first noted that convergent lady beetle *Hippodamia convergens* consumed ten fifth instar nymphs of TPP during one and one-half hours. In the United States, *Scymnus loewii* was a generalist predator with a geographical range that overlapped that of TPP, suggesting it may represent an “old association” between ladybird and TPP (O’Connell *et al.* 2012). In New Zealand, O’Connell *et al.* (2012) found a “new species association” between the ladybirds *Cryptolaemus montrouzieri* and *Cleobora mellyi* and TPP; predatory ladybird larvae and adults consumed mixed instars of TPP nymphs in the lab. Compared with two larger coccinellid species (*C. montrouzieri* and *C. mellyi*)

with higher consumption rates, the small coccinellid, *S. loewii*, might have less potential as it has a lower consumption rate of TPP and lower effectiveness when the leaf surface is covered trichomes (O’Connell *et al.* 2012). The ladybird with the greatest potential for biocontrol, *C. mellyi*, is well established in New Zealand (Berndt *et al.* 2010; O’Connell *et al.* 2012). A glasshouse microcosm study showed that it was able to significantly decrease TPP densities on potato three weeks after the ladybird was released and maintained lower TPP density for 10 weeks (Pugh *et al.* 2015). Moreover, buckwheat can be used as a supplemental food source for increasing *C. mellyi*’s survival when TPP density was low in early-season (Pugh *et al.* 2015). Therefore, the single application of *C. mellyi* without TPP also reduced the number and size of tubers which indicated that further research need to be conducted on the potential of *C. mellyi* against TPP on different crops.

In New Zealand, the ladybirds *C. undecimpunctata* and *H. conformis* were relatively rare during a two-year investigation of potato plants, but they were also capable of predated on all life stages of TPP in the laboratory conditions (MacDonald *et al.* 2016; Walker *et al.* 2011). However, the laboratory studies showed that *C. undecimpunctata* might displace resident biological control agents by intra-guild predation since it attacked the brown lacewing *Micromus tasmaniae* (MacDonald & Walker 2012). So *H. conformis* might be important natural enemies of TPP in crops in New Zealand.

1.4.4 Predatory lacewing and hoverfly

The dominant predator of TPP in potatoes at Pukekohe, New Zealand were predatory lacewing, *M. tasmaniae* (during spring and summer) and predatory hoverfly, *Melanostoma fasciatum* in mid-summer (MacDonald & Walker 2012; Walker *et al.* 2011). In choice assays the nymphs and adults of these two native predators could eat TPP eggs, nymphs or adults when *Myzus persicae* aphids were also present and even their small predatory larvae were able to consume more than 12 TPP young nymphs in 24 h (MacDonald *et al.* 2016). These two commonly occurring predators may be important natural enemy sources for TPP in potato crops in New Zealand.

1.4.5 Predatory mites

A novel predator-prey interaction between predatory mites and TPP has been studied only in the last three years (Davidson *et al.* 2016; Geary *et al.* 2016; Patel & Zhang 2017a; b; Xu & Zhang 2015). The first predatory mite used for TPP control was *Amblydromalus limonicus* which primarily predate on TPP eggs and first instar nymph on capsicum and tomato in the laboratory (Xu & Zhang 2015). One of the top selling biocontrol agents, *Neoseiulus cucumeris*, proved to be a predatory mite of TPP eggs and first instar nymphs (Patel & Zhang 2017a; b). Compared with *N. cucumeris*, *A. limonicus* proved to be a better predatory mite against TPP (Patel & Zhang 2017a; b). In previous studies, lab assays show that *A. limonicus* females consumed more TPP eggs and first instar nymphs and produced more eggs than *N. cucumeris* (Patel & Zhang 2017b). Moreover, functional and numerical response tests indicated that *A. limonicus* had a higher attack rate and a shorter handling time on TPP eggs and first instar nymphs than the response of *N. cucumeris* (Patel & Zhang 2017a). The generalist predatory mite *Anystis baccarum* L. has also been observed attacking and eating TPP nymphs in an organic potato crop (Geary *et al.* 2016). However, *A. baccarum* only produces two to three generations per year, and it enters diapause when kept in a high temperature (28 °C) in summer. These shortcomings might limit the application of *A. baccarum* for pest control in the field (Wu 1994). In addition, *A. limonicus* could develop to an adult when fed on psyllid sugars, and this supplemental sugar may be used as alternative diet for *A. limonicus* when prey is scarce (Xu & Zhang 2015). In addition to the control of TPP, *A. limonicus* has been commercially applied in the control of thrips and whiteflies in protected crops in Europe and a mass production system was developed in January 2012 (Knapp *et al.* 2013; Mcmurtry *et al.* 2013). However, the mass-rearing of *A. limonicus* is not yet available commercially in New Zealand due to the lack of suitable food sources and the patent protection of *Carpoglyphus lactis* L. used for mass-rearing *A. limonicus* (Tepzz & Marc 2012).

1.4.6 Entomopathogenic fungi

Entomopathogenic fungi (EPF) are the predominant pathogens of Hemiptera which can penetrate the insect's integument (Lacey *et al.* 2009). In Mexico, the United States and New Zealand some fungal entomopathogens (13 *Beauveria bassiana*

strains, eight *Metarhizium anisopliae* strains, five *Isaria (Paecilomyces) fumosorosea* strains and four *Lecanicillium muscarium* strains) have been evaluated for the control of TPP (Table 1.2) (Ai-Jabr 1999; Lacey *et al.* 2009, 2011; Mauchline & Stannard 2013; Pérez-González & Sánchez-Peña 2017; Rios-velasco *et al.* 2014; Sánchez-Peña *et al.* 2007a; Tamayo-Mejía *et al.* 2014, 2015; Villegas-Rodriguez *et al.* 2014, 2017). Apart from two *I. fumosorosea* (Pfr 97 and 36 isolates) and *M. anisopliae* (F52 and DWR 346) tested on potato plants, most strains were tested on pepper and tomato plants (Table 1.2). Currently, higher levels of TPP nymph and adult mortality caused by EPF were observed compared with untreated controls under laboratory or semi-greenhouse conditions (Table 1.2). However, there are very limited field trials of EPF against TPP. In potato fields, the commercially produced fungal formations (*I. fumosorosea* Pfr 97 and *M. anisopliae* F52) significantly reduced TPP eggs, nymphs and ZC symptoms (Lacey *et al.* 2011). This resulted in higher tuber yield. Compared with the higher mortality in laboratory assays, infection rates of adults could be influenced in the field or greenhouse conditions which may be associated with great humidity and temperature range, ultraviolet radiation, wind, irrigation and rainfall (Conceschi *et al.* 2016). Besides, the environmental requirements of EPF for their infection and growth rate was very restrictive. The application of EPF in the greenhouse and field should consider the local weather conditions associated with the germination, infection, and survival of EPF (Mauchline & Stannard 2013; Vidal *et al.* 2007). More long-term field and greenhouse studies are necessary to determine the result of EPF application under laboratory and semi-greenhouse conditions. Due to the role of TPP in the rapid transmission of Lso, the economic threshold of TPP for effective TPP/ZC management is considered to be >3 TPP per trap per week in the Pukekohe region, New Zealand (Walker *et al.* 2015). In order to increase the control effectiveness on TPP and ZC, EPF should be sprayed early after the appearance of TPP or combined with other biological control agents (Lacey *et al.* 2011). Although EPF could be used as a part of IPM programs against TPP, EPF exerts a strong negative effect on non-target organisms (Dogan *et al.* 2017; Lacey *et al.* 2015; Mauchline & Stannard 2013; Seiedy *et al.* 2012a). So the compatibility of EPF with other biological control agents should be assessed before large-scale application.

The pathogenicity of *H. citriformis* on TPP adults was first reported in Mexico. However, the control potential of *H. citriformis* against TPP is still unknown (Casique-Valdez *et al.* 2011). Compared with other entomopathogenic fungi (*Beauveria*, *Metarhizium* and *Isaria*), *Hirsutella* has a longer life cycle which might limit the horizontal transmission of the fungus among TPP in the fields (Casique-Valdez *et al.* 2011). Apart from a record of fungal infections of TPP by *Z. radicans* (Cranshaw 2013), *Z. radicans* could cause 40, 74 and 76% infection rates of TPP adults after 24, 72 and 120h of incubation in the laboratory condition (Torres Acosta *et al.* 2016).

1.5 Interactions between entomopathogenic fungi and predatory mites

In integrated pest management (IPM) programs, combinations of multiple biological control agents, such as entomopathogenic fungi and predatory mites, have become an increasingly popular way to enhance the efficiency of biocontrol and further reducing the application of broad-spectrum insecticides (Numa Vergel *et al.* 2011; Wu *et al.* 2015, 2016d). Here I review previous studies about the compatibility between EPF and predatory mites to identify their interactions in the laboratory or greenhouse conditions. I herein review all studies (n=33) that examined the applications of EPF on predatory mites or on preys which fed by predatory mites.

1.5.1 Diversity of EPF and predatory mite biocontrol agents

Ten entomopathogenic fungal species and 31 strains have been evaluated on different predatory mites under high humidity conditions, and these selected strains normally were highly virulent against pests (Table 1.3). *B. bassiana* was the most evaluated EPF species on predatory mites. The majority of strains were not specific strains, and there are only five commercial strains such as *B. bassiana* (BotaniGard® 22 WP, BotaniGard® ES, and Naturalis®), *M. anisopliae* (Met52® EC), and *V. lecanii* (MicroGermin® Plus). Commercial strains of EPF have been applied in a variety of pests on crops around the world. It is insufficient to assess the interactions between commercial EPF and commercial natural enemies (Lacey *et al.* 2015).

Table 1.2. Reports of entomopathogenic fungi strains evaluated for the control of tomato/potato psyllids in the United States, Mexico, and New Zealand.

Entomopathogenic fungi	Strains	Host of strains	Targeted stages	Crops	Conditions	Country	References
<i>Beauveria bassiana</i>	–	-	Nymphs	Tomato	Lab and greenhouse	United States	(Al-Jabr 1999)
	UA-3	Grasshopper	Nymphs	Pepper and tomato	Greenhouse	Mexico	(Sánchez-Peña <i>et al.</i> 2007)
	UA-21	Soil	Nymphs	Pepper and tomato	Greenhouse	Mexico	(Sánchez-Peña <i>et al.</i> 2007)
	UA-15	Soil	Nymphs	Pepper and tomato	Greenhouse	Mexico	(Sánchez-Peña <i>et al.</i> 2007)
	Exotic GHA strain (BotaniGard® ES, USA)	-	Nymphs and adults	Pepper and tomato	Lab and greenhouse	New Zealand	(Mauchline & Stannard 2013)
	Exotic GHA strain (BotaniGard® 22WP, USA)	-	Nymphs and adults	Pepper	lab	New Zealand	(Mauchline & Stannard 2013)
	Exotic GHA strain (Mycotrol® O, USA)	-	Nymphs and adults	Pepper	lab	New Zealand	(Mauchline & Stannard 2013)
	Bb-CIAD1	Soil	Nymphs	Tomato	Lab	Mexico	(Rios-velasco <i>et al.</i> 2014)
	BB09 (Commercial strain)	-	Nymphs	Pepper	Greenhouse	Mexico	(Villegas-Rodriguez <i>et al.</i> 2014, 2017)
	BB37	<i>Melanoplus</i> sp.	Nymphs	Pepper	Lab	Mexico	(Tamayo-Mejía <i>et al.</i> 2014)
	BB40	<i>Phyllophaga</i> sp.	Nymphs	Pepper	Lab	Mexico	(Tamayo-Mejía <i>et al.</i> 2014, 2015)
	BB42	<i>Lygus</i> spp.	Nymphs	Pepper	Lab and field	Mexico	(Tamayo-Mejía <i>et al.</i> 2014, 2015, Villegas-Rodriguez <i>et al.</i> 2014, 2017)
	Exotic GHA strain (Mycotech Industries, USA)	-	Nymphs	Pepper	Lab and field	Mexico	(Tamayo-Mejía <i>et al.</i> 2014, 2015)
<i>Isaria (Paecilomyces) fumosorosea</i>	Pfr 97	<i>Bemisia tabaci</i>	Nymphs and adults	Potato	Lab and field	United States	(Lacey <i>et al.</i> 2009, 2010, 2011)
	36 isolate	<i>Diaphorina citri</i>	Nymphs and adults	Potato	Lab	United States	(Lacey <i>et al.</i> 2009)
	Exotic FE 9901 strain (NoFly™ WP, USA)	-	Nymphs and adults	Pepper	Lab	New Zealand	(Mauchline & Stannard 2013)
	Exotic Apopka 97 strain (PreFeRal® 20WG, Belgium)	-	Nymphs and adults	Pepper	Lab	New Zealand	(Mauchline & Stannard 2013)
	Isolate F129	<i>Galleria mellonella</i>	Nymphs and adults	Pepper	Lab	New Zealand	(Mauchline & Stannard 2013)

<i>Metarhizium anisopliae</i>	UA-12	Soil	Nymphs	Pepper and tomato	Greenhouse	Mexico	(Sánchez-Peña <i>et al.</i> 2007)
	UA-11	Soil	Nymphs	Pepper and tomato	Greenhouse	Mexico	(Sánchez-Peña <i>et al.</i> 2007)
	F 52	<i>Cydia pomonella</i>	Nymphs and adults	Potato	Lab and field	United States	(Lacey <i>et al.</i> 2009, 2010, 2011)
	DWR 346	Soil	Nymphs and adults	Potato	Lab	United States	(Lacey <i>et al.</i> 2009)
	Exotic F52 strain (Met52® EC, Australia)	-	Nymphs and adults	Pepper and tomato	Lab and greenhouse	New Zealand	(Mauchline & Stannard 2013)
	Ma-CIAD1	Soil	Nymphs	Tomato	Lab	Mexico	(Tamayo-Mejía <i>et al.</i> 2014)
	MA25	Scarabaeidae	Nymphs	Pepper	Greenhouse	Mexico	(Villegas-Rodríguez <i>et al.</i> 2014, 2017)
	MA28 (Commercial strain)	<i>Phyllophaga</i> sp.	Nymphs	Pepper	Lab and greenhouse	Mexico	(Tamayo-Mejía <i>et al.</i> 2014; Villegas-Rodríguez <i>et al.</i> 2014, 2017)
<i>Lecanicillium muscarium</i>	Isolate F421	<i>Scolytopa australis</i>	Nymphs and adults	Pepper	Lab	New Zealand	(Mauchline & Stannard 2013)
	Isolate F425	<i>Scolytopa australis</i>	Nymphs and adults	Pepper	Lab	New Zealand	(Mauchline & Stannard 2013)
	Isolate F426	<i>Scolytopa australis</i>	Nymphs and adults	Pepper	Lab	New Zealand	(Mauchline & Stannard 2013)
	eNtocide L™ (Biotelliga Limited, NZ)	-	Nymphs and adults	Pepper and tomato	Lab and greenhouse	New Zealand	(Mauchline & Stannard 2013)
<i>Hirsutella citriformis</i>	HC8D13	<i>Diaphorina citri</i>	Adults	Pepper	Lab	Mexico	(Casique-Valdez <i>et al.</i> 2011)
	HC8D17	<i>Diaphorina citri</i>	Adults	Pepper	Lab	Mexico	(Casique-Valdez <i>et al.</i> 2011)
<i>Zoophthora radicans</i>	-	<i>B. cockerelli</i>	Adults	-	-	United States	(Cranshaw 2013)
	-	<i>Bagrada hilaris</i>	Nymphs and adults	Pepper	Greenhouse and field	Mexico	(Torres Acosta <i>et al.</i> 2016)

“-” indicated no record in the reference.

Table 1.3. Reports in the interactions between entomopathogenic fungi strains and predatory mite.

Predators	Entomopathogenic fungi (strain)	Compatibility	Shared prey	References
<i>Amblydromalus limonicus</i>	<i>Lecanicillium lecanii</i> (Łub-05), <i>Paecilomyces lilacinus</i> (Pl-2/08)	M*	-	(Pytlak <i>et al.</i> 2014)
	<i>B. bassiana</i> (Dv-1/07), <i>I. fumosorosea</i> (Borek-04)	T*	-	(Pytlak <i>et al.</i> 2014)
<i>Amblyseius swirskii</i>	<i>B. bassiana</i> (ANT-03, GHA strain (BotaniGard® 22 WP), GZGY-1-3, SCWJ-2, SDDZ-9, SZ-26, WLMQ-32)	N*	<i>Frankliniella occidentalis</i> , <i>Tetranychus urticae</i>	(Lin <i>et al.</i> 2017; Saito & Brownbridge 2018; Wu <i>et al.</i> 2016b)
	<i>B. bassiana</i> (CQBb111)	N*, disseminate spores (↑)	<i>Diaphorina citri</i>	(Zhang <i>et al.</i> 2015b)
	<i>M. anisopliae</i> (Met52® EC)	N*, control efficiency in combination (√), predatory mite populations in combination (√)	<i>F. occidentalis</i>	(Saito & Brownbridge 2018)
	<i>B. bassiana</i> (DEBI008)	N*, predation behavior (↓)	<i>T. vaporariorum</i>	(Seiedy 2015)
	<i>B. bassiana</i> (GHA strain, BotaniGard® 22 WP)	M* in adults, N* in juvenile, fecundity (↓)	-	(Midthassel <i>et al.</i> 2016)
	<i>B. bassiana</i> (J.B., F)	M*	<i>T. vaporariorum</i>	(Seiedy <i>et al.</i> 2015)
<i>Gaeolaelaps gillespiei</i>	<i>B. bassiana</i> (GHA strain (BotaniGard® 22 WP)), <i>M. anisopliae</i> (Met52® EC)	M*, control efficiency in combination (√)	<i>F. occidentalis</i> ,	(Saito & Brownbridge 2016)
<i>Ipheseius degenerans</i>	<i>M. anisopliae</i> (ESC 1),	N*	-	(Ludwig & Oetting 2001)
	<i>B. bassiana</i> (JW-1 (Naturalis®)), <i>Lecanicillium (Verticillium) lecanii</i> (MicroGermin® Plus)	M*	-	(Ludwig & Oetting 2001)
	<i>B. bassiana</i> (GHA strain (BotaniGard® ES))	T*	-	(Ludwig & Oetting 2001)
<i>Neoseiulus barkeri</i>	<i>B. bassiana</i> (SZ-26)	Life table parameters (√)	<i>F. occidentalis</i>	(Wu <i>et al.</i> 2014)
	<i>Acremonium hansfordii</i> (Ahy1)	Survival rate(√), oviposition(√), longevity(√), and fecundity (√)	<i>T. urticae</i>	(Shang <i>et al.</i> 2018)
	<i>B. bassiana</i> (SZ-26)	Developmental time (↑), longevity (↑), predation rates (↓), oviposition (↓), efficiency in combination (↓); efficiency in combination (↑) at appropriate interval	<i>F. occidentalis</i>	(Wu <i>et al.</i> 2015, 2016a, 2017)
<i>Neoseiulus californicus</i>	<i>B. bassiana</i> (GZGY-1-3, Naturalis®, SCWJ-2, SDDZ-9, SZ-26, WLMQ-32)	N*	<i>T. urticae</i>	(Castagnoli <i>et al.</i> 2005; Wu <i>et al.</i> 2016b)
	<i>B. bassiana</i> (DSM 15126), <i>P. fumosoroseus</i> (DSM 12256)	N*, oviposition (↓), control efficiency in combination (√)	<i>T. urticae</i>	(Numa Vergel <i>et al.</i> 2011)
	<i>B. bassiana</i> (Naturalis®)	Oviposition (↓)	-	(Castagnoli <i>et al.</i> 2005)
	<i>M. anisopliae</i> (V275)	T*	<i>T. urticae</i>	(Dogan <i>et al.</i> 2017)
<i>Neoseiulus cucumeris</i>	<i>B. bassiana</i> (GHA strain (BotaniGard® 22 WP), GZGY-1-3, Naturalis-L, SCWJ-2, SDDZ-9, SZ-26, WLMQ-32)	N*	<i>F. occidentalis</i>	(Jacobson <i>et al.</i> 2001a; Saito & Brownbridge 2018; Wu <i>et al.</i> 2016b)
	<i>B. bassiana</i> (CQBb111)	N*, control efficiency in combination (↑)	<i>D. citri</i>	(Zhang <i>et al.</i> 2015b)

	<i>M. anisopliae</i> (Met52 [®] EC)	N*, control efficiency of thrips in combination (√), control efficiency of thrips in combination (↑), predatory mite populations in combination (√)	<i>F. occidentalis</i> , <i>T. urticae</i>	(Saito & Brownbridge 2018)
	<i>B. bassiana</i> (ANT-03)	Mortality (↓)	-	(Lin <i>et al.</i> 2017)
<i>Neoseiulus womersleyi</i>	<i>B. bassiana</i> (GZGY-1-3, SCWJ-2, SDDZ-9, SZ-26, WLMQ-32)	N*	<i>T. urticae</i>	(Wu <i>et al.</i> 2016b)
<i>Phytoseiulus longipes</i>	<i>Neozygites floridana</i>	Predation rates (↓), predation behaviour (√), oviposition (↓)	<i>T. evansi</i> , <i>T. urticae</i>	(Wekesa <i>et al.</i> 2007)
	<i>M. anisopliae</i> (ICIPE 78)	Efficiency in combination (√) Predatory mite populations in combination (√)	<i>T. evansi</i>	(Automated <i>et al.</i> 2018; Maniania <i>et al.</i> 2016)
<i>Phytoseiulus persimilis</i>	<i>Beauveria bassiana</i> (GZGY-1-3, J.B., SCWJ-2, SDDZ-9, SZ-26, WLMQ-32)	N*	<i>T. urticae</i>	(Seiedy & Moezipour 2017; Wu <i>et al.</i> 2016b)
	<i>Lecanicillium muscarium</i> (V24)	M*	<i>T. urticae</i>	(Donka <i>et al.</i> 2008, 2009)
	<i>Metarhizium anisopliae</i> (<i>anisopliae</i>) (V275)	T*	<i>T. urticae</i>	(Dogan <i>et al.</i> 2017)
	<i>B. bassiana</i> (DSM 15126)	N*, control efficiency in combination (√)	<i>T. urticae</i>	(Numa Vergel <i>et al.</i> 2011)
	<i>B. bassiana</i> (DEBI008)	Predation rates (↓), predation behaviour (↓), longevity (↓), oviposition (↓), life table parameters (↓)	<i>T. urticae</i>	(Seiedy 2014; Seiedy <i>et al.</i> 2012b, 2013)
	<i>B. bassiana</i> (GZGY-1-3)	Predation rates (√), predation behavior (↓)	<i>T. urticae</i>	(Wu <i>et al.</i> 2018b)
	<i>B. bassiana</i> (GHA strain (BotaniGard [®] ES))	N*, developmental time (√), longevity (√) and oviposition (↓) treated on eggs, longevity (↓) and oviposition (↓) treated on adults, control efficiency in combination (↑)	<i>T. urticae</i>	(Shaef & Lim 2017; Ullah & Lim 2017)
	<i>B. bassiana</i> (F)	M*, longevity (√)	<i>T. urticae</i>	(Seiedy & Moezipour 2017)
	<i>B. bassiana</i> JW-1 (Naturalis [®])	M* in adults, N* in eggs, oviposition (↓), T* in the laboratory, N* in the greenhouse	-	(Duso <i>et al.</i> 2008; Ludwig & Oetting 2001)
	<i>B. bassiana</i> (432.99 (Naturalis-L))	Efficiency in combination (↑) Predatory mite populations in combination (↓)	<i>T. urticae</i>	(Chandler <i>et al.</i> 2005)
<i>Stratiolaelaps scimitus</i>	<i>B. bassiana</i> (ANT-03)	N*	-	(Lin <i>et al.</i> 2017)
	<i>B. bassiana</i> (GHA strain (BotaniGard [®] 22 WP))	N*, efficiency in combination (√)	<i>F. occidentalis</i>	(Saito & Brownbridge 2016)
	<i>B. bassiana</i> (XJWLMQ-32)	N*, development (√), reproduction (√), longevity (√)	-	(Sun <i>et al.</i> 2018)
	<i>M. anisopliae</i> (Met52 [®] EC)	N*, control efficiency in combination (√)	<i>F. occidentalis</i>	(Saito & Brownbridge 2016)
<i>Typhlodromalus aripo</i>	<i>Neozygites tanajoae</i>	Predation behavior (↓), longevity (↓), control efficiency in combination (↓)	<i>Mononychellus tanajoa</i>	(Agboton <i>et al.</i> 2013)

N*—no pathogenicity or lower mortality (mortality <30%), M*—moderately harmful (mortality between 30–79%), T* harmful to predatory mites (mortality between 80–99% or >99%), “-” indicated no record in the reference.

Symbols indicate increase or positive effect (↑), decrease or negative effect (↓), no significant effect (√) on performance of predatory mites in the laboratory when sprayed by EPF, immersed into EPF, fed on EPF-treated preys or the control efficiency of combining predatory mites and EPF in the greenhouse, in comparison to a control or the single application of predatory mites or EPF.

The compatibility of EPF had been assessed on 12 predator mite species. Most previous evaluations focus on the compatibility between EPF *B. bassiana* and commercially-available predatory mites, *P. persimilis*, *A. swirskii*, and *N. cucumeris* (Table 1.3). These evaluations mainly are laboratory assessments and only few studies have been conducted in the greenhouse conditions. Shared prey/hosts of EPF and predatory mites are related to generalist feeding habitat of phytoseiid mites. *Tetranychus urticae* and *F. occidentalis* are the most shared prey species under the evaluations (Table 1.3). As a result, bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*) are the most used host plants for evaluating the compatibility between EPF and predatory mites in the laboratory and greenhouse.

1.5.2 Compatibility

The International Organisation for Biological and Integrated Control (IOBC) established a classification system to assess the side-effect of pesticides on natural enemies in the laboratory condition (Boller *et al.* 2005). According to the toxicity classes by the IOBC system, most EPF strains showed no pathogenicity or lower mortality (mortality <30%) against predatory mites; 12 EPF strains were moderately harmful (mortality between 30–79%) to predatory mites; five EPF strains were harmful to predatory mites when EPF caused 80–99% or even >99% mortality (Table 1.3). Seven predatory mite species were susceptible to EPF species (Table 1.3).

1.5.3 Behavior and predation

EPF mediate predation and behavior of predatory mites on shared prey (Seiedy *et al.* 2013; Wu *et al.* 2018b). Predatory mites might recognize the presence of EPF by detecting volatile chemicals produced by fungi (Baverstock *et al.* 2005). For example, *P. persimilis* females showed a significant avoidance response to leaves which were sprayed with EPF (Seiedy 2014; Seiedy *et al.* 2013; Wu *et al.* 2018b). When *P. persimilis* females were released on leaf treated with EPF, the mites spent significant longer time in self-grooming behavior than those mites released on leaf disks without conidia (Wu *et al.* 2018b). The avoidance response of the fungus and long time spent in self-grooming behavior by predators might decrease the influence of intra-guild interactions and allow coexistence in the same environment.

The predatory mites *P. persimilis* and *A. swirskii* spent significantly longer time in searching, handling, and feeding and had a lower predation rate when fed on *Trialeurodes*

vaporariorum and *T. urticae*-treated with *B. bassiana* (Seiedy 2015; Seiedy *et al.* 2012b). However, the predation rates of *P. persimilis* females on *T. urticae* were not affected by *B. bassiana* when *T. urticae* treated by *B. bassiana* in 24h (Wu *et al.* 2018b). Their choice tests indicated that the behavior response of *P. persimilis* was not affected by treating with *B. bassiana* conidia after 12h. However, these tests were only conducted in 12 or 24h. When *N. barkeri* fed on *B. bassiana* treated *F. occidentalis*, the predation rates and intrinsic rate of increase of predatory mite could be significantly negatively affected by *B. bassiana* (Wu *et al.* 2015). Therefore, it is critical to study the sublethal effect of EPF on predator's behavior over their entire life span.

1.5.4 Life table parameters

Predatory mites might be exposed to EPF when the mites were directly sprayed by EPF or fed on contaminated prey (Seiedy *et al.* 2012a). Even though EPF showed no pathogenicity or lower mortality (mortality <30%) against predatory mites, predatory mites might suffer from sublethal effects of EPF. For example, *B. bassiana* (SZ-26) at 1×10^8 conidia/mL caused low corrected mortality (4%) of *N. barkeri*, but the developmental time, longevity, fecundity and population parameters of *N. barkeri* were significantly affected by EPF when fed on *B. bassiana*-treated thrips (Wu *et al.* 2014, 2015). Similarly, the fecundity and life table parameters of *A. swirskii* and *P. persimilis* adults were negatively affected by EPF when the mites were directly applied by *B. bassiana* GHA strain at 1×10^8 conidia/mL, although there were no significant differences in the corrected mortality of adults after 10 days (Midthassel *et al.* 2016; Saito & Brownbridge 2018; Ullah & Lim 2017). At a lower concentration (1×10^6 conidia/mL), *B. bassiana* (DEBI008) also significantly reduced the longevity, fecundity and population parameters of *P. persimilis* when fed on infected *T. urticae* (Seiedy *et al.* 2012a). These sublethal effects not only negatively influenced the performance of the parental predatory mite generation but also affected the fitness of the offspring generation (Castagnoli *et al.* 2005). *B. bassiana*-treated parental *N. californicus* and *P. persimilis* significantly reduced egg hatching rates of offspring (Castagnoli *et al.* 2005; Pozzebbon & Duso 2010). But, contrary to these findings, egg hatching rates of *P. persimilis* and *A. swirskii* were not affected by the treatments of females infected with *B. bassiana* (Duso *et al.* 2008; Midthassel *et al.* 2016; Seiedy *et al.* 2012a). In order to provide a comprehensive evaluation of the susceptibility of phytoseiid mites to EPF, it is critical to assess the sublethal effect of EPF on the performance of parental and offspring generations.

1.5.5 Control efficiency

Greenhouse studies of the simultaneous applications of entomopathogenic fungi and predatory mites concluded that the majority of EPF have no negative effects on predators (Automated *et al.* 2018; Jacobson *et al.* 2001a; Numa Vergel *et al.* 2011; Wu *et al.* 2016a). Some studies showed that EPF were not pathogenic or had lower mortality to predators in the laboratory. However, this compatibility relationship did not mean that two types of biological control agents were compatible and could significantly improve the control efficiency compared to a single application in the greenhouse (Automated *et al.* 2018; Midthassel *et al.* 2016; Numa Vergel *et al.* 2011). The combined application of *B. bassiana* and the predatory mite *N. cucumeris* did not increase the control efficiency of *F. occidentalis* in cucumber plants (Jacobson *et al.* 2001a). The release of *N. californicus* in the rose with the first application of *P. fumosoroseus* and after *B. bassiana* did not significantly reduce *T. urticae* compared with a single application (Numa Vergel *et al.* 2011). Moreover, the combined efficiency of entomopathogenic fungi *M. anisopliae* with predatory mites *P. longipes* or *G. gillespiei* on pest *T. evansi* or *F. occidentalis* was not higher than a single application (Automated *et al.* 2018; Maniania *et al.* 2016; Saito & Brownbridge 2016). Similarly, a single application of *B. bassiana* or *N. barkeri* significantly reduced thrip populations more than the concomitant use of two agents (Wu *et al.* 2016a). However, if *N. barkeri* was released two weeks after spraying *B. bassiana*, the combined control efficiency was slightly higher than the single application of *B. bassiana* (Wu *et al.* 2017). Combining the application of *P. persimilis* and *B. bassiana* also significantly reduced the number of *T. urticae* on greenhouse tomato crops (Chandler *et al.* 2005). To achieve the maximum efficiency of combined treatments, we might consider the interactions among EPF, predators, preys and crops in the laboratory, greenhouse and field conditions (Midthassel *et al.* 2016).

1.6 Rearing of phytoseiid mites

Augmentative biological control (ABC) is defined as releasing large numbers of natural enemies (parasitoids, predators and, micro-organisms) to augment natural enemy populations and rapidly reduce pest infestations (Collier & Van Steenwyk 2004). Although ABC to suppress arthropod pests has been utilised for many years, it has not been as universally accepted and commercially applied in agricultural systems compared with chemical insecticides (Barratt *et al.* 2018; Collier & Van Steenwyk 2004). For example, in 2015, the global market of biological control agents (US\$ 1.7 billion) was less than 2% of the global

pesticide market (US\$ 58.46 billion) (Barratt *et al.* 2018). One of the main reasons is that ABC releases are much more expensive than pesticides (Collier & Van Steenwyk 2004). Large-scale regular releases of natural enemies require the commercial mass production of natural enemies.

Phytoseiid mites are important biological control agents of phytophagous mites and small soft-bodied insects like whiteflies, thrips and psyllids (McMurtry *et al.* 2013; Patel & Zhang 2017a, b; Xu & Zhang 2015). Four predatory mites are listed in the 10 most important invertebrate biological control agents in ABC (Van Lenteren 2012). Current methods of mass production of most important predatory mites require multiple organisms, including, predators, natural prey (spider mites), and host plants (Khanamani *et al.* 2017b; Rao *et al.* 2018). Maintaining multiple organisms may cause high costs in labor and rearing facilities which results in high market prices of predatory mites (De Clercq *et al.* 2005b; Rao *et al.* 2018). Moreover, mass rearing of predatory mites on natural prey often require a lot of time to prepare and wait for the predator-prey population growth (Khanamani *et al.* 2017c). One approach to reducing high-cost rearing techniques is by using factitious foods, artificial diets or pollens. The availability of an effective alternative diet for predatory mites could reduce the market prices of ABC.

1.6.1 Artificial diets

Some studies have examined the development and reproduction of predatory mites on artificial diets. Some generalist phytoseiid mites (Type II, III or IV) can develop and survive on some artificial diets. However, most predatory mite females failed to reproduce or their fecundity was much lower than those mites fed on natural diets (Abou-Awad *et al.* 1992; Itagaki & Koyama 1986; Kennett & Hamai 1980; McMurtry & Scriven 1966; Ochieng' *et al.* 1987; Ogawa & Osakabe 2008; Shehata & Weismann 1972; Shih *et al.* 1993). When phytoseiid mite adult females were switched from artificial diets (90 days maintenance periods) to natural prey, they still produced eggs. This indicates that artificial diets are able to be used as an alternative food source for predatory mites to facilitate long-term survival (Ogawa & Osakabe 2008).

Adding insect hemolymph to artificial media might improve their acceptability and nutritional quality for predators, and these enriched artificial diets could enhance the

fecundity and survival of predators (Grenier & De Clercq 2003; Nguyen *et al.* 2013). A basic artificial diet which added 20% hemolymph from oak silkworm pupae (*Antheraea pernyi*) significantly improved the fecundity and intrinsic rate of increase (r_m) of *A. swirskii* compared with those mites fed on a basic artificial diet composed of egg yolk, sucrose, yeast extract, tryptone, and honey (Nguyen *et al.* 2013). Adding pupal hemolymph of *A. pernyi* to the diet was more expensive and resulted in lower reliability for the commercial mass rearing of phytoseiid mites (Heslin *et al.* 2005; Nguyen *et al.* 2014; Xie *et al.* 1997). Other easily available and cheap nutrients—decapsulated dry cysts of the brine shrimp *Artemia franciscana* and Mediterranean flour moth *Ephestia kuehniella* eggs with basic artificial diet have been tested on the performance of *A. swirskii*. *A. swirskii* fed on artificial diet with the addition of *A. franciscana* cysts performed better than those mites fed on artificial diet with *E. kuehniella* eggs after six generations of continuous rearing (Nguyen *et al.* 2014). Khanamani *et al.* (2017b) evaluated 10 different artificial diets on life table parameters of *N. californicus*, and found that enriching an artificial diet with *E. kuehniella* eggs, *A. franciscana* cysts, and maize pollen resulted in higher intrinsic rate of increase of *N. californicus* than an artificial diet with other food sources. However, when fed on a liquid artificial diet with *A. franciscana* cysts, the developmental and reproductive parameters of *N. californicus*, *N. cucumeris*, *A. andersoni*, and *A. limonicus* performed less well compared with these predatory mites fed on natural diets of *T. urticae* or *T. latifolia* pollen (Nguyen *et al.* 2015b). Compared with the average market price of *A. franciscana* cysts, the black soldier fly *Hermetia illucens* maybe a much cheaper source because their larvae can feed on waste materials (Hale 1973). *A. swirskii* fed on a basic artificial diet enriched with 20 % *H. illucens* prepupae haemolymph had a faster development and higher fecundity than those mites offered with a basic artificial diet (Nguyen *et al.* 2015a). Artificial diets supplemented with arthropod components or pollen fully supported the development and reproduction of predatory mites which had potential applications in reducing the mass-rearing cost of predatory mites and providing supplemental foods to maintaining their populations in the protected crops after release (Nguyen *et al.* 2015b).

1.6.2 Pollen

Pollen is an important food source and satisfies the nutritional needs for phytoseiid mites, because it consists of important nutrients, such as proteins, carbohydrates, lipids, free amino acids, vitamins, flavonoids and minerals (Riahi *et al.* 2016; Wäckers 2005). Previous studies

reported that the developmental and reproductive parameters of phytoseiid mites may be influenced by different pollen species. These predatory mites include *A. limonicus* (Goleva *et al.* 2015; Lee & Zhang 2016; Nguyen *et al.* 2015b; Samaras *et al.* 2015; Vangansbeke *et al.* 2014a), *A. andersoni* (Lorenzon *et al.* 2012; Nguyen *et al.* 2015b), *A. herbicolus* (Rodríguez-Cruz *et al.* 2013), *A. swirskii* (Delisle *et al.* 2015a; Goleva *et al.* 2014, 2015; Goleva & Zebitz 2013; Kumar *et al.* 2014), *Cydnoseius negevi* (Alatawi *et al.* 2018), *Euseius concordis* (Lopes *et al.* 2018), *E. finlandicus* (Broufas & Koveos 2000), *E. ovalis* (Nguyen & Shih 2010, 2012), *E. stipulates* (Calabuig *et al.* 2018; Pina *et al.* 2012), *G. aculeifer* (Navarro-Campos *et al.* 2016), *I. degenerans* (Calabuig *et al.* 2018; Van Rijn & Tanigoshi 1999; Tsolakis *et al.* 2016), *Kampimodromus aberrans* (Lorenzon *et al.* 2012), *N. cucumeris* (Delisle *et al.* 2015a; Goleva *et al.* 2015; Nguyen *et al.* 2015b; Van Rijn & Tanigoshi 1999), *N. californicus* (Khanamani *et al.* 2017a; b; c; d; Marafeli *et al.* 2014; Nguyen *et al.* 2015b; Pina *et al.* 2012), *N. womersleyi* (Nguyen & Shih 2010, 2012), *S. scimitus* (Navarro-Campos *et al.* 2016; Xie *et al.* 2018), *Typhlodromus athenas* (Kolokytha *et al.* 2011a; b), *T. bagdasarjani* (Riahi *et al.* 2016), *T. laurentii* (Tsolakis *et al.* 2016), *T. pyri* (Lorenzon *et al.* 2012), *T. transvaalensis* (Cañarte *et al.* 2017). Based on the reproduction parameters of predatory mites on different pollen species, high crude protein content (above 25%) of pollen was considered as the best indicator for the nutritive quality of pollen for the performance of phytoseiid mites (Höcherl *et al.* 2012; Khanamani *et al.* 2017c; Riahi *et al.* 2016). Carbohydrates may be another indicator for the nutritional quality of pollen for predatory mites, and the high protein and sugar content of pollen may be related to the high reproductive performance of *N. californicus* (Khanamani *et al.* 2017c). Also, toxic secondary metabolites in pollen may influence the survival rates of predatory mites, for example, pollen of castor bean (*R. communis*) caused 100 % pre-adult mortality of the predatory mites *A. cabonus*, *N. cucumeris*, *T. balanites*, *T. bagdasarjani*, and *T. sennarensis* (Momen 2004; Ranabhat *et al.* 2014; Riahi *et al.* 2016). These toxic compounds in castor bean pollen may be produced as a chemical defense by plants against herbivores or plant pathogens (Detzel & Wink 1993). In addition, the suitability of different pollen species for predatory mites may be caused by the different morphological characteristics, such as their different shapes, spines, apertures, sizes and exine patterns (Khanamani *et al.* 2017c). Pollen grain size may influence predatory mite handling. For example, large size pollen required great effort from predatory mites to pick up and rupture individual pollen grains (Cañarte *et al.* 2017; Papadopoulos & Papadoulis 2008).

The feeding preference of phytoseiid mites was associated not only with the suitability of pollen, but also with the morphological, physiological and behavioral characteristics of phytoseiid mites (Van Rijn & Tanigoshi 1999). Phytoseiid mites can be classified into four different lifestyle types, based on their feeding habits, morphological traits and relative importance for biological control (McMurtry and Croft (1997) and McMurtry *et al.* (2013). Type I are highly specialized predators of Tetranychidae and Tydeoidea, including species that do not consume pollen at all, e.g. *Phytoseiulus* spp., *Paraseiulus* spp. and *Typhlodromina* spp.. Type II are selective predators of tetranychid mites, represented by *Galendromus* spp., *Neoseiulus* spp. and some *Typhlodromus* spp.. Type III are generalist predators, which consume pollen, but prefer or perform better on a wide range of prey, including the majority of *Typhlodromus* spp. and *Amblyseius* spp. and other species. Type IV are specific pollen feeders and generalist predators, represented by *Euseius* spp. that perform better on pollen.

1.6.3 Factitious food

Some phytoseiid mites could be mass-reared on factitious foods, i.e., an organism that generalist phytoseiids would not encounter in their natural habitat but predatory mites are capable of consuming and developing their populations on these food sources in laboratory conditions (Zhang 2003). Factitious foods comprise living, frozen, lyophilized, or irradiated insects, mites, and crustaceans (Riddick 2009). Currently, a variety of Lepidopteran eggs and storage mite species have been routinely applied in the commercial mass rearing of various insect predators (De Clercq *et al.* 2005a; Van Lenteren 2003). Also, decapsulated dry cysts of the brine shrimp *A. franciscana* (Branchiopoda: Artemiidae) can be used as a supplemental diet for the mass rearing of predatory mites (De Clercq *et al.* 2005a).

Lepidopteran eggs with high nutritional value in protein and amino acids are important food source for various arthropod predators (Hassan *et al.* 2017; Vangansbeke *et al.* 2014b). Some previous studies showed that the eggs of the Mediterranean flour moth *E. kuehniella* (Lepidoptera: Pyralidae) could support population growth of some phytoseiid mites (Delisle *et al.* 2015a; Hassan *et al.* 2017; Kishimoto 2015; Momen & El-Laithy 2007; Navarro-Campos *et al.* 2016; Nguyen *et al.* 2014; Vangansbeke *et al.* 2014b; Vantornhout *et al.* 2004; Xie *et al.* 2018). *I. degenerans* had a longer developmental time on *E. kuehniella* eggs than those mites reared on pollen of apple (*Malus domestica*), almond (*P. dulcis*), plum (*P. domestica*), castor bean and spider mites (*T. urticae*) (Vantornhout *et al.* 2004). When fed on

E. kuehniella eggs, *A. zaheri* and *N. barkeri* mites successfully developed from larvae to adulthood, but *T. balanites* mites could not reach the deutonymphal stage. *N. barkeri* females developed faster and reproduced more eggs than *A. zaheri* females when fed on *E. kuehniella* eggs (Momen & El-Laithy 2007). *E. kuehniella* eggs could support the development and reproduction of *A. swirskii* after six generations of continuous rearing, although there were significant differences in the total number of eggs and intrinsic rate of increase of females between generations (Nguyen *et al.* 2014). When offered factitious foods for *A. limonicus* on bean leaf discs, *A. limonicus* showed the fastest development, highest fecundity and largest body size on *E. kuehniella* eggs than *A. franciscana* cysts and *T. latifolia* pollen (Vangansbeke *et al.* 2014b). *E. kuehniella* eggs could support the development and reproduction of *N. cucumeris* and *A. swirskii* and resulted in shorter developmental time and higher survival rates compared with their targeted pest species *F. occidentalis* larvae (Delisle *et al.* 2015a). In order to find a suitable alternative food for the mass rearing of phytoseiid mites in Japan, the development and oviposition of eight native phytoseiid mites were tested on *E. kuehniella* eggs. The larvae of both *A. eharai* and *T. transvaalensis* developed into adults when fed on *E. kuehniella* eggs, and their females deposited significantly more eggs than those mites fed on tea (*Camellia sinensis*) pollen (Kishimoto 2015). Although *N. californicus* and *E. sojaensis* larvae fed on *E. kuehniella* eggs could still develop to adults, the fecundity of both mites was significantly lower than the mites fed on tea pollen. For another four predatory mite species (*A. tsugawai*, *Phytoseius nipponicus*, *T. vulgaris*, and *N. womersleyi*), either only a few larvae developed into adults or a few females deposited eggs when fed on *E. kuehniella* eggs (Kishimoto 2015). The development and reproduction of *Cosmolaelaps keni*, *Laelaspis astronomicus*, and *Protogamasellopsis denticus* was evaluated on eggs of *E. kuehniella*, and found that *E. kuehniella* eggs were more suitable factitious food for *C. keni* and *P. denticus* than *L. astronomicus* based on their reproduction parameters (Hassan *et al.* 2017). The soil-dwelling predatory mites *G. aculeifer* and *S. scimitus* fed on *E. kuehniella* eggs could successfully develop to adult, and the number of eggs laid by *G. aculeifer* and *S. scimitus* when fed on *E. kuehniella* eggs was similar with those females fed on *F. occidentalis* or *Tyrophagus curvipenis* (Navarro-Campos *et al.* 2016; Xie *et al.* 2018). Although rice moth *Corcyra cephalonica* eggs were shown to support development and reproduction of *A. largoensis* under laboratory conditions, other mites tested (*N. barkeri*, *C. negevi* and *A. swirskii*) failed to develop beyond the protonymph stage when fed with these eggs (Nasr *et al.* 2015). One other cheap food source, peach fruit fly *Bactrocera zonata* eggs,

could provide commensurate nutritional effects on survivorship, development and reproduction of *A. largoensis*, *N. barkeri*, and *P. kadii*, but *A. swirskii* and *C. negevi* failed to develop beyond the protonymphs stage on *B. zonata* eggs.

Astigmatid mites have been largely used as factitious prey in the mass production of different generalist phytoseiid mites (Barbosa & de Moraes 2016). *Acarus farris* (Ramakers & Van-Lieburg 1982) and *Tyrophagus putrescentiae* (Workman *et al.* 1994) were the first astigmatids to be commercially used for mass-rearing phytoseiid mite *N. cucumeris* (Gerson *et al.* 2003). *C. lactis* may be also an appropriate factitious prey for *N. cucumeris* (Ji *et al.* 2015). *Suidasia medanensis*, *C. lactis* and *Thyreophagus entomophagus* could be used as factitious prey in the mass production of *A. swirskii* (Bolckmans *et al.* 2017; Fidgett & Stinson 2008; Ji *et al.* 2015; Midthassel *et al.* 2013; Nasr *et al.* 2015). When fed on *T. putrescentiae*, *A. swirskii* had the longest developmental time and lowest fecundity and lifespan among factitious foods (*E. kuehniella* eggs and cysts of *A. franciscana*) which indicated that *T. putrescentiae* might be the less favourable factitious prey for this predatory mite (Riahi *et al.* 2017b). Astigmatid mites *Lepidoglyphus destructor* and *A. siro* were suitable factitious prey for mass-rearing of *N. californicus* (Simoni *et al.* 2006). Astigmatina species *Austroglycyphagus lukoschusi* and *Blomia tropicalis* were suitable prey for rearing *N. californicus* (Barbosa & de Moraes 2015). *T. putrescentiae* was used as an alternative prey in the commercial mass-rearing of *N. barkeri* (Ramakers 1983). *C. lactis* is used in the commercial production of *A. limonicus* in Europe (Knapp *et al.* 2013). *C. lactis* may be a suitable extra-guild prey for the mass production of *A. eharai* (Ji *et al.* 2015). In order to find a perhaps more efficient prey for the commercial mass-rearing of *S. scimitus* and *Protogamasellopsis zaheri*, 11 astigmatid species (*Acalvolia squamata*, *Aeroglyphus robustus*, *Aleuroglyphus ovatus*, *Cosmoglyphus oudemansi*, *T. cracentiseta*, *T. putrescentiae*, *B. tropicalis*, *Chortoglyphus arcuatus*, *Dermatophagoides pteronyssinus*, *Glycyphagus domesticus*, and *S. nesbitti*) were evaluated on these two predatory mites. They found that *T. putrescentiae* and *A. ovatus* were the most suitable food sources with higher oviposition rates of *S. scimitus* (3.4 and 2.3 eggs/female/day) and *P. zaheri* (8.5 eggs/female/day) (Barbosa & de Moraes 2016). The daily oviposition rates of *S. scimitus* on *T. putrescentiae* and *A. ovatus* were higher than other studies in this mite when fed on *A. siro* (Ali & Brennan 1997), on *T. putrescentiae* (Enkegaard *et al.* 1997; Shereef *et al.* 1980) and on *Rhizoglyphus echinopus*

(Hoda *et al.* 1986; Shereef *et al.* 1980). Oviposition rates of *P. zaheri* females on *A. ovatus* was higher than those females fed on *T. putrescentiae* and *R. echinopus* (Castilho *et al.* 2009).

A. franciscana cysts are a cheap food source for some predatory mites in laboratory conditions (Nguyen *et al.* 2014; Vangansbeke *et al.* 2014b; Vantornhout *et al.* 2004). *A. franciscana* cysts could support good performance on the development and reproduction of *A. limonicus* (Vangansbeke *et al.* 2014b). Also, *A. franciscana* cysts allow development, reproduction, and predation rates of *A. swirskii* to the same extent as *E. kuehniella* eggs after six generations of continuous rearing (Nguyen *et al.* 2014). *I. degenerans* successfully completed development and laid legs on decapsulated *A. franciscana* cysts (Vantornhout *et al.* 2004). When fed on encapsulated *A. franciscana* cysts, *I. degenerans* cannot develop beyond the protonymph which indicates that their protonymphs were not capable of piercing the alveolar layer of fresh *Artemia* cysts (Van Stappen 1996). However, *Artemia* sp. cysts were not a suitable factitious food for *S. scimitus* with high mortality occurring in the deutonymph stage (Xie *et al.* 2018). Compared with *E. kuehniella* eggs for the mass rearing of predatory mites, *A. franciscana* cysts have their advantages, because the market price of decapsulated *A. franciscana* cysts is less than 10 percent of those of frozen *E. kuehniella* eggs (Nguyen *et al.* 2014).

1.6.4 Supplemental food for predatory mites

Various studies have shown that supplemental food for phytoseiid mites can increase biological control of plant pests (Duarte *et al.* 2015; Nomikou *et al.* 2010). The presence of alternative food can reduce the strength of competitive interactions in arthropod food webs, such as cannibalism, intraguild predation (IGP), and hyperpredation (Pozzebon *et al.* 2015). For example, the addition of cattail pollen significantly reduced predatory mite *E. stipulatus* cannibalism and IGP between *E. stipulatus* and the predatory mite *I. degenerans*, and cattail and apple pollen could reduce the hyperpredation of *E. stipulatus* on the aphid predator *Aphidoletes aphidimyza* (Calabuig *et al.* 2018). Similar results were reported in IGP between predatory mites *T. manihoti* and *E. fustis* fed maize pollen (Onzo *et al.* 2005) and IGP between the predatory mite *N. cucumeris* and the anthocorid bug *O. laevigatus* when fed strawberry pollen (Shakya *et al.* 2009). The presence of non-prey food allowed preventive introductions of predatory mites and increased the density of predators at low densities of pests, and subsequently resulted in a reduction of pest densities (Van Rijn & Sabelis 1990).

The initial application of supplemental pollen on pepper seedlings significantly established and maintained higher densities of *A. swirskii* compared with the control without pollen (Kumar *et al.* 2015). Also, the addition of *T. angustifolia* pollen and powdered artificial diet resulted in significantly high *A. swirskii* densities on potted chrysanthemum plants (*Dendranthema X grandiflorum*) and ivy plants (*Hedera helix*) (Vangansbeke *et al.* 2016a). Similar results were found whereby *T. latifolia* pollen significantly increased *A. swirskii* populations on cucumber plants and significantly reduced whitefly *B. tabaci* adults and immatures (Nomikou *et al.* 2010). The release of the predatory mite *A. herbicolus* on young chili pepper with weekly supplementation of honey bee (*Apis mellifera*) pollen sustained a high population of predatory mites and protected chili peppers from damage from the broad mite *Polyphagotarsonemus latus* (Duarte *et al.* 2015). The presence of *T. latifolia* pollen significantly reduced the predation rates of the predatory mite *A. swirskii* and *E. ovalis* on poinsettia thrips *Echinothrips americanus* in laboratory conditions. Furthermore, greenhouse trials combining *A. swirskii* and *T. latifolia* pollen still significantly reduced the densities of *E. americanus* compared with a single application (Ghasemzadeh *et al.* 2017).

The addition of supplementary food does not always enhance the outcome for predatory mites, but instead may reduce the control efficiency due to apparent competition. For example, combining the predatory mite *E. ovalis* with *T. latifolia* pollen did not significantly enhance the control of *E. americanus* (Ghasemzadeh *et al.* 2017). Similarly, in the presence of almond pollen (*Prunus amygdalis*) *A. swirskii* consumed fewer *T. urticae* immatures than those predatory mites without almond pollen (Riahi *et al.* 2017a). Although, supplementing *Pinus brutia* pollen and *T. latifolia* pollen had a positive effect on predatory mite populations, this positive effect did not increase the control efficiency of *A. swirskii* on thrips *F. occidentalis* in eggplants cultivar “Cukurova topagi” (Kutuk 2017) or the control efficiency of *A. limonicus* on *F. occidentalis* and whitefly *T. vaporariorum* (Hoogerbrugge *et al.* 2011). Therefore, there are still some risks in the application of supplemental food on crops (Kutuk 2017). The combination of predatory mites and supplemental food may be influenced by the species of phytoseiids, the nutritional quality of food, and the type of crop leaves (Ghasemzadeh *et al.* 2017; Kutuk 2017).

1.6.5 Application method of supplemental food

Currently, much attention has focused on the conservation of generalist predatory mites on crops (Nomikou *et al.* 2010; Weintraub *et al.* 2009). Supplementary food can be added in times of prey scarcity in the greenhouse (Nguyen *et al.* 2015b). Direct application by dusting or placing the food on leaves was not so common in commercial systems because they are too labor intensive (Adar *et al.* 2014; Nomikou *et al.* 2002). Although applying corn pollen with electrostatic applicator on sweet pepper could improve *A. swirskii* populations and reduce thrips *F. occidentalis*, this application needed large amounts of pollen with high costs (Weintraub *et al.* 2009). Large amount of pollen may not stick on leaves and instead is wasted when it falls on the ground (Adar *et al.* 2014). One slow-release system relies on “sachets” hanging on plants; these sachets consist of predatory mites and factitious prey that fed on a food source (Jacobson *et al.* 2001b). Maintaining predatory mite populations on leaves can be influenced by other factors, for example, the availability of oviposition sites and shelters (Adar *et al.* 2014; Mcmurtry *et al.* 2013). Plant leaf surfaces with various structures (trichomes and domatia) could influence the retention and performance of predatory mites (Pozzebon *et al.* 2015; Schmidt 2014). Conservation of phytoseiids on plants was associated with these structures which trapped fungal spores or pollen for predatory mites or acted as a refuge to avoid cannibalism or intraguild predation (Schmidt 2014). Provision of oviposition sites using cotton patches with cattail pollen on bean plants allowed better establishment of *A. swirskii* populations and enhanced egg production (Loughner *et al.* 2011). Rayon jute twine covered with corn and oak pollen significantly promoted *E. scutalis* and *A. swirskii* mobile mites and eggs on pre-flowering pepper plants (*C. annuum*) (Adar *et al.* 2014). Cotton threads with *T. orientalis* pollen resulted in high population levels of *A. limonicus* which in turn could significantly reduce greenhouse whitefly *T. vaporariorum* on bell pepper in the greenhouse (Lee & Zhang 2018).

1.7 Tri-partite complexity among hemipterans, ants and natural enemies

Mutualism is defined as a reciprocally beneficial interaction between two organisms (Herre *et al.* 1999). Some hemipterans consume very large quantities of phloem sap from plants, consequently producing a lot of honeydew which is available for hemipteran-tending ants to feed on (Stadler & Dixon 2005). Soft-bodied Hemipterans result in less efficient defense

against natural enemies. In turn, hemipteran-tending ants provide services that protect Hemiptera by reducing the survival and abundance of natural enemies, changing their spatial distribution on plants or even killing natural enemies (Herbert & Horn 2008; Styrsky & Eubanks 2007). For example, the treehopper *Publilia concave* benefited from the ant *Formica obscuriventris* attendance by protection from salticid spiders (Orales 2000). Indirect effects of tending ants increase hemipteran populations and decrease the control efficiency by natural enemies. In order to avoid predation, animals display plastic responses to predators or their cues, including behavioral plasticity and altering life-history traits (Tigreros *et al.* 2017). Theoretical and empirical work on predation risk indicates that animals exhibit plastic responses to variation in risk by the differential allocation of feeding and antipredator-efforts—animals are expected to allocate more to antipredator efforts when the predation risk is high, but feed more in low-risk situations (Lima *et al.* 1999). Predatory ladybirds *Adalia bipunctata* show an avoidance response against aphid-tending ants *Lasius niger* by moving away from patches and reducing oviposition in these patches with *L. niger* or their semiochemicals (Oliver *et al.* 2008). Myrmecophilous ladybird *Coccinella magnifica* larvae choose to feed on non-tended pea aphids *Acyrtosiphon pisum* or carry aphids away to avoid or counter aphid-tending ants *Formica rufa* (Sloggett & Majerus 2003). Moreover, predatory hoverfly *Episyrphus balteatus* females show significant avoidance of hemipteran-tending ants *Cataglyphis aenescens* and *F. cunicularia* in aphids *Myzus persicae*. The performance measures (survival rates and fecundity) of *E. balteatus* are significantly negatively influenced by ant presence (Amiri-Jami *et al.* 2017).

The mutualistic relationships between ants and hemipterans in published references mostly concern aphids and mealybugs, and only a few of them focus on psyllids (Navarrete *et al.* 2013; Stadler & Dixon 2005). The ants *Pheidole megacephala* Fabricius and *Crematogaster striatula* Emery build shelter-like structure with detritus and soil to protect eggs of the psyllid *Diaphorina enderleini* Klimaszewski from competitors (Alén *et al.* 2011). In addition, parasitism of the Asian citrus psyllid *D. citri* Kuwayama nymphs by *T. radiata* (Waterston) is significantly affected by ants *P. megacephala* and *Brachymyrmex obscurior* Forel (Navarrete *et al.* 2013). Moreover, the ant *Lasius flavus* (Fabr.) provided protection for the hawthorn psyllids *Cacopsylla peregrina* Forster, *C. melanoneura* Forster and *C. crataegi* (Schrank) against two parasitoids, *Prionomitus tiliaris* (Dalman) and *P. mitratus* (Dalman) (Novak 1994). Mutualistic ants could protect psyllids by providing enemy-free space, but little

information is available on the indirect effects of ant odour on the performance of natural enemies (Novak 1994).

1.8 Thesis objectives

The overall aim of this thesis is to develop more effective control tools for the crop pest, tomato/potato psyllid *B. cockerelli* (TPP) by exploring the effects of diet and habitat shelter on the performance of the generalist predatory mite *A. limonicus*, and the interactions between TPP, entomopathogenic fungi, and predatory mites. It also evaluates the effect of ants on the life table parameters and predation rates of *A. limonicus*. My PhD thesis has six main objectives:

Chapter 2 evaluates the performance of a New Zealand strain of the generalist predatory mite *A. limonicus* on *Typha orientalis* pollen, factitious food and artificial diet in the laboratory to determine the best diet for the augmentation of *A. limonicus*. The main objective of this chapter was to compare the development, survival, and reproduction of *A. limonicus* on *T. orientalis* pollen, *Ephestia kuehniella* Zeller eggs, either untreated, punctured, or coated with bee honey solution (10%), and an artificial diet enriched with the pupal hemolymph of *E. posvittana*. More specifically, I hypothesized that *A. limonicus* fed on *T. orientalis* pollen would develop faster and lay more eggs than those mites fed on *E. kuehniella* eggs and artificial diet. Chapter 2 was published in *International Journal of Acarology*.

Chapter 3 investigates how habitat dispersion, diet and density influence the per capita population growth of the predatory mite, *A. limonicus*. The major objective of this chapter was to assess how variable habitat dispersion (dispersed patches, general patches, and aggregated patches), two diets (*T. orientalis* pollen and *E. kuehniella* eggs) and initial predator density—one or two *A. limonicus* founder females—on the population growth of *A. limonicus* in 7 days. More specifically I predicted that (i) dispersed patches would result in higher *A. limonicus* populations than the other two types of patches; (ii) feeding on *T. orientalis* pollen would improve *A. limonicus* populations more than *E. kuehniella* eggs; (iii) starting with one founder female would result in a higher predatory mite density than beginning with two founder females. Chapter 3 was published in *Experimental and Applied Acarology*.

Chapter 4 screens 16 New Zealand native strains of entomopathogenic fungi at a concentration of 1×10^7 conidia mL^{-1} against TPP nymphs and adults in ten days and assesses the lethal and sublethal effects of the most virulent strain on TPP through life table analysis under laboratory conditions. The main objectives of this chapter were to: (1) find out the most virulent strain of entomopathogenic fungi based on mortality and median lethal times (LT_{50}) in TPP first instar nymphs and young adults; (2) understand the sublethal effects of most virulent strain on the performance of the parental and offspring TPP generations. I predicted that some *Beauveria bassiana* and *Metarhizium anisopliae* strains would cause higher mortality and shorter LT_{50} values against TPP. Sublethal concentrations of the most virulent strain could significantly negatively influence the life table parameters and predation rates of TPP. Chapter 4 was submitted to *Crop Protection*.

Chapter 5 investigates the compatibility of the most virulent strain of fungus identified in Chapter 4 with the predatory mite *A. limonicus* in laboratory conditions. First, I hypothesized that *A. limonicus* females were susceptible to entomopathogenic fungi. And then, I assessed the toxicity of the most virulent strain of the fungus on the females of *A. limonicus* through concentration-response bioassays (ranging from 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , to 1×10^8 conidia mL^{-1}). Thus, the sublethal concentrations of the most virulent strain would be assessed on the life table parameters and predation potential of both parental and offspring *A. limonicus* generations for TPP. I expected that the sublethal concentrations of fungi would not affect the performance of two *A. limonicus* generations. Chapter 5 was submitted to *Ecotoxicology and Environmental Safety*.

Chapter 6 evaluates the combined use of two predatory mites with either entomopathogenic fungus (suspensions and dry conidia) or with supplementary food (*T. orientalis* pollen) as a control of TPP in bell pepper for 7 weeks in the greenhouse. I predicted that the application of *A. limonicus* would significantly reduce more TPP populations than *N. cucumeris*, and entomopathogenic fungi would not negatively influence predatory mites populations. Consequently, I expected that the combined use of *A. limonicus* with entomopathogenic fungus (suspensions and dry conidia) or with *T. orientalis* pollen would cause significantly decreased TPP populations and better growth of bell pepper plant than other treatments. Chapter 6 was published in *Pest Management Science*.

Chapter 7 examines the influence of the odour of hemipteran-tending ants on the performance of the predatory mite *A. limonicus* when feeding on TPP. I investigated the effects of ant odour on the development, survival, reproduction and predation rates of *A. limonicus*. The age-stage, two-sex life table theory was used to compare the life table parameters and predation rates of *A. limonicus* in the absence and presence of ant odour. I expected that the presence of ant odour would delay the developmental time and decrease the oviposition of *A. limonicus*, and the population parameters of *A. limonicus* would be negatively affected by ant odour. Moreover, ant odour would decrease the predation rates of *A. limonicus* on TPP. Chapter 7 was published in *Pest Management Science*.

Chapter 8 provides a synthesis of the preceding chapters and highlights the salience messages from my studies for achieving the Integrated Pest Management of TPP in New Zealand and overseas. The limitations of this study and future research directions/applications are also discussed within the context of the research findings.

Chapter 2

Development, survival and reproduction of a New Zealand strain of *Amblydromalus limonicus* (Acari: Phytoseiidae) on *Typha orientalis* pollen, *Ephestia kuehniella* eggs and an artificial diet

Publication status: Published

Liu, J.F. & Zhang, Z.Q. (2017) Development, survival and reproduction of a New Zealand strain of *Amblydromalus limonicus* (Acari: Phytoseiidae) on *Typha orientalis* pollen, *Ephestia kuehniella* eggs and an artificial diet. *International Journal of Acarology*, 43(2), 153–159.

<https://doi.org/10.1080/01647954.2016.1273972>

2.1 Introduction

Predatory mites (Phytoseiidae) are among the most important biocontrol agents for greenhouse pests, and 3 of the 10 highest selling biocontrol agents are mites: *Amblyseius swirskii*, *Neoseiulus cucumeris* and *Phytoseiulus persimilis* (Van Lenteren 2012). While *P. persimilis* is a type-I specialist predator of *Tetranychus* spider mites, *A. swirskii* and *N. cucumeris* are type-III generalist predators used for the control of small insects such as thrips and whiteflies (McMurtry *et al.* 2013). Another generalist species, *Amblydromalus limonicus*, was recently commercialized, primarily for thrips and whitefly control in Europe (Choraży *et al.* 2016; Knapp *et al.* 2013; Medd & Greatrex 2014). This important species was recently reported to have formed a new association with a psyllid species in New Zealand and is believed to represent an opportunity for the biocontrol of this new group of pests (Davidson *et al.* 2016; Xu & Zhang 2015).

The tomato/potato psyllid (TPP), *Bactericera cockerelli* (Hemiptera: Triozidae), has been an invasive pest species in Solanaceae crops in New Zealand since May 2006 (Teulon *et al.* 2009), causing serious damages to crops, in particular potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), bell peppers (*Capsicum annuum*), and eggplants (*Solanum melongena*) (Mauchline & Stannard 2013; Yang & Liu 2009). Moreover, *B. cockerelli* vectors *Candidatus Liberibacter solanacearum* and induces a disease known as “Zebra Chip” (ZC) (Liefing *et al.* 2008). The economic impact of *B. cockerelli* as well as the disease vectored by this species have been substantial for New Zealand primary industries: in 2008 export receipts of New Zealand capsicums were reduced by approximately \$NZ5.22 million (Teulon *et al.* 2009), and the cost of *B. cockerelli* and *Candidatus Liberibacter solanacearum* to New Zealand potato industry has been more than \$NZ120 million (Ogden 2011).

The control of *B. cockerelli* mainly depends on frequent pesticide applications (Mauchline & Stannard 2013; Walker *et al.* 2015). However, biological control is a sustainable pest control strategy in various protected crops. The predatory mite *A. limonicus* has been commercialized as an efficient biological control agent of whiteflies and thrips in protected crops (Hoogerbrugge *et al.* 2011; Knapp *et al.* 2013; Messelink *et al.* 2006; Vangansbeke *et al.* 2014c; Xu & Zhang 2015). It can also feed on pollens of various plants, leaf tissue, extrafloral nectar, mealybug honeydew, psyllid sugar and the spores of fungi (Goleva *et al.*

2015; Leman & Messelink 2015; McMurtry & Croft 1997; McMurtry & Scriven 1965; Vangansbeke *et al.* 2014a; b). Xu and Zhang (2015) and Davidson *et al.* (2016) discovered that a female *A. limonicus* could consume an average of 2.5 eggs, 2.1 nymph I or 4.4 *B. cockerelli* psyllids per day and suggested that it has good potential as a new biological control agent for tomato/potato psyllids, especially via preventive releases in early crop seasons, coupled with augmentation of predators using alternative diets. *A. limonicus* is currently not produced by any biocontrol companies in New Zealand. There is a need to find factitious and artificial diets from local sources for augmentation of *A. limonicus* in New Zealand.

The storage mite, *Carpoglyphus lactis* L., is conventionally used as the primary food source in the commercial production of *A. limonicus* (Bolckmans *et al.* 2017). However, this species causes health risks to production workers in the mass-rearing of predatory mites, and sometimes causes allergies in farmers when released in protected crops, resulting in allergic contact dermatitis and asthma (Bolckmans *et al.* 2017; Fernandez-Caldas *et al.* 2007; Halliday 2003). So the utilization of an artificial diet is considered another option for cost-effective rearing of predator mites to eliminate the application of storage mites (Riddick 2009). Some previous studies found that predatory mites of the Phytoseiidae did not perform well in terms of development and reproduction when fed basic artificial diet (Kennett & Hamai 1980; McMurtry & Scriven 1966). However, when basic artificial diets were supplemented with the dry, decapsulated cysts extracted from brine shrimp (*Artemia franciscana* Kellogg) and the haemolymph extracted from the black soldier fly (*Hermetia illucens*), predatory mites had a shorter immature duration and a higher fecundity than those mites fed only by a basic artificial diet (Nguyen *et al.* 2013, 2015a). In addition, these enriched artificial diets, like pollen, can be applied in the greenhouse to provide food for predatory mite populations (e.g. Vangansbeke *et al.* 2016a).

Several studies have demonstrated that *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) egg is an alternative food source for predatory mites (Buitenhuis *et al.* 2015; Delisle *et al.* 2015a; Nguyen *et al.* 2014; Vangansbeke *et al.* 2014b; c). The development and predation of *Dicyphus hesperus* was better on *E. kuehniella* eggs with plant feeding or access to water than on *E. kuehniella* eggs alone (Gillespie & McGregor 2000). Only 34% of *A. limonicus* could develop to adulthood on *E. kuehniella* eggs in an artificial plastic arena of the Munger cell without bean leaf (Vangansbeke *et al.* 2014b). It seems that access to water by predatory

mites is vital to their ability to utilize *E. kuehniella* eggs successfully, especially for the juvenile stages of predatory mites (Delisle *et al.* 2015a).

Although some researchers have done experiments on the development and reproduction of *A. limonicus* fed on factitious and artificial diets, none focused on the New Zealand strain of *A. limonicus* nor examined feeding on *E. kuehniella* eggs by *A. limonicus* without access to water, which was studied here, along with a treatment of coating eggs with honey solution in an effort to decrease the hardening of the egg shell. In the present study, we assessed the suitability of factitious and artificial diets as alternative food sources for *A. limonicus*, with a focus on locally available species. Another objective of this study was to evaluate the development and survival of *A. limonicus* fed on *E. kuehniella* eggs under different ambient relative humidity (RH), with or without access to water. This study also provided a basis for further studies on the factors affecting cannibalism in *A. limonicus* reared using different media (Lee & Zhang 2016).

2.2 Material and methods

2.2.1 Mite cultures and diets

A stock culture of *A. limonicus* used in this study was obtained from greenhouse capsicums in south Auckland in 2012, and maintained in a cabinet set at 25 ± 1 °C, $85 \pm 5\%$ RH, and a 16:8 h light: dark (L:D) photoperiod. Predatory mites were reared on black plastic arena (7×7 cm) on a wet sponge in a plastic tray containing water to prevent mite escape and provide them with access to water (Lee & Zhang 2016; Overmeer 1985). Some 3cm pieces of black sewing thread dusted with fresh *Typha orientalis* pollen were placed around the margin of rearing arenas to serve as a feeding station and an oviposition substrate. Fresh pollen-dusted threads were added every other day, and mouldy threads were removed frequently.

Fresh pollen of *T. orientalis*, was collected from a reserve in St Johns, Auckland. They were dried in an oven at 35 °C for 2 days, and then stored at -18 °C for the experiment (Kar *et al.* 2015)

Frozen eggs of *E. kuehniella* were obtained from Bioforce Limited (south Auckland), which imported these from the Beneficial Insectary in Guelph, Ontario, Canada. Dried decapsulated cysts of brine shrimp were purchased from a local pet shop. Both were stored at -18 °C. Bee

honey was manuka honey (Comvita® UMF® 5+) produced in New Zealand and a solution (10%) was used to coat moth eggs and as a control in experiment 1.

2.2.2 Preparation of artificial diet

The preparation of the artificial diet was modified from the method used by Nguyen *et al.* (2013), and the basic artificial diet used the following formula: honey (5%), sucrose (5%), tryptone (5%), yeast extract (5%), egg yolk (10%), decapsulated shrimp cysts (10%), and distilled water (60%). This diet was stored in 2 mL Eppendorf tubes at $-18\text{ }^{\circ}\text{C}$. The final diet used in this study consisted of 80% basic artificial diet supplemented with 20% haemolymph of leaf roller pupae, *Epiphyas posvittana* (Lepidoptera: Tortricidae). The preparation of the pupal haemolymph of *E. posvittana* also followed the method of Nguyen *et al.* (2013). The aqueous supernatant extracted from the pupal haemolymph was stored in Eppendorf tubes (size 2 mL) and freeze dried at $-80\text{ }^{\circ}\text{C}$. They were later stored in a freezer at $-18\text{ }^{\circ}\text{C}$ before addition to the basic artificial diet. The freeze-dried final diet was stored at $-18\text{ }^{\circ}\text{C}$ before use.

2.2.3 Experimental arenas

A Munger cell modified from Nguyen *et al.* (2013) was used to rear individual mites in experiments. The main differences in the modified cell are as follows. (1) The three transparent plexiglass slides were 25 mm wide, 38 mm long and 3 mm thick. (2) The middle hole (the cell) in the top two slides had a 6 mm diameter on the upper surface but a 3 mm diameter on the lower surface to allow better observation of mites in the cell. Between the middle slide and the bottom slide was placed one bean leaf disc (25 × 25 mm) and one tissue paper (25 mm wide, 38 mm long) beneath it; the tissue paper was moistened by allowing one strip to extend into a water pan below the plastic shelf holding the rearing cell. Between the top and middle slide was a thin cling film, which was punctured by an insect pin to allow air exchange; the punctured holes were small enough to prevent mites from escaping. Four metal clips were used to fix the Munger cells tightly from four directions. As the rearing space only consisted of a small surface of bean leaf discs and plexiglass sides, *A. limonicus* could not freely access to water in experiment 1. Due to the transpiration of the leaf disc, the RH immediately next to the leaf disc was higher than the ambient RH, as a stable boundary layer was formed (see e.g. Gaede 1992, Ferro & Southwick 1984).

Experiment 2 had two kinds of bean leaf discs. The no-puncture leaf disc in treatment one

was the same as those in experiment 1, while leaf-puncture discs had 9 small holes made by an insect pin (size 00) in treatment two. By puncturing the bean leaf discs, *A. limonicus* had the opportunity to drink water from the tissue paper below the leaf. The bean leaf discs was clipped tightly by the plexiglass slides and metal clips, which meant *A. limonicus* was not able to get water from the bean leaf directly in treatment one.

2.2.4 Experiment 1: development and reproduction of *A. limonicus*

Experiment 1 was conducted in a heated room at 25 ± 1 °C, $50 \pm 5\%$ RH and a photoperiod of 16:8 h light: dark (L:D). To obtain synchronized eggs of *A. limonicus*, approximately 100 pairs of newly developed adults of both sexes were collected from the stock colonies and maintained in black plastic arenas in a new tray, which had fresh pollen-dusted threads. After 8 h of oviposition, newly deposited eggs were transferred individually to the Munger cells, with *ad libitum* one of the following diets: (1) a droplet of bee honey solution (10%), (2) *E. kuehniella* eggs, (3) *E. kuehniella* eggs coated with bee honey solution (10%), (4) *E. kuehniella* eggs punctured with an insect pin, (5) artificial diet powder and (6) pollen of *T. orientalis*. The factitious and artificial diets were replenished every other day.

The duration of immature stages and the survival rate of *A. limonicus* were counted every 24 h under a microscope until all of them developed to adulthood. As *A. limonicus* immatures could not complete their development when they were reared on bee honey solution (10%), this treatment only had 30 replicates, while other treatments had 70–125 replicates each. The successful development of each *A. limonicus* stage was assessed by checking the presence of exuviae of that stage inside each rearing cell. The exuviae were removed after checking, to avoid duplicated counts.

When *A. limonicus* immatures completed development, a female mated with a male that fed on the same diet as the female breeding mate. After the *A. limonicus* female started to produce eggs, the male was removed. The preoviposition, oviposition period, longevity and fecundity of *A. limonicus* females were recorded until all females died. The sex ratio of the offspring was observed by transferring 50 eggs from each treatment to new Munger cells for rearing to adulthood, fed on the same diets as their parents. A small number of predatory mites (approximately 5%) either escaped or died accidentally, and these were not included in the final analysis. Some females died before oviposition, and they were included in the

calculation of developmental time and immature survival, but not in the calculation of reproductive parameters.

2.2.5 Experiment 2: influence of ambient relative humidity on the developmental time and immature survival of *A. limonicus* on *E. kuehniella* eggs

Although *A. limonicus* could develop to adulthood on the bean leaf discs when immatures were reared on *E. kuehniella* eggs, *A. limonicus* immatures in their experiment were reared cells with access to water (Vangansbeke *et al.* 2014b). However, in a heated greenhouse, for example, *A. limonicus* could not have constant access to water on the leaves of plants. In addition, when *A. limonicus* only fed on *E. kuehniella* eggs and water, but with no leaves, 60% of mite larvae failed to develop to adults. The lack of bean leaf disc was one of the reasons for the high mortality of larvae, which failed to pierce the chorion of *E. kuehniella* eggs (Vangansbeke *et al.* 2014b). However, *A. limonicus* had established the population in a Munger cell only with *E. kuehniella* eggs and available water in our laboratory (Chapter 3). So it was necessary to test the effect of different ambient relative humidities on the developmental time and survival rate of *A. limonicus* on *E. kuehniella* eggs.

Because *A. limonicus* could not utilize *E. kuehniella* eggs at $50 \pm 5\%$ ambient relative humidity (experiment 1), this experiment was conducted under two different ambient relative humidities ($50 \pm 5\%$ and $90 \pm 5\%$ RH), with punctured and intact leaf discs to test whether or not *A. limonicus* immatures could successfully puncture *E. kuehniella* egg chorion and develop to adults, with or without access to water. This study observed the developmental time and survival rate of *A. limonicus* fed on *E. kuehniella* eggs with a leaf-puncture disc or no-leaf-puncture disc under 90% or 50% RH, respectively. Methods for observing the development and survival of *A. limonicus* were the same as those for experiment 1. The number of individuals tested under two ambient relative humidities with punctured and intact leaf discs were 18, 14, 13, and 15, respectively.

2.2.6 Statistical analysis

Data were analysed using SPSS Statistic 17.0 to evaluate the effect of factitious and artificial diets on the developmental time and female reproductive parameters of *A. limonicus*. Before

analyses, all data were checked with a Kolmogorov-Smirnov test for normality; when the data were normally distributed, they were analysed with one-way analysis of variance (ANOVA), and the heteroscedasticity of variances was also checked. If the data were not normally distributed, a non-parametric test (Kruskal-Wallis) was used for these data, followed by Mann-Whitney U tests. Developmental times of *A. limonicus* fed on factitious and artificial diets were compared using one-way ANOVA (Tukey's b test), and significance levels were 0.05. Similar analyses were also applied to evaluate the female reproductive parameters and longevity of *A. limonicus*. The immature survival percentages and offspring sex ratio of *A. limonicus* on different diets and under two different relative humidities with punctured and intact leaf discs were analysed using chi-square analysis with SPSS ($p < 0.05$).

2.3 Results

2.3.1 Experiment 1: development and reproduction of *A. limonicus* on different diets

Immature survival. The survival of juveniles of *A. limonicus* was significantly different when fed on factitious and artificial diets (Table 2.1). Most *A. limonicus* individuals could develop to adulthood when provided with *T. orientalis* pollen, artificial diet, and punctured eggs of *E. kuehniella*, respectively.

None of the *A. limonicus* individuals completed the deutonymphal stage when only 10% bee honey solution was provided in the Munger cells.

Most *A. limonicus* immatures were unable to puncture intact *E. kuehniella* eggs, which were hardened under 50% ambient RH. As a result, less than a quarter of the protonymphs and deutonymphs developed to the next stage. However, coating *E. kuehniella* eggs with 10% bee honey reduced hardening of the eggs and resulted in more than a sixfold increase in total immature survival (Table 2.1).

Developmental time. The developmental times of *A. limonicus* protonymphs and deutonymphs were significantly influenced by the four diets, but there was no obvious difference in developmental time of *A. limonicus* larvae with different diets (Table 2.2). Offering *T. orientalis* pollen accelerated the development of immature *A. limonicus*, which was significantly shorter than the developmental time with the other three diets. There was no

significant difference between the developmental time of *A. limonicus* fed on *E. kuehniella* punctured and artificial diet, except for in the deutonymphs.

Table 2.1. Immature survival of *A. limonicus* reared on 6 diets at 25 °C.

Diet	Egg	Larva	Protonymph	Deutonymph	Egg to adult
Bee honey	90.3% (n = 28)	64.3% (n = 18)	33.3% (n = 6)	0.0% (n = 0)	0.0% (n = 0)
<i>E. kuehniella</i>	91.8% (n = 78)	53.9% (n = 42)	21.4% (n = 9)	22.2% (n = 2)	2.4% (n = 2)
<i>E. kuehniella</i> + 10% bee honey	91.8% (n = 89)	74.2% (n = 66)	43.9% (n = 29)	48.3% (n = 14)	14.4% (n = 14)
<i>E. kuehniella</i> punctured	90.0% (n = 63)	90.5% (n = 57)	84.2% (n = 48)	77.1% (n = 37)	52.9% (n = 37)
Artificial diet	93.6% (n = 117)	90.6% (n = 106)	84.9% (n = 90)	78.9% (n = 71)	56.8% (n = 71)
<i>T. orientalis</i>	95.0% (n = 95)	90.5% (n = 86)	90.7% (n = 78)	84.6% (n = 66)	66.0% (n = 66)
χ^2	2.11	58.204	112.804	44.397	147.141
df	5	5	5	5	5
<i>p</i>	0.834	< 0.001	< 0.001	< 0.001	< 0.001

χ^2 , df and *p* values were analysed using chi-square analysis with SPSS (*p* < 0.05).

Table 2.2. Developmental time of *A. limonicus* fed on four diets at 25 °C.

Diet	N ^a	Developmental time (mean ± SE; days) ^b				
		Egg	Larva	Protonymph	Deutonymph	Total immature
<i>E. kuehniella</i> + 10% bee honey	31	2.1 ± 0.1a	1.4 ± 0.1a	3.4 ± 0.6a	3.4 ± 0.8a	10.4 ± 1.1a
Artificial diet	125	2.2 ± 0.1a	1.3 ± 0.1a	2.1 ± 0.1b	3.4 ± 0.2a	9.0 ± 0.3b
<i>E. kuehniella</i> punctured	70	1.9 ± 0.1a	1.6 ± 0.1a	2.4 ± 0.2b	2.4 ± 0.2b	8.2 ± 0.3b
<i>T. orientalis</i>	100	2.1 ± 0.1a	1.2 ± 0.1a	1.2 ± 0.1c	1.6 ± 0.1b	6.1 ± 0.2c

a: Initial number of tested individuals.

b: Means followed by the same letter are not significantly different (*p* > 0.05; Tukey's b test after one-way ANOVA).

Table 2.3. Reproductive parameters of *A. limonicus* fed on three diets at 25 °C.

Diets	Preoviposition period (days) ^a	Oviposition period (days) ^a	Fecundity (eggs per female) ^a	Oviposition rate (eggs per female per day) ^a	Female longevity (days) ^a	Offspring sex ratio (%)
<i>E. kuehniella</i> punctured	2.8 ± 0.4ab	8.9 ± 1.6a	11 ± 2.7a	1.2 ± 0.1a	17.2 ± 1.7a	69 ± 3.4
Artificial diet	3.1 ± 0.3a	4.9 ± 1.1b	3.8 ± 0.7b	0.9 ± 0.1a	13.5 ± 1.4a	63 ± 4.2
<i>T. orientalis</i>	2.1 ± 0.2b	7.2 ± 0.6ab	14.6 ± 1.7a	2.1 ± 0.1b	13.4 ± 1.0a	67 ± 3.5

a: Means followed by the same letter are not significantly different (*p* > 0.05; Tukey's b test after one-way ANOVA).

Reproduction and longevity. Diets significantly influenced the reproduction of *A. limonicus* (Table 2.3). The duration of the preoviposition period was significantly affected by diet, and *A. limonicus* females reared on *T. orientalis* pollen had a shorter preoviposition period than those reared on an artificial diet. There was a significantly higher fecundity when *A.*

limonicus females were reared on *T. orientalis* pollen than on artificial diet. The daily oviposition rate of *A. limonicus* fed on *T. orientalis* pollen was higher than for those on an artificial diet and punctured *E. kuehniella* eggs. However, diets had no significant effect on the female longevity and offspring sex ratio of *A. limonicus* ($\chi^2 = 0.238$, $df = 2$, $p > 0.05$; Table 2.3).

Table 2.4. Immature survival of *A. limonicus* on *E. kuehniella* eggs under two different ambient relative humidities with punctured and intact leaf discs at 25 °C.

Diet	Egg	Larva	Protonymph	Deutonymph	Total immature
Leaf-puncture (90%)	100% (n = 18)	83% (n = 15)	47% (n = 7)	100% (n = 7)	39% (n = 7)
Leaf-puncture (50%)	100% (n = 14)	100% (n = 14)	79% (n = 11)	100% (n = 11)	79% (n = 11)
No-leaf-puncture (90%)	100% (n = 13)	85% (n = 11)	100% (n = 11)	91% (n = 10)	77% (n = 10)
No-leaf-puncture (50%)	93% (n = 14)	57% (n = 8)	38% (n = 3)	67% (n = 2)	13% (n = 2)
χ^2	3.051	8.757	12.155	5.107	16.361
df	3	3	3	3	3
<i>p</i>	0.384	0.033	0.007	0.164	0.001

χ^2 , df, and *p* values were analysed using chi-square analysis with SPSS ($p < 0.05$)
n was the number of survival individuals.

Table 2.5. Developmental time of *A. limonicus* on *E. kuehniella* eggs with different ambient relative humidities 25 °C.

Diet	N*	Developmental time (mean ± SE; days)**				
		Egg	Larva	Protonymph	Deutonymph	Total immature
Leaf-puncture (90%)	18	2.3 ± 0.3a	1.6 ± 0.2a	1.9 ± 0.3ab	2.3 ± 0.5a	8.0 ± 0.4a
Leaf-puncture (50%)	14	2.2 ± 0.2a	1.9 ± 0.2a	1.9 ± 0.4ab	1.6 ± 0.3a	7.6 ± 0.4a
No-leaf-puncture (90%)	13	1.8 ± 0.1a	1.2 ± 0.1a	2.5 ± 0.3a	1.7 ± 0.3a	7.2 ± 0.4a
No-leaf-puncture (50%)	15	2.0 ± 0.0a	1.5 ± 0.5a	1.0 ± 0.0b	3.0 ± 2.0a	7.5 ± 2.5a

*, Initial number of tested individuals.

**, Means followed by the same letter are not significantly different ($p > 0.05$; Tukey's b test after one-way ANOVA).

2.3.2 Experiment 2: influence of ambient relative humidity on the development and immature survival of *A. limonicus* on *E. kuehniella* eggs at 25 °C

Ambient relative humidity and access to water via punctured leaf significantly affected the survival rates of *A. limonicus*, except at the egg and deutonymphal stages (Table 2.4). The survival rates of *A. limonicus* were 78.6% and 76.9% when predatory mites had access to water or the ambient RH was under 90%, respectively, and these rates were higher than those under lower or higher ambient relative humidity with access to water.

There was no significant difference in the developmental time of each *A. limonicus* stage under different ambient relative humidity with or without access to water, except for the protonymphal stage (Table 2.5).

2.4 Discussion

This study attempts to provide basic data for augmentation and preventive release of *A. limonicus* with factitious and artificial diets when this predatory mite is applied to the biological control of the tomato/potato psyllid, *B. cockerelli*, in New Zealand (Davidson *et al.* 2016; Xu & Zhang 2015). Our focus is on finding diets available locally in New Zealand, which has strict biosecurity regulations (e.g. the importation of pollen is not allowed). We showed in our laboratory experiments that the punctured eggs of *E. kuehniella* and *T. orientalis* pollen are suitable food sources for the development and reproduction of *A. limonicus* on bean leaf discs without access to water. Access to water or high ambient RH influenced the immature stage survival of *A. limonicus* fed on *E. kuehniella* eggs: *A. limonicus* achieved a higher survival rate under 90% ambient RH or with access to water.

2.4.1 Water availability for *A. limonicus* feeding on *E. kuehniella* eggs

Water availability has a significant effect on the survival, development and reproduction of predatory mites (Blommers *et al.* 1977; Mori & Chant 1966; Sabelis 1981). Low relative humidity influenced the survival rates of phytoseiids. The eggs of *A. swirskii*, *P. macropilis* and *P. persimilis* were not able to hatch under 50% RH, and the hatching rate of *A. anonymus* on bean leaf discs was only 8.4 % under 50 % RH, while provision of access to water for *A. idaeus* and *A. anonymus* females prolonged their survival time by at least 3 days at 70% RH (Van Dinh *et al.* 1988; Ferrero *et al.* 2010). *Typhlodromus balanites* failed to develop beyond the protonymphal stage when fed on *E. kuehniella* egg without access to water (Momen & El-Laithy 2007). *A. limonicus* eggs could not hatch at 50% RH (Bakker *et al.* 1993; van Houten *et al.* 1995; McMurtry & Scriven 1965). In our study, the rates of egg eclosion were over 93% at 50% ambient RH. This is due to the fact that other studies placed eggs in plastic containers only without leaf or leaf disc on web paper tissue. In this study, *A. limonicus* eggs were placed on a surface of bean leaf discs, whose transpiration would increase RH in the small cell (e.g. Ferro & Southwick 1984, Gaede 1992).

Vangansbeke *et al.* (2014a; b) reported that *A. limonicus* had a higher survival rate and fecundity fed on *E. kuehniella* eggs on a bean leaf disc and provided with access to water through a tissue paper wick. However, in the current study, 97.65% of *A. limonicus* failed to develop to adulthood fed on *E. kuehniella* eggs which were only living on a bean leaf disc without access to water under 50% ambient RH. In our experiment 2, more than three-quarters of *A. limonicus* completed development to adulthood on *E. kuehniella* eggs on a bean leaf disc with access to water (water provided via punctured leaf in the enclosed cell) or under a higher ambient relative humidity (90%). This indicated that the lack of access to water or lower ambient relative humidity might influence the ability of *A. limonicus* to pierce the chorion of the *E. kuehniella* eggs, particularly for the juvenile stages of predatory mites (Delisle *et al.* 2015a). Our study further showed that only early juvenile stages of *A. limonicus* were affected (Table 2.4); the deutonymphs are larger, with better-developed chelicerae, making them better able to pierce the chorion of the *E. kuehniella* eggs than larvae and protonymphs.

During the augmentative rearing and preventive release of *A. limonicus*, it may not be easy to provide access to water or high relative humidity for *A. limonicus* so that they can puncture *E. kuehniella* eggs successfully. Instead, it would be useful if some methods could be found for changing the penetrability of *E. kuehniella* eggs. Honey solution could maintain the moisture of the shell's horny layer (Jiménez *et al.* 1994). In the present study, the survival rates of immature *A. limonicus* fed with *E. kuehniella* eggs coated with bee honey solution were over 6 times higher than for those fed on *E. kuehniella* eggs without honey coating, which could help *A. limonicus* to pierce *E. kuehniella* eggs more easily.

2.4.2 The performances of *A. limonicus* on different diets

When researchers study the effect of factitious and artificial diets on the development and reproduction of predatory mites in the laboratory, they usually provide a favourable microenvironment for mites, such as a relatively higher RH or access to water, and then the developmental time will be shorter, and survival rate and reproductive parameters will be higher. However, it is very difficult for predatory mites to have access to water or a higher RH during some periods in heated greenhouses. In order to provide basic knowledge for establishing an *A. limonicus* population in the plant system and developing long-term and sustainable pest control in greenhouses, this study simulated the greenhouse condition in the

laboratory where *A. limonicus* could not access water under 50% ambient RH. Diet significantly affected the immature survival rate of *A. limonicus*, except for the egg stage (which is a non-feeding stage and should not be affected by diet), and survival and fecundity were lower than in previous experiments in which *A. limonicus* had access to water (Nguyen *et al.* 2015b; Samaras *et al.* 2015; Vangansbeke *et al.* 2014a; b; c).

The *T. orientalis* pollen and artificial diet enriched with the pupal haemolymph of *E. posvittana* in the present study supported the development of *A. limonicus*, with 66.0% and 56.8% of the predatory mites reaching adulthood, respectively, even under a low ambient RH (50%). These rates are relatively low due to a low ambient RH. A further study at higher humidity levels (75% and 85% RH) showed *T. orientalis* pollen could support population development of *A. limonicus* in the laboratory (Lee & Zhang 2016), and *T. orientalis* pollen was used in a greenhouse experiment to augment the population development of *A. limonicus* against greenhouse whiteflies (*Trialeurodes vaporariorum*) on capsicums in a greenhouse (Lee & Zhang 2018).

The developmental time of *A. limonicus* on *T. orientalis* pollen was the shortest among all the diets and was similar to that reported by Samaras *et al.* (2015). The oviposition rate of *A. limonicus* on *T. orientalis* pollen (2.1 eggs/female/day) was higher than that reported by Van Houten *et al.* (1995) (1.5 eggs/female/day) on sweet pepper pollen; by Vangansbeke *et al.* (2014b) on *T. latifolia* (1.80 eggs/female/day); and by Nguyen *et al.* (2015b) (1.9 eggs/female/day).

In order to simulate practical application methods, dry artificial diet powder was dusted on the leaf disc directly but not dissolved in water, total juvenile development of *A. limonicus* was 8.97 days, which is longer than that reported by Nguyen *et al.* (2015b). The oviposition rate of *A. limonicus* was lower than those mites on the artificial diet designed by Kennett and Hamai (1980) (1.22 eggs/female/day) and Nguyen *et al.* (2015b) (1.90 eggs/female/day). These differences may be caused by a number of factors: the different composition and solution of artificial diet, or the humidity inside the rearing cell (no access to water in this study). On the other hand, multiple mating could increase the oviposition rate of predatory mites; in this study only one male was provided for female once, and then the male was

removed when a female started to lay eggs, so the oviposition rate of *A. limonicus* in this study was generally lower than in other studies with multiple mating.

The role of *E. kuehniella* eggs as an alternative food source for the development and reproduction of predatory mites is well documented (Delisle *et al.* 2015a; Nguyen *et al.* 2014; Vangansbeke *et al.* 2014b; c). However, when fed with eggs of *E. kuehniella* under 50% ambient RH, the survival rate of *A. limonicus* was very low (2.75%) in the first experiment, while more than half of *A. limonicus* could develop to adulthood when fed with punctured eggs of *E. kuehniella*. The lower humidity might increase the evaporation of *E. kuehniella* egg fluids punctured by insect pin in the Munger cells; as a result some *A. limonicus* were not able to get enough food to develop and produce eggs before *E. kuehniella* egg fluids dried up, and the reproductive parameters of *A. limonicus* were not high compared with those of mites fed on *T. orientalis* pollen. Thus *E. kuehniella* eggs provide enough nutrition for the development and reproduction of *A. limonicus*, but low humidity could reduce its use by predatory mites

New Zealand forbids importing any pollen from other countries due to the outbreak of serious disease (*Pseudomonas syringae*) in the New Zealand kiwifruit industry following the import of kiwifruit pollen from overseas (Everett *et al.* 2011; Greer & Saunders 2012). However, *T. orientalis* pollen is easily collected in large quantities in wetlands in New Zealand, and *Neocypholaelaps novaehollandiae* could complete development and reproduction when fed on *T. orientalis* pollen (Kar *et al.* 2015). This pollen has also been determined to be an optimal diet for the mass rearing of *A. victoriensis* and *Typhlodromus doreenae* in Australia (James 1993; James & Whitney 1993). So *T. orientalis* pollen can be chosen as an alternative diet for the augmentative rearing and preventive release of *A. limonicus* in New Zealand.

2.5 Conclusion

Under the lower ambient relative humidity (50%) and without access to water, factitious and artificial diets significant influenced the survival rates, development and reproduction of *A. limonicus*. Access to water or high ambient relative humidity (90%) is the major factor influencing the ability of *A. limonicus* to puncture *E. kuehniella* eggs, especially for immature mites. Due to the strict biosecurity regulations covering the importation of pollen to New

Zealand, locally available *T. orientalis* pollen would be a suitable food source for the augmentation of *A. limonicus* and could also be applied as an alternative diet for the preventive releases in early crop seasons. The results of our study could also be useful to understand the importance of access to water in the rearing process of predatory mites when ambient relative humidity is not very high. Further studies are needed to evaluate the effect of access to water and factitious diets on the performance of *A. limonicus* in greenhouses.

Chapter 3

Population development of the predatory mite

Amblydromalus limonicus is modulated

by habitat dispersion, diet and density of

conspecifics

Publication status: Published

Liu, J.F., Beggs, J.R., & Zhang, Z.Q. (2018) Population development of the predatory mite *Amblydromalus limonicus* is modulated by habitat dispersion, diet and density of conspecifics. *Experimental and Applied Acarology*, 76(1), 109–121.

<https://doi.org/10.1007/s10493-018-0292-5>

3.1 Introduction

Cannibalism, the killing and eating of conspecific individuals sharing the common resource, can provide a range of positive benefits at the population level; enhanced development and reproduction of the survivors, as well as reduction in intraspecific competition and predation (Richardson *et al.* 2010). Cannibalism can also strongly influence the persistence of animal population structure and dynamics, and it is a common interaction in ecological communities, especially in predator populations (Croft & Croft 1996; Polis 1981; Schausberger 2003; Schausberger & Croft 2000a; b).

The intensity of cannibalistic interactions is influenced by a variety of factors, such as habitat dispersion, diet, and population density (Van Buskirk 1989; Ji *et al.* 2016; Schausberger 2003). Microenvironment strongly affects the retention and performance of predator-prey systems on crops. In leaves, microenvironments such as trichomes and domatia increase leaf surface area to some extent, and the increased area influences the dispersal response of predator populations. For example, predatory mites prefer to spend more time and produce more eggs within open domatia (English-Loeb *et al.* 2002; Faraji *et al.* 2002). This type of habitat dispersion decreases cannibalism of immature animals by creating a refuge and reducing encounter rates between adults and immatures (Ferreira *et al.* 2008; Ghazy *et al.* 2016; Janssen *et al.* 2007). In addition, leaves with domatia can enhance the reproduction of predators at low relative humidity (30–38%) which suggests that domatia provide a climate shelter for predators in an extreme environment (Grostal & O’Dowd 1994).

Recent research for improving biological control has identified diet augmentation in crops as a promising technique for increasing populations of natural enemies (Adar *et al.* 2014; Duarte *et al.* 2015; Leman & Messelink 2015). For example, trichomes and domatia with highly folded cuticle and branching fibrils can trap pollen or fungal spores (Schmidt 2014; Tilney *et al.* 2012). These additional food sources are very important in maintaining phytoseiid persistence when prey is scarce, especially for generalist predators (Roda *et al.* 2003; Schmidt 2014; Sudo & Osakabe 2013). The application of pollen to crops can strongly increase the control efficiency of predatory mites *Amblyseius swirskii*, *A. herbicolus*, *Amblydromalus limonicus*, *Euseius scutalis*, *Iphiseius degenerans*, *Neoseiulus californicus*, and the predatory bug *Orius insidiosus* (Adar *et al.* 2014; Delisle *et al.* 2015b; Duarte *et al.* 2015; Janssen & Sabelis 2015; Khanamani *et al.* 2017a; Lee & Zhang 2016; Leman &

Messelink 2015; Loughner *et al.* 2011; Van Rijn *et al.* 2002; Vangansbeke *et al.* 2014c; a; Wong & Frank 2013). In addition to pollen, Mediterranean flour moth *Ephestia kuehniella* eggs, dry decapsulated *Artemia* sp. cysts, powdered artificial diet and grape powdery mildew may also serve as suitable alternative food for predatory bugs (*Macrolophus pygmaeus*, *O. laevigatus*, *O. majusculus*) and predatory mites (*A. swirskii*, *A. limonicus*, *Typhlodromus pyri* and *A. andersoni*) to improve pest control (Oveja *et al.* 2016; Pozzebon *et al.* 2015; Vangansbeke *et al.* 2016a; b). Moreover, the use of alternative food sources on the plant can also be used to augment some omnivorous pests such as western flower thrips, *Frankliniella occidentalis* Pergande (Leman & Messelink 2015; Vangansbeke *et al.* 2014c, 2016b). It could be a novel method to enhance the effectiveness of biocontrol by altering habitat structure and providing supplemental food (Adar *et al.* 2014; Leman & Messelink 2015; Loughner *et al.* 2011). For example, more *A. swirskii* are found on the leaves of bean and impatiens when they were provided with pollen and fibres that mimic trichomes (Loughner *et al.* 2011). Similarly, addition of pollen on twine in pepper (*Capsicum annuum*) plants significantly increased populations of both *E. scutalis* and *A. swirskii* (Adar *et al.* 2014).

Density-dependent cannibalism also affects the survival and size structure of predator populations; the interaction is more intense under higher than lower density conditions (Van Buskirk 1989; Collins & Cheek 1983; Ji *et al.* 2016; Walls 1998; Wildy *et al.* 2001). When population densities are high, some adults regulate brood size through inducing filial cannibalism and exhibiting phenotypic plasticity (Bartlett 1987; Creighton 2005). A high-density population naturally causes a shortage of habitat and food, and conspecifics with a higher encounter rate suffer from a higher frequency of cannibalism.

The predatory mite *A. limonicus*, a type III generalist predator, is commercialized as an efficient biocontrol agent of whiteflies and thrips (Ghasemzadeh *et al.* 2017; Hoogerbrugge *et al.* 2011). This predator also feeds on a wide range of non-prey food such as pollens of various plants, mealybug honeydew, extrafloral nectar, psyllid sugar and fungal spores (Hoogerbrugge *et al.* 2011; Knapp *et al.* 2013; McMurtry & Croft 1997; McMurtry & Scriven 1965; Messelink *et al.* 2006; Patel & Zhang 2017a; b; Vangansbeke *et al.* 2014c; Xu & Zhang 2015). Cannibalism is found to be widespread in generalist phytoseiid mites (Schausberger & Croft 2000a). Increasing habitat structural complexity with rice husks and adding *T. orientalis* pollen can reduce the cannibalistic behaviour of *A. limonicus* (Lee &

Zhang 2016). *A. limonicus* females display diet-dependent cannibalistic behaviour on their own eggs when fed on *T. angustifolia* pollen, or on eggs of *E. kuehniella* and *Ceratitis capitata*, but not on other prey, such as, *F. occidentalis*, *Trialeurodes vaporariorum*, or *Carpoglyphus lactis* (Vangansbeke *et al.* 2014a).

Previous studies have focused mainly on how the dispersal of predator-prey interactions influences the persistence and stability of predator-prey systems (Huffaker 1958; Huffaker *et al.* 1963). However, relatively little is known about the influence of dispersion of the microenvironment in leaves on predator abundance and performance (Crowley 1981; Huffaker 1958; Huffaker *et al.* 1963). This is one of a series of our studies on the biology and ecology of *A. limonicus*, which is much less known than other biocontrol agents (Lee & Zhang 2016; Patel & Zhang 2017a; b; Xu & Zhang 2015). In this study we conducted laboratory experiments to test the population development of *A. limonicus* when influenced by three levels of habitat dispersion (dispersed, general and aggregated patches) with two supplemental diets and two different initial densities of *A. limonicus* females. We measured the population growth of *A. limonicus* on days 1, 3, 5 and 7.

3.2 Methods

3.2.1 Mite cultures and diets

A stock colony of *A. limonicus* was maintained as described in section 2.2.1. All experiments were conducted in a climate room at 25 ± 1 °C, 16:8 h light:dark and $85 \pm 5\%$ relative humidity (RH) at Landcare Research, Auckland, New Zealand. In order to create space for oviposition sites and for decreasing cannibalism, black sewing threads were covered with glass coverslips (1×1 cm). In the experiments all mites were gravid females and their ages were between 7 and 9 days. Diets were prepared as described in section 2.2.1.

3.2.2 Experimental set-up

The experiment consisted of three levels of habitat dispersion (dispersed patches, general patches and aggregated patches) in a modified Munger cell (Li & Zhang 2016). The top and bottom slides were of the same size: 25 mm wide, 40 mm long, and 2 mm thick. The top slide had a 5-mm diameter hole covered with a piece of cling film punctured using a fine insect pin (Li & Zhang 2016) to improve air ventilation. The middle slide was 40 mm wide, 40 mm long, and 3 mm thick, and the middle hole had a 15-mm-diameter cylinder-shaped cell. There

was a plastic bag (5 cm) with water-saturated black tissue paper (15 mm diameter) between the middle slide and bottom slide. The middle hole was thus closed by the plastic bag with black tissue paper, creating a platform for the experiment. Chapter 2 showed that water is vital for *A. limonicus* piercing the chorion of *E. kuehniella* eggs; therefore nine small holes were punctured with an insect pin (size 00) through the side of the plastic bag facing the platform, which enabled mites access to water.

The habitat within the rearing unit was varied by different arrangements of eight pieces of threads (5 mm long). The gravid female is commonly considered the most cannibalistic life stage to larvae, probably because of its larger size and higher energy requirements during reproduction (Schausberger 2003). The other cannibalistic life stages, protonymphs and deutonymphs, were also found to prefer larvae to eggs (Schausberger & Croft 2000a). As it is very difficult to penetrate egg chorion, phytoseiid larvae are considered to be more vulnerable to cannibalism (Ghazy *et al.* 2016; Schausberger 2003). Some larvae develop strategies to avoid being cannibalized, for example, keeping quiet or hiding in the crevasses (Schausberger 2003). In the first treatment (dispersed patches), eight individual pieces of thread were placed along the margin of middle hole, ensuring they did not touch each other. This habitat provided the experimental area with eight small shelters that formed dispersed spaces for mite hiding/oviposition, and therefore could both attract mites to lay eggs and reduce encounter between immatures and adults. In the second treatment (general patches), four pieces of thread were stacked into one pile in the shape of #, and two piles were placed separately in the experimental area. To further increase the encounter rates of mites, the third treatment (aggregated patches) consisted of two overlapping #-shaped piles. In this study three different treatments both had eight patches, and we only changed the distribution of eight patches in each Munger cell (Figure 3.1).

3.2.3 Experimental procedure

To obtain a cohort of predators with the same age, 50 paired *A. limonicus* adults were transferred to a new black plastic plate with *T. orientalis* pollen and new pieces of black sewing thread, and the adults were allowed to lay eggs for 12 h before removal. The *A. limonicus* eggs of the same day were maintained on plastic plates with *T. orientalis* pollen for 7 days when they became adults. One or two gravid *A. limonicus* founder females were transferred individually to cells with different habitat dispersion, either provisioned with *T.*

orientalis pollen or *E. kuehniella* eggs. *Typha orientalis* pollen was dusted on threads and supplied daily for 3 days. *Ephestia kuehniella* eggs were placed near threads and also supplied for each of 3 days. The eggs, larvae, nymphs, and adults were counted under a stereomicroscope on days 1, 3, 5 and 7. The experiments were conducted as a complete three-way factorial design with $2 \times 2 \times 3 = 12$ factor combinations. As each factor combination was replicated $15 \times$, this yielded 180 individual experiments.

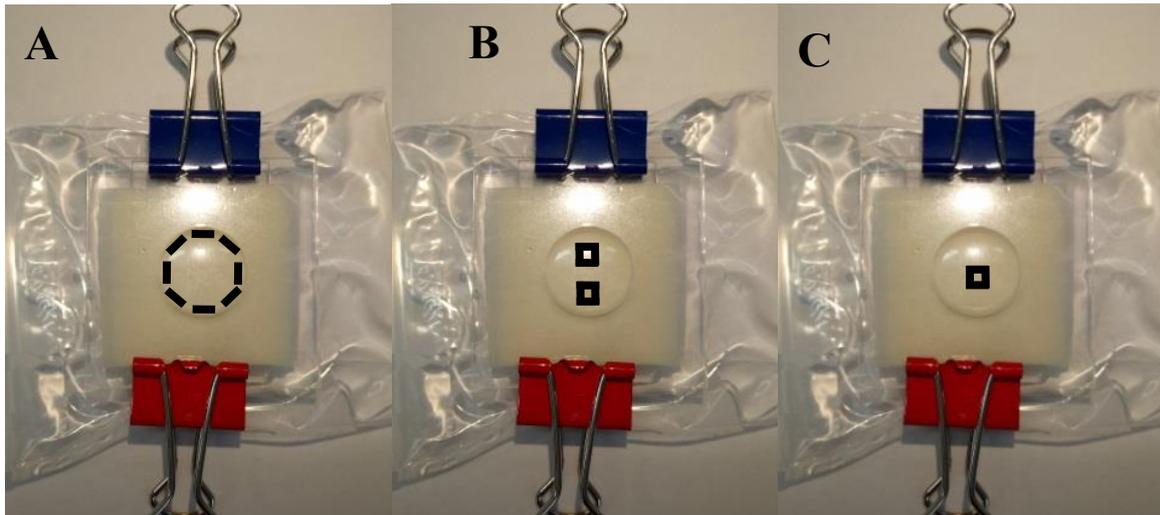


Figure 3.1. Three different arrangements of 8 pieces of threads in a Munger cell. A—Dispersed patches, B—General patches, C—Aggregated patches.

3.2.4 Statistical analysis

Data were analysed with SPSS v.17.0 (Chicago, IL, USA) to evaluate the effect of habitat dispersion, diet, and the initial density of females on the per capita population growth of *A. limonicus*. All experimental data were checked for normality with a Kolmogorov-Smirnov test. When the data were found to be normally distributed, we used a four-way ANOVA to evaluate the effect of experimental factors and their interactions on population density of *A. limonicus*. If four-way ANOVA showed a significant *P* value, the differences among habitat dispersion was tested with using Tukey's honestly significant difference (HSD) test, and the comparisons among diet and density were tested using independent samples *t* test. Before the analysis, all data on the number of *A. limonicus* were $\log(x+1)$ -transformed to meet the assumptions of ANOVA. All data on the number of *A. limonicus* started with two *A. limonicus* females is only half of the total density of mites (so that per capita growth is compared).

3.3 Results

Dispersed habitat patches resulted in a significant higher total number of *A. limonicus* than general patches from day 3 to 7 when fed on either of the diets and at both initial densities of *A. limonicus* females except with two founder females on *E. kuehniella* eggs (Tukey's HSD, Figure 3.2). The total number of *A. limonicus* fed on *T. orientalis* pollen was higher than the number of mites fed on *E. kuehniella* eggs after day 7 for each of the three habitat types (dispersed, general, aggregated patches) and two density levels (one or two founders) ($P \leq 0.009$). The total population density of mites that started with one founder was higher than the population density of mites that began with two founders when fed on either of *T. orientalis* pollen ($P = 0.002$) or *E. kuehniella* eggs ($P = 0.029$) in the dispersal patches. However, there was no significant difference in the general (pollen: $P = 0.05$, eggs: $P = 0.079$) and aggregated (pollen: $P = 0.26$, eggs: $P = 0.26$) patches.

Table 3.1. ANOVA results for the influence of habitat dispersion (aggregated, general and dispersed patches), diet (*T. orientalis* pollen, *E. kuehniella* eggs), and density (1 or 2 founder females) on population growth of *A. limonicus*.

	Immature				Female			
	SS	df	F	P	SS	df	F	P
Habitat	1.617	2	44.871	<0.001	0.083	2	4.167	0.016
Diet	0.575	1	31.907	<0.001	1.073	1	107.916	<0.001
Density	1.291	1	71.644	<0.001	0.104	1	10.504	0.001
Time	25.396	3	469.712	<0.001	12.453	3	417.483	<0.001
Habitat * diet	0.194	2	5.376	0.005	0.066	2	3.329	0.036
Habitat * density	0.063	2	1.741	0.176	0.072	2	3.611	0.028
Habitat * time	0.192	6	1.771	0.102	0.101	6	1.686	0.122
Diet * density	0.003	1	0.181	0.67	0.083	1	8.354	0.004
Diet * time	0.208	3	3.838	0.01	1.461	3	48.986	<0.001
Density * time	0.054	3	1.002	0.391	0.118	3	3.943	0.008
Habitat * diet * density	0.005	2	0.138	0.871	0.115	2	5.759	0.003
Habitat * diet * time	0.176	6	1.632	0.136	0.081	6	1.354	0.231
Habitat * density * time	0.099	6	0.915	0.483	0.127	6	2.127	0.048
Diet * density * time	0.067	3	1.236	0.296	0.121	3	4.05	0.007
Habitat * diet * density * time	0.313	6	2.898	0.009	0.201	6	3.365	0.003

A four-way ANOVA was used to evaluate the variation in population growth of *A. limonicus* among habitat, diet, density, time and their interactions (Table 3.1). The main effects indicated that habitat, diet, density, and time influenced the number of immature and female *A. limonicus* (four-way ANOVA: $P < 0.05$). Significant interactions between habitat

dispersion and diet were detected on the immature and female of *A. limonicus* (four-way ANOVA: $P < 0.05$; Table 3.1). Dispersed patches resulted in a higher number of immature (one founder: 9.6 ± 0.77 , two founders: 19.73 ± 1.64) and female *A. limonicus* (one founder: 7.27 ± 0.51 , two founders: 9.73 ± 0.52) than aggregated patches (immature, with one founder: 5.73 ± 0.56 , with two founders: 12.87 ± 0.79 ; female, with one founder: 4.87 ± 0.36 , with two founders: 6.0 ± 0.77) when *T. orientalis* pollen was used (Tukey's HSD; immature, one founder: $P = 0.003$, two founders: $P < 0.001$; female, one founder: $P = 0.001$, two founders: $P < 0.001$), whereas this positive effect of habitat was only seen in the number of immature *A. limonicus* with two founders (Tukey's HSD: $P = 0.001$) when predators were fed *E. kuehniella* eggs, regardless of the initial density of one or two *A. limonicus* females.

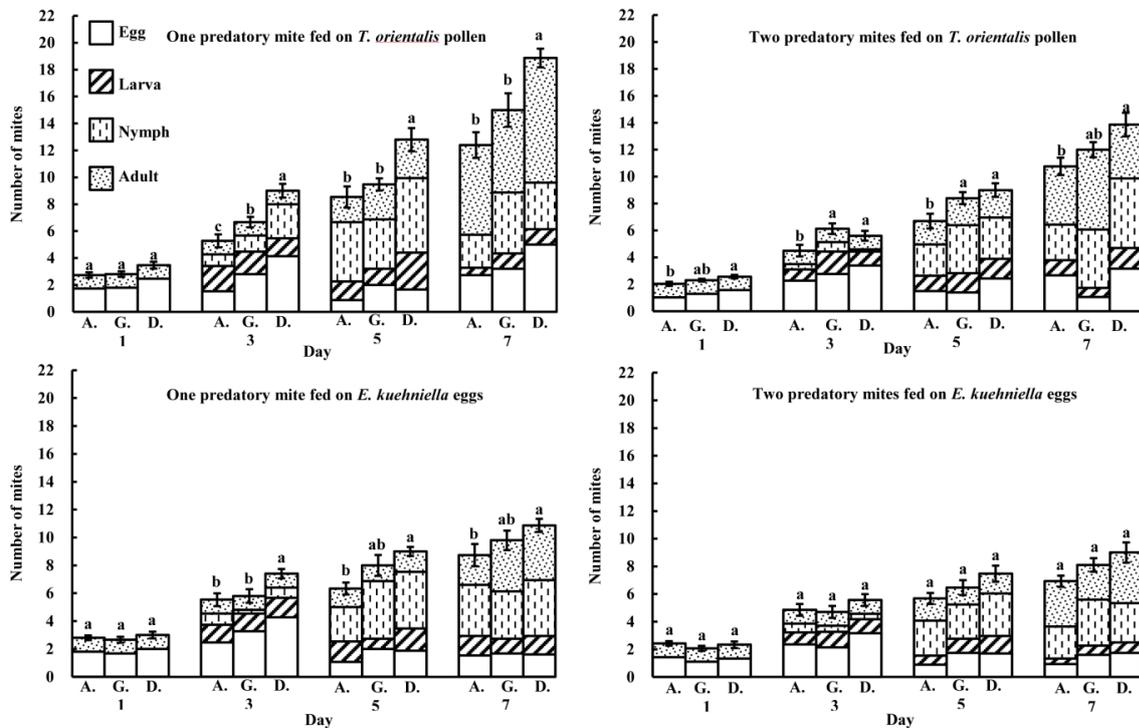


Figure 3.2. Effects of three different habitat dispersions on population density of *A. limonicus* over 7 days. Treatment differences that are not significantly different are indicated by the same letter above the respective bars ($P > 0.05$; Tukey's HSD test after one-way ANOVA). A.—Aggregated patches; G.—General patches; D.—Dispersed patches. Each treatment has 15 replicates.

There were significant interactions in habitat dispersion*density, diet*density and habitat * diet * density for the number of female but not for immature *A. limonicus* (four-way ANOVA; Table 1). Compared with starting with two *A. limonicus* females (dispersed patches: 4.87 ± 0.26 ; aggregated patches: 3.00 ± 0.38), beginning with one *A. limonicus* female on *T. orientalis* pollen produced significantly more females in dispersed (7.27 ± 0.51) and

aggregated (4.87 ± 0.36) patches (t test; dispersed patches: $t = 6.163$, $P < 0.001$; aggregated patches: $t = 3.601$, $P = 0.001$), while there were no differential effects on female *A. limonicus* when fed on *E. kuehniella* eggs.

3.4 Discussion

Previous studies have documented the effect of habitat distribution, diet, and density in the persistence of predatory mite populations. Pozzebon *et al.* (2015) found that increasing habitat structural complexity (access to domatia) and additional food (grape powdery mildew) increased the abundance of generalist predatory mites, *T. pyri* and *A. andersoni*, on plants. But it is very difficult to achieve the large-scale application of creating complex habitat structure on leaves in a greenhouse. However, it is easier to spread or spray the pieces of cotton thread to the leaves and create dispersed spaces for mite hiding/oviposition. There are few studies on how habitat dispersion, diet and density influence *A. limonicus* populations. In this study we aimed to examine the effects of (1) habitat dispersion, (2) diet, and (3) density on the population growth of *A. limonicus*. We found that (1) dispersed patches of resources resulted in a higher total number of *A. limonicus* than two less dispersed habitats, (2) diet had a significant effect on the population growth of *A. limonicus*, and (3) starting with one gravid *A. limonicus* founder female on *T. orientalis* pollen achieved a higher number of females in dispersed patches compared with starting with two females. These findings extend our knowledge of how interactions between phytoseiids and habitat dispersion/diet/density could alter the effectiveness of biological control. They also confirm that habitat dispersion can influence the strength of cannibalism and that *T. orientalis* pollen was a better nutritional quality supplemental diet for *A. limonicus* compared with *E. kuehniella* eggs. This information is important to improve the establishment of predatory mites in greenhouses.

3.4.1 Habitat dispersion

Dispersed patches consisting of eight small cotton threads (coated with pollen or *E. kuehniella* eggs) strongly increased the population growth of immature and female *A. limonicus* compared with aggregated patches, especially when mites were fed on *T. orientalis* pollen. Dispersed patches provided more spaces for gravid female mites, and these independent spaces increased the retention and performance of females in the limited microenvironment condition. This not only provided a favourable oviposition site for

predatory mite females that normally tended to produce eggs on the artificial trichomes of threads, but also acted as a shelter for mites to decrease encounters between immature and gravid female predatory mites (Adar *et al.* 2014; Loughner *et al.* 2011). In nature, the abundance of predatory mites is strongly associated with the presence of domatia and trichomes (Choraży *et al.* 2016; Loughner *et al.* 2010). In this study each cotton thread patch functioned as artificial domatia to provide hiding sites for mites, and the patch also had abundant hair on the surface similar to the leaf trichomes. Other research has demonstrated the population abundance and persistence of the predatory mites *T. pyri* and *A. andersoni* were influenced by the distribution of open domatia (Pozzebon *et al.* 2015). *Typhlodromus pyri* and *A. andersoni* gravid females prefer to spend more time and oviposit more eggs on leaves with accessible domatia or trichomes compared with leaves with fewer domatia or trichomes (English-Loeb *et al.* 2002; Roda *et al.* 2001). Similarly, *Phytoseius plumifer* females oviposit more eggs on fig leaves with plenty of glandular hairs than on sour orange (*Citrus aurantium*) smooth leaves (Rasmy & El-Banhawy 1974). In this study *A. limonicus* gravid females exhibited similar behaviour to produce more eggs in dispersed patches than in aggregated patches when fed on two diets and either started with an initial density of either one or two *A. limonicus* females. In the greenhouse we also found that *A. limonicus* normally hides in the domatia of capsicum (personal observation).

Apart from the domatia and pubescence in leaves, some researches have shown that *T. pyri* females spent more time and laid more eggs on cotton fibers or *Tetranychus urticae* webbing in a glabrous bean leaf than on a leaf without fibres or webbing (Roda *et al.* 2001). Similar results also showed that the addition of a small cotton fibre on *Gossypium hirsutum* leaves not only increased the densities of the predator bug *Geocoris* sp., but also decreased herbivorous mites and increased the overall yield of *G. hirsutum* (Agrawal & Karban 1997). Different artificial material may also influence the establishment of predatory mite populations. Cotton fibres are the common material used to help establish oviposition sites in the previous studies (Agrawal & Karban 1997; Lee & Zhang 2016; Loughner *et al.* 2011; Roda *et al.* 2001). Compared with jute material, cotton patches effectively increased and maintained *A. swirskii* populations on bean leaves (Loughner *et al.* 2011). Rayon-jute twine was considered as a better oviposition site to mimic natural domatia and leaf pubescence for *E. scutalis* and *A. swirskii* than polypropylene twine (Adar *et al.* 2014).

The densities of *A. limonicus* female fed *E. kuehniella* eggs in aggregated patches was not different from those in other patches, which means that cannibalism in this condition was more complex than in dispersed or general patches. The open domatia of coffee (*Coffea arabica*) and sweet pepper leaves (*C. annuum*) reduced larval cannibalism in *Iphiseiodes zuluagai*, *A. herbicolus*, and *I. degenerans* (Ferreira *et al.* 2008). Small patches provided more refuges for *A. limonicus* larvae and decreased larval cannibalism by females (personal observations).

3.4.2 Diet-dependent development

Providing *T. orientalis* pollen yielded more *A. limonicus* regardless of the initial density of one or two *A. limonicus* females. The role of pollen and *E. kuehniella* eggs has been well documented as alternative food sources for generalist predatory mite, *A. limonicus* (Nguyen *et al.* 2015b; Samaras *et al.* 2015; Vangansbeke *et al.* 2014b). Samaras *et al.* (2015) evaluated the nutritional suitability of the pollen of cattail (*T. latifolia*), pine (*Pinus brutia*), Calabrian corn (*Zea mays*), and olive (*Olea europea*) for *A. limonicus*: *A. limonicus* females developed faster on *T. latifolia* and *O. europea* pollen, and *T. latifolia* pollen resulted in higher intrinsic rates of increase than the other pollens. Besides, feeding on cattail pollen could prolong the developmental time and decrease the reproduction of *A. limonicus* compared with *E. kuehniella* eggs (Vangansbeke *et al.* 2014b).

The differences in developmental time and oviposition rate of *A. limonicus* between *E. kuehniella* eggs versus *T. latifolia* pollen were possibly due to (1) different *A. limonicus* strains having different development and reproduction parameters (Nguyen *et al.* 2015b). (2) improved ability of *A. limonicus* to puncture *E. kuehniella* eggs by access to water or under a high ambient relative humidity (Chapter 2), and (3) diet-dependent cannibalism in *A. limonicus* (Vangansbeke *et al.* 2014a). *Amblydromalus limonicus* females are known to consume their own eggs when reared on *E. kuehniella* eggs and *T. angustifolia* pollen (Vangansbeke *et al.* 2014a), and there was also a similar result in this study: cannibalism was more complex in the aggregated patches, which significantly reduced the population growth of predatory mites when fed on *T. orientalis* pollen and *E. kuehniella* eggs from days 3 to 7, except when beginning with two *A. limonicus* females on *E. kuehniella* eggs. In addition, the different distribution of diets provisioned in different habitat dispersion might influence mite access to diets and further result in the population growth of *A. limonicus*.

3.4.3 Density-dependent development

Per capita population growth was negatively affected by the initial density of mites probably because some factors depress mite population growth, such as mutual interference, habitat space or food limitation (Rotem & Agrawal 2003). Per capita growth rate of *T. urticae* decreased when fed on *Leonurus cardiaca* with the increasing density of conspecific mites (Rotem & Agrawal 2003). The results of our experiment indicate that beginning with two *A. limonicus* founder females fed on *T. orientalis* pollen strongly decreased the population growth of *A. limonicus* females compared with populations beginning with one *A. limonicus* female, but this effect only occurred on *T. orientalis* pollen, not on *E. kuehniella* eggs. Compared with *T. orientalis* pollen, *A. limonicus* females might not show a good appetite for *E. kuehniella* eggs even if a female did not need to compete with other females and had unlimitedly access to *E. kuehniella* eggs.

3.5 Concluding remarks

In our study, dispersed patches significantly increased the population growth of predatory mites. This finding indicates that increasing the dispersion of artificial shelters on the leaves' surface may stimulate the control efficiency of *A. limonicus* in greenhouses. Our results provide a novel method to maintain the population stability of predators controlling pests. However, our experiments on population growth of mites were only conducted in Munger cells in the laboratory. Future work should therefore study the application of dispersed patches and *T. orientalis* pollen on population dynamics of predatory mites in controlling pests in a greenhouse environment.

Chapter 4

**Lethal and sublethal effects of
entomopathogenic fungi on tomato/potato
psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera:
Triozidae) in capsicum**

Publication status: Submitted to *Crop Protection* on 12 April, 2019

4.1 Introduction

The tomato/potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is one of the most important agricultural insect pests of bell pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), potato (*Solanum tuberosum* L.), and tomato (*Lycopersicon esculentum* L.) in the United States, Mexico, and Central America (Mustafa *et al.* 2015; Yang & Liu 2009). It was first reported in New Zealand in May 2006, but had already established populations throughout North and South Islands (Thomas *et al.* 2011). This phloem-feeding insect causes damage by direct feeding on solanaceous plants, or vectoring a serious plant pathogen “*Candidatus Liberibacter solanacearum* (Lso)”, which causes Zebra Chip disease, or excreting honeydew, which can facilitate the development of sooty mold on leaf surfaces (Yao *et al.* 2016). The number of psyllid generations per year usually varies from three to seven depending on the different host plants and temperature (Munyaneza 2012). Moreover, TPP is a highly polyphagous insect and can survive on a wide range of hosts from 20 plant families, including most cultivated crops and non-crop species such as groundcherry (*Physalis* spp.), nightshade (*Solanum* spp.), and matrimony vine (*Lycium* spp.) (Munyaneza 2012; Thinakaran *et al.* 2015). Outbreaks of TPP and liberibacter infection can result in widespread plant mortality and has caused millions of dollars’ worth of losses per year in the potato industry, as well as reducing the quality of crops for export from USA, Mexico and New Zealand (Mauchline & Stannard 2013; Munyaneza *et al.* 2007).

Conventional synthetic pesticides have been widely used to control TPP and reduce the incidence of Zebra Chip (ZC). Since the arrival of TPP in the Pukekohe region (North Island, New Zealand) the average number of insecticide applications has increased to 16 on potatoes (Wright *et al.* 2017). In Texas, growers used 18 types of insecticides to control Zebra Chip and TPP in the 2009–2011 crops (Guenther *et al.* 2012). In Mexico, up to 30 applications of insecticides were delivered with different modes of action (Almeyda-León *et al.* 2008). The increasing number of insecticide applications by producers in greenhouses and fields had increased the development of resistance in psyllid populations; for example, in California and northern Mexico psyllid populations have developed resistance to imidacloprid and spinosad (Almeyda-León *et al.* 2008; Liu & Trumble 2007). In addition, synthetic pesticides are costly, can seriously harm human health, and cause environmental problems including the elimination of populations of natural enemies (Carvalho 2017). Recently, several synthetic pesticides (fenitrothion and phorate) have been banned in New Zealand, and overseas

pesticides need to be de-registered which means that some synthetic pesticides are becoming unavailable to growers (Glare & O’Callaghan 2017). New Zealand growers and companies are forced to seek alternatives, for example, the rapidly growing biopesticide market may prove the purchasing requirements from NZ growers (Glare & O’Callaghan 2017).

Biopesticides are an eco-friendly alternative to chemical pesticides (Roberts & Hajek 1992). Entomopathogenic fungi (EPF) are virulent against a wide spectrum of plant insect pests and can provide ready-made components in IPM programmes (Lacey *et al.* 2015). EPF conidia adhere to and germinate on the host cuticle, and then penetrate the host cuticle with enzymes during the invasion process (Qu & Wang 2018). When conidia enter the host hemocoel, they disturb the physiological balance, produce secondary metabolites, largely compete for host nutrients, and finally kill the host (Qu & Wang 2018). A number of fungal entomopathogens have been evaluated or released to control *B. cockerelli* in the laboratory, greenhouse and fields in Mexico, United States and New Zealand; for example, thirteen *Beauveria bassiana* strains have been evaluated against *B. cockerelli* nymphs and adults: *Metarhizium anisopliae* (eight strains), *Isaria (Paecilomyces) fumosorosea* (five strains) and *Lecanicillium muscarium* (four strains) (Ai-Jabr 1999; Lacey *et al.* 2009, 2011; Mauchline & Stannard 2013; Pérez-González & Sánchez-Peña 2017; Rios-velasco *et al.* 2014; Sánchez-Peña *et al.* 2007a; Tamayo-Mejía *et al.* 2014, 2015; Villegas-Rodríguez *et al.* 2014, 2017). Considerable levels of TPP nymphs and adults mortality caused by EPF were observed compared with untreated control under laboratory or semi-greenhouse conditions. In the fields, *M. anisopliae* (F52[®]) and *Isaria fumosorosea* (Pfr 97[®]) significantly reduced psyllid damage and the prevalence of Zebra Chip symptoms on potato (Lacey *et al.* 2011). Therefore, EPF could provide good insecticidal activity to rapidly and substantially reduce psyllids populations and ZC disease (Lacey *et al.* 2009, 2011).

In New Zealand, Mauchline and Stannard (2013) screened six exotic bioinsecticides (*B. bassiana*, *I. fumosorosea*, and *M. anisopliae*) and four native fungal isolates (*Lecanicillium muscarium*) on *B. cockerelli* adults and nymphs. Exotic BotaniGard[®] ES (*B. bassiana*) resulted in the greatest reduction in *B. cockerelli* in the lab and greenhouse trials. However, there is a high environmental risk to introducing a non-native fungal pathogen to New Zealand, because the effects of exotic fungal pathogens are unknown for non-target organisms (Hokkanen & Hajek 2003). Also, it has the limitation in importing biopesticides

containing microorganism from overseas, because these biopesticides need the approvals from the Ministry for Primary Industries (MPI) and Environmental Protection Agency (EPA) (Glare & O’Callaghan 2017). In addition, some native fungi strains demonstrated good activity against multiple local solanaceous crop pests (Sánchez-Peña *et al.* 2007). In this study, we first screened 16 NZ fungi strains against TPP adults and nymphs in the laboratory and the stains were obtained from the International Collection of Microorganisms from Plants (ICMP). Our aim is to screen the existing NZ EPF strains to identify the most virulent strain for use in TPP integrated pest management.

A comprehensive measurement of the deleterious effects of EPF on psyllids requires not only assessment of acute lethal effects but also consideration of possible sublethal effects (Farooq 2016; Jarrahi & Safavi 2016b; Wu *et al.* 2016c; Yuan *et al.* 2018). A virulent EPF strain should have higher mortality and shorter median lethal time on TPP and then it might decrease Lso transmission by psyllids. Because *B. cockerelli* adults only need a two-week Lso latent period, and are able to successfully transmit Lso to new host plants after the acquisition access periods of 24 and 72-h on infected host plants (Sengoda *et al.* 2014). *B. bassiana* (Bb-CIAD1) and *M. anisopliae* (Ma-CIAD1) quickly result in 50% mortality of TPP nymphs in 6.4 and 4.8 days, respectively (Rios-velasco *et al.* 2014). Moreover, sublethal effects can be defined as impacts (either physiological or behavioural) on individuals that survive exposure to a concentration of a chemical (Wang *et al.* 2018). Such effects may have important consequences on life table parameters, such as shortening or prolonging development rates, and reducing longevity or fecundity (Cui *et al.* 2018; Jarrahi & Safavi 2016a; b; Pelizza *et al.* 2013; Seyed-Talebi *et al.* 2012; Torrado-León *et al.* 2006; Wang *et al.* 2014, 2018; Zhang *et al.* 2015a). However, currently there is little information on the sublethal effects of EPF on *B. cockerelli*. Therefore, it is critical to assess the sublethal effects of EPF through a life table study to better understand the flow-on effects to the population dynamics of TPP colonies.

We conducted experiments aiming to select a virulent NZ entomopathogenic fungus from 16 NZ EPF strains based on their mortality and median lethal time on *B. cockerelli* nymphs and adults. The most virulent strain (*B. bassiana* ICMP 8701) was selected based on higher mortality and shorter LT₅₀ of *B. cockerelli* at 1×10^7 mL⁻¹ in 10 days. The lethal and sublethal effects of *B. bassiana* ICMP 8701 on *B. cockerelli* were then evaluated through a life table

study for two generations. To the best of our knowledge, this is the first study on the sublethal effects of EPF for the control of TPP in a transgenerational experiment.

4.2 Materials and Methods

4.2.1 Host plant

Capsicum (variety 'Giant Bell' Yates) were seeded first in a black transplant tray (10*20cm) with soil (Big Value Potting Mix, New Zealand) in a greenhouse under ambient Auckland conditions. After 10 days, the seedlings were individually transferred to 1-L plastic pots with 2 kg soil, 15 g compound fertilizer (Slowenne 212, Valagro S.p.A. Zona Ind. le 66041 Atessa (Chieti), Italy), and 150 mL vermiculite. The plants were watered every 3 days. When the capsicum achieved 10–12 fully expanded leaves (after one month), the top fully expanded leaves were used in the bioassay.

A leaf was inserted into a plastic vial (5 ml) filled with sterile distilled water. Blue TACK (10 mm*10 mm, Bostik Australia Pty Ltd, Australia) was used to fix the plastic vial to the bottom of a 1-L cup (china) (Yang *et al.* 2013). Each cup was covered with a 200-mesh polyethylene screen on the cup lid (80 mm diameter hole).

4.2.2 Tomato/potato psyllid colony

In this study, experiments were conducted on TPP first instar nymphs and adults. The *B. cockerelli* colony was derived from a population reared in captivity by Plant & Food Research Limited (New Zealand) since July 2015. I reared a laboratory colony in a fine-mesh cage (100×70×70 cm, mesh size: 0.125mm) with capsicum 'Giant Bell' Yates products in a climate-controlled greenhouse in Auckland. In order to obtain TPP individuals of the same age for use in bioassays, nearly 200 psyllid adults were transferred to a cage with two capsicum plants using an aspirator, and the adults were allowed to mate and lay eggs for 48 hours, after which adults were removed (Patel & Zhang 2017a; b). The infested capsicum was maintained in the cage. The capsicum leaves infested with the first instar nymphs (5 days) or adults (23 days) were used in the bioassay.

4.2.3 Entomopathogenic fungi strains

The sixteen EPF strains used in this study (Table 4.1) were obtained from the International Collection of Microorganisms from Plants (ICMP, Auckland New Zealand). Most entomopathogenic fungi strains were collected in New Zealand from 1960s and 1970s, kept deep frozen in liquid nitrogen at $-196\text{ }^{\circ}\text{C}$ and re-cultured periodically. The fungal isolates were recovered from storage in liquid nitrogen, grown on potato dextrose agar (PDA) media for 14 days at $18 \pm 1\text{ }^{\circ}\text{C}$ and 12:12h (L: D) photoperiod. Fungal suspensions were prepared by scraping the conidia from the agar plate and then adding them to 10-ml sterile reverse osmosis water with 0.05% Tween 80 (Merck KGaA, Darmstadt, Germany) in the screw cap test tube. The quality of the 16 strains was evaluated by counting the conidial concentration in a hemacytometer (Neubauer Improved, Germany) and adjusting to 1×10^7 conidia mL^{-1} . The germination rate of conidia from each strain was assessed by counting germinated spores on PDA (0.1 ml) poured onto petri dishes 90×15 mm (Thermo Fisher Scientific). A sterile microscope cover slip was placed on the surface of each plate. Conidial viability was checked by examining 100 random conidia per plate after 24 h of incubation at $18 \pm 1\text{ }^{\circ}\text{C}$ under a light microscope. Spores were checked for viability before every experiment, and viability over 90% was considered an acceptable level for each experiment.

4.2.4 Pathogenicity of fungal stains against psyllid first instar nymphs and adults

This experiment was conducted to identify the virulence of 16 isolates to infect TPP first instar nymphs and adults. It was very difficult to transfer first instar nymphs with a fine brush because they adhere closely to the capsicum leaves, so the capsicum leaves infested with TPP were cut into short pieces of 1–2 cm in length, with each piece containing appropriately 10 individuals. Adults were anaesthetised using low temperature and transferred to filter paper by a fine brush. Twenty psyllid first instar nymphs and 2-day-old adults were immersed in 1 mL fungal suspension containing 1×10^7 conidia mL^{-1} for approximately 10 s and the suspensions were drawn away by Venturi vacuum pump (Rocker 300) as described by Mauchline and Stannard (2013). The control groups were immersed in sterile water containing 0.05% Tween 80. After treatment, infected first instar nymphs and adults were placed on filter papers (42.5 mm) for 20 min to remove excess inoculum. TPP first instar nymphs and adults were subsequently transferred to a cup containing a fresh capsicum leaf. Capsicum leaves were washed using tap water and allowed to dry for 10 min before use in a

treatment. Cups were incubated in a cabinet set at 25 ± 1 °C, $85 \pm 5\%$ RH, and 16:8 h light:dark (L:D). Each treatment was replicated five times (20 first instar nymphs or adults/replicate). The experiments were conducted in completely randomized block design.

For 10 days, we observed the symptoms of EPF infections daily under a stereoscope microscope. Dead psyllids were removed and placed on a new capsicum leaf in a petri dish containing a piece of moistened filter paper. The Petri dish was sealed with a piece of cling film and punctured by an insect pin (size 00) to allow air exchange. In order to avoid saprophytic fungal growths, capsicum leaf was washed by sterile water and we also used new filter paper and Petri dishes to do the experiment. It was incubated at 25 °C for 3–5 days, and the cause of death of psyllids by EPF was re-confirmed by post-mortem, the fungal growth on the infected psyllid.

Table 4.1. List and origin of entomopathogenic fungal strains against *Bactericera cockerelli*.

Accession No. ¹	Culture	Host	Site origin (Collected date)
ICMP 2080	<i>Beauveria bassiana</i> (Balsamo) Vuillemin	<i>Declana floccosa</i> (Walker)	Dargaville, Northland (1967)
ICMP 6887	<i>Beauveria bassiana</i> (Balsamo) Vuillemin	<i>Aenetus virescens</i> (Doubleday)	Wainuiomata, Wellington (1980)
ICMP 8147	<i>Beauveria bassiana</i> (Balsamo) Vuillemin	<i>Vespa germanica</i> (Fabricius)	Kerikeri, Northland (1983)
ICMP 8701	<i>Beauveria bassiana</i> (Balsamo) Vuillemin	<i>Hyperodes bonariensis</i> (Kuschel)	Wairakei, Taupo (1984)
ICMP 18372	<i>Beauveria bassiana</i> (Balsamo) Vuillemin	Milk powder ex dairy factory	Auckland (2010)
ICMP 19153	<i>Beauveria bassiana</i> (Balsamo) Vuillemin	—	New Zealand
ICMP 5539	<i>Beauveria pseudobassiana</i> S.A.Rehner & Humber	<i>Graphognathus leucoloma</i> (Boheman)	Mt Albert, Auckland (1977)
ICMP 5541	<i>Beauveria pseudobassiana</i> S.A.Rehner & Humber	<i>Graphognathus leucoloma</i> (Boheman)	Mt Albert, Auckland (1977)
ICMP 18977	<i>Beauveria pseudobassiana</i> S.A.Rehner & Humber	<i>Amphipsalta zelandica</i>	Omana Regional Park, Auckland (2011)
ICMP 15464	<i>Isaria cicadae</i> Miquel	Cicada nymph	Waitakere Ranges, Auckland
ICMP 3259	<i>Metarhizium anisopliae</i> Petch	<i>Heteronychus arator</i> (Fabricius)	New Zealand
ICMP 5439	<i>Metarhizium anisopliae</i> Petch	<i>Stethaspis longicornis</i> (Arrow)	Leigh, Auckland (1965)
ICMP 149	<i>Metarhizium novozealandicum</i> (Driver & Milner) Kepler, Rehner & Humber	<i>Oxycanus</i> sp.	Palmerston North, Wanganui (1961)
ICMP 3095	<i>Metarhizium novozealandicum</i> (Driver & Milner) Kepler, Rehner & Humber	<i>Costelytra zelandica</i>	Palmerston North, Wanganui (1969)
ICMP 3258	<i>Metarhizium novozealandicum</i> (Driver & Milner) Kepler, Rehner & Humber	<i>Wiseana</i> sp.	New Zealand
ICMP 2113	<i>Metarhizium novozealandicum</i> (Driver & Milner) Kepler, Rehner & Humber	Caterpillar	Christchurch, Mid Canterbury (1967)

¹Access number of the International Collection of Microorganisms from Plants (ICMP, New Zealand: <https://www.landcareresearch.co.nz/resources/collections/icmp>).

4.2.5 Effects of different concentrations on susceptibility of adults

The most virulent of the 16 EPF strains was *B. bassiana* ICMP 8701; this strain had the highest mortality and lowest LT_{50} values against *B. cockerelli* first instar nymphs and 2-day old adults in 10 days. Therefore, this strain was selected to conduct concentration-dependent mortality response assays at five concentrations: 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 conidia mL^{-1} . Treatments and control were prepared as described above. The experiment was conducted in cups using the same adult TPP treatments adults described above. Control TPP adults were immersed in sterile water containing 0.05% Tween 80. Twenty 2-day-old TPP adults were maintained as described above and TPP mortality and symptoms of fungal infection were observed daily for 10 days. Dead TPP adults were transferred to petri dish as described in the last experiment for the reconfirmation of infection. The experiment was completely randomized and replicated five times.

4.2.6 Evaluation of sublethal effects of *B. bassiana* on reproduction of *B. cockerelli* adults

Although *B. cockerelli* first instar nymphs and adults could cause economic damage by feeding on solanaceous plants, *B. cockerelli* adults can also vector a serious pathogen “*Candidatus Liberibacter solanacearum*” which causes Zebra Chip disease. This experiment focused on evaluating the sublethal effects of EPF on *B. cockerelli* adults. Based on concentration-mortality response assays, 2-day-old *B. cockerelli* adults were treated with sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701. The 2-day-old *B. cockerelli* adults ($n=180$) were collected using an aspirator and maintained in cups for use in this study. Pairs of 2-day-old TPP females and males were placed in separate cups and treated with sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* following the methods described in the previous experiment. Control paired TPP were immersed with sterile water containing 0.05% Tween 80. Each treatment had 30 pairs of TPP adults. The survival, fecundity and lifespan of *B. cockerelli* adults were recorded daily.

4.2.7 Evaluation of sublethal effects of *B. bassiana* on offspring of the treated *B. cockerelli* adults

To evaluate any possible carry-over activity of *B. bassiana* on the *B. cockerelli* offspring, we collected the eggs from the fifth day of oviposition from the treated and control females and

determined the life table parameters of offspring. We used no more than 10 eggs on each leaf (Thinakaran *et al.* 2015; Yang *et al.* 2013). Approximately 100 eggs (a total of 11 capsicum leaves) were placed in each cup for each treatment (LC₁₀ and LC₃₀) and control. The laboratory experiment was designed and conducted as described in the above experiment.

In order to standardise the visual assessment to observe the development and survival of *B. bassiana*, we marked the location of eggs and nymphs on the leaf surface using a non-toxic coloured marker pen because *B. cockerelli* nymphs were sessile on capsicum leaves (Yang *et al.* 2013). The duration of incubation period was recorded, and developmental time, moulting, and survival of each *B. cockerelli* were observed daily.

Newly emerged *B. cockerelli* adults (<6 h) were collected and paired with an adult of the opposite sex in a separate cup with fresh capsicum leaf for longevity and fecundity studies. Each leaf was replaced with a new one daily. The number of eggs laid by females was counted under a microscope. The longevity of females and males in each treatment and control was recorded daily until the death of the last adult.

4.2.8 Data analyses

We used SPSS 17.0 (Chicago, IL, USA) to calculate the concentrations of entomopathogenic fungal strains that were lethal and sublethal to *B. cockerelli* first instar nymphs and adults. Data of *B. cockerelli* mortality were corrected for control mortality (Abbott 1925) and all mortality percentages were transformed using arcsine square root transformation before analysis of variance (ANOVA) (Steel *et al.* 1980). Mean comparisons of *B. cockerelli* first instar nymphs and adult mortality in 16 isolates and the sublethal effects of *B. bassiana* on preoviposition period, oviposition period, fecundity, and longevity of *B. cockerelli* adults were made using a Tukey HSD test. Lethal time to 50% mortality (LT₅₀), respective 95% confidence intervals (CI), and lethal concentration to 10%, 30% and 50% mortality (LC₁₀, LC₃₀ and LC₅₀) values were estimated using the probit analysis method for correlation data (Throne *et al.* 1995). The demographic raw data for all individuals (*B. cockerelli* offspring) were analysed according to the age-stage, two-sex life table theory (Chi 1988; Chi & Liu 1985). For the population parameters, the variance and standard errors of developmental time, fecundity, and longevity of *B. cockerelli* were calculated via 100,000 bootstraps, which were calculated in the TWSEX-MSChart computer program (Chi 2017c; Efron & Tibshirani

1993). The differences among developmental time, fecundity, and longevity of *B. cockerelli* were compared by paired bootstrap test. Both bootstrap and paired bootstrap tests can be analysed in the TWOSEX-MSChart computer program (Chi 2017c). The curves for survival rates, developmental times, fecundity, reproductive values, and life expectancy were constructed using SigmaPlot 13.0 software.

4.3 Results

4.3.1 Pathogenicity of fungal isolates

The mortality levels and median lethal times (LT_{50}) of TPP first instar nymphs and adults varied significantly among the 16 fungal strains at 1×10^7 conidia mL^{-1} ($F=2.844$; $df= 15, 64$; $P=0.002$; $F=5.163$, $df= 15, 64$; $P<0.0001$). The control mortalities of TPP first instar nymphs and adults with 0.05% Tween 80 were only between 16.9 and 14.3%, respectively. After the 10-day incubation period all fungal strains caused mortality levels above 50% to TPP first instar nymphs and adults (Table 4.2). There was a significant difference in survival among *Metarhizium*, *Beauveria*, and *Isaria* fungal species after 10 days for TPP first instar nymphs and adults ($F=4.170$; $df= 2, 77$; $P=0.019$; $F=10.772$; $df= 2, 77$; $P<0.0001$). Analysis of the interactions between fungal species and TPP stages ($F=1.432$; $df= 2, 154$; $P=0.242$) and between fungal isolates and TPP stages ($F=0.675$; $df= 15, 128$; $P=0.805$) did not show significant difference (Table 4.2). Among *Metarhizium*, *Beauveria*, and *Isaria* isolates, TPP first instar nymphs and adults were susceptible to *B. bassiana* ICMP 8701 (Table 4.2). According to a probit analysis, median lethal time (LT_{50}) of TPP first instar nymphs and adults varied from 3.1 to 4.4 days and 2.9 to 8.7 days (respectively) among fungal strains (Table 4.2). Compared with the other fungal pathogens, *B. bassiana* ICMP 8701 had shorter LT_{50} values against TPP first instar nymphs (3.1 days) and adults (2.9 days). Based on the mortality and LT_{50} of *B. cockerelli* among 16 entomopathogenic fungal isolates in 10 days, *B. bassiana* isolate ICMP 8701 caused the high levels of mortality and rapidly killed psyllids (both first instar nymphs and adults). This isolate was therefore selected for experimental studies on the lethal and sublethal effects on TPP.

Table 4.2. Mortalities of 16 entomopathogenic fungal isolates against *Bactericera cockerelli* first instar nymphs and adults at 1×10^7 mL⁻¹ after 10 days. The isolate which caused the highest mortality (in bold) was selected for further study.

Fungal species	Isolate	First instar nymphs		Adults	
		% Mortality \pm SE	LT ₅₀ (days) (95% CI)	% Mortality \pm SE	LT ₅₀ (days) (95% CI)
<i>B. bassiana</i>	ICMP 2080	90.38 \pm 3.61ab	3.1 (2.8–3.4)	94.17 \pm 3.19a	2.9 (2.7–3.2)
<i>B. bassiana</i>	ICMP 6887	92.78 \pm 1.29ab	3.3 (3.0–3.5)	93.00 \pm 1.17abc	3.3 (3.0–3.6)
<i>B. bassiana</i>	ICMP 8147	84.36 \pm 1.47ab	3.7 (3.5–3.9)	87.17 \pm 1.17abcd	3.7 (3.4–3.9)
<i>B. bassiana</i>	ICMP 8701	95.19\pm1.20a	3.1 (2.9–3.3)	97.67\pm1.43a	2.9 (2.7–3.2)
<i>B. bassiana</i>	ICMP 18372	83.39 \pm 5.76ab	3.6 (3.3–3.8)	89.72 \pm 5.22ab	3.5 (3.0–3.8)
<i>B. bassiana</i>	ICMP 19153	86.97 \pm 3.61ab	3.4 (3.1–3.7)	90.67 \pm 2.33abcd	3.7 (3.4–3.9)
<i>B. pseudobassiana</i>	ICMP 5539	78.91 \pm 6.70ab	3.8 (3.5–4.1)	82.61 \pm 6.17abcd	4.0 (3.6–4.4)
<i>B. pseudobassiana</i>	ICMP 5541	92.58 \pm 1.29ab	3.2 (3.0–3.3)	94.78 \pm 2.27a	3.0 (2.7–3.3)
<i>B. pseudobassiana</i>	ICMP 18977	87.97 \pm 2.69ab	3.4 (3.2–3.6)	89.89 \pm 2.17abcd	3.4 (3.1–3.7)
<i>I. cicadae</i>	ICMP 15464	77.15 \pm 6.70ab	4.3 (4.0–4.6)	66.16 \pm 6.55cd	6.4 (5.9–7.0)
<i>M. anisopliae</i>	ICMP 3259	93.99 \pm 3.80a	3.1 (2.8–3.4)	95.33 \pm 1.17a	2.9 (2.7–3.2)
<i>M. anisopliae</i>	ICMP 5439	84.36 \pm 1.14ab	3.3 (3.0–3.5)	87.17 \pm 2.18abcd	3.3 (3.1–3.6)
<i>M. novozealandicum</i>	ICMP 149	73.54 \pm 4.50ab*	4.2 (3.7–4.7)	61.94 \pm 8.64d	8.7 (7.5–10.6)
<i>M. novozealandicum</i>	ICMP 3095	79.55 \pm 4.08ab	3.7 (3.5–4.0)	87.17 \pm 6.50abc	3.8 (3.4–4.2)
<i>M. novozealandicum</i>	ICMP 3258	68.73 \pm 7.70b	4.4 (3.9–4.9)	69.67 \pm 7.69bcd	4.4 (3.9–5.0)
<i>M. novozealandicum</i>	ICMP 2113	84.69 \pm 6.17ab	4.0 (3.6–4.5)	86.37 \pm 3.47abcd	4.5 (4.2–4.9)

Means within a row followed by the same lower-case do not differ significantly (Tukey's HSD multiple range test, $P > 0.05$).

4.3.2 Toxicity effect of *B. bassiana* ICMP 8701 on *B. cockerelli* adults

Concentration-dependent mortality responses for the effect of *B. bassiana* ICMP 8701 on *B. cockerelli* adults estimated that the LC₅₀ value of *B. bassiana* was 4.2×10^4 (2.3×10^4 – 7.1×10^4) conidia mL⁻¹. The average LC₁₀ and LC₃₀ values were estimated to be 4.6×10^2 (1.1×10^2 – 1.3×10^3) and 6.7×10^3 (2.6×10^3 – 1.3×10^4) conidia mL⁻¹, respectively (Table 4.3).

4.3.3 Sublethal effects of *B. bassiana* ICMP 8701 on longevity and fecundity of *B. cockerelli* adults

Two sublethal concentrations of *B. bassiana* (LC₁₀ and LC₃₀) significantly influenced the longevity and fecundity of *B. cockerelli* adults in the parental generation (Figure 4.1). At sublethal concentrations, *B. bassiana* did not significantly shorten the preoviposition period

of *B. cockerelli* ($F=0.155$; $df=2, 87$; $P=0.856$), but significantly reduced the oviposition period of females ($F=14.949$; $df=2, 80$; $P<0.001$). The LC_{10} and LC_{30} treatment shortened the longevity of *B. cockerelli* adults ($F=43.442$; $df=2, 177$; $P<0.001$), and the number of eggs laid by *B. cockerelli* adults was reduced significantly in the LC_{10} and LC_{30} treatment ($F=26.959$; $df=2, 87$; $P<0.001$).

Table 4.3. Acute toxicity of *B. bassiana* ICMP 8701 against *B. cockerelli* adults at 10 days post-treatment.

Isolate	N	conidia mL ⁻¹				Slope ±SE	X ²
		LC ₁₀	LC ₃₀	LC ₅₀	LC ₉₀		
<i>B. bassiana</i> (ICMP 8701)	500	4.6×10 ² (1.1×10 ² –1.3×10 ³)	6.7×10 ³ (2.6×10 ³ –1.3×10 ⁴)	4.2×10 ⁴ (2.3×10 ⁴ –7.1×10 ⁴)	3.9×10 ⁶ (2.1×10 ⁶ –9.1×10 ⁶)	0.652±0.061	17.893

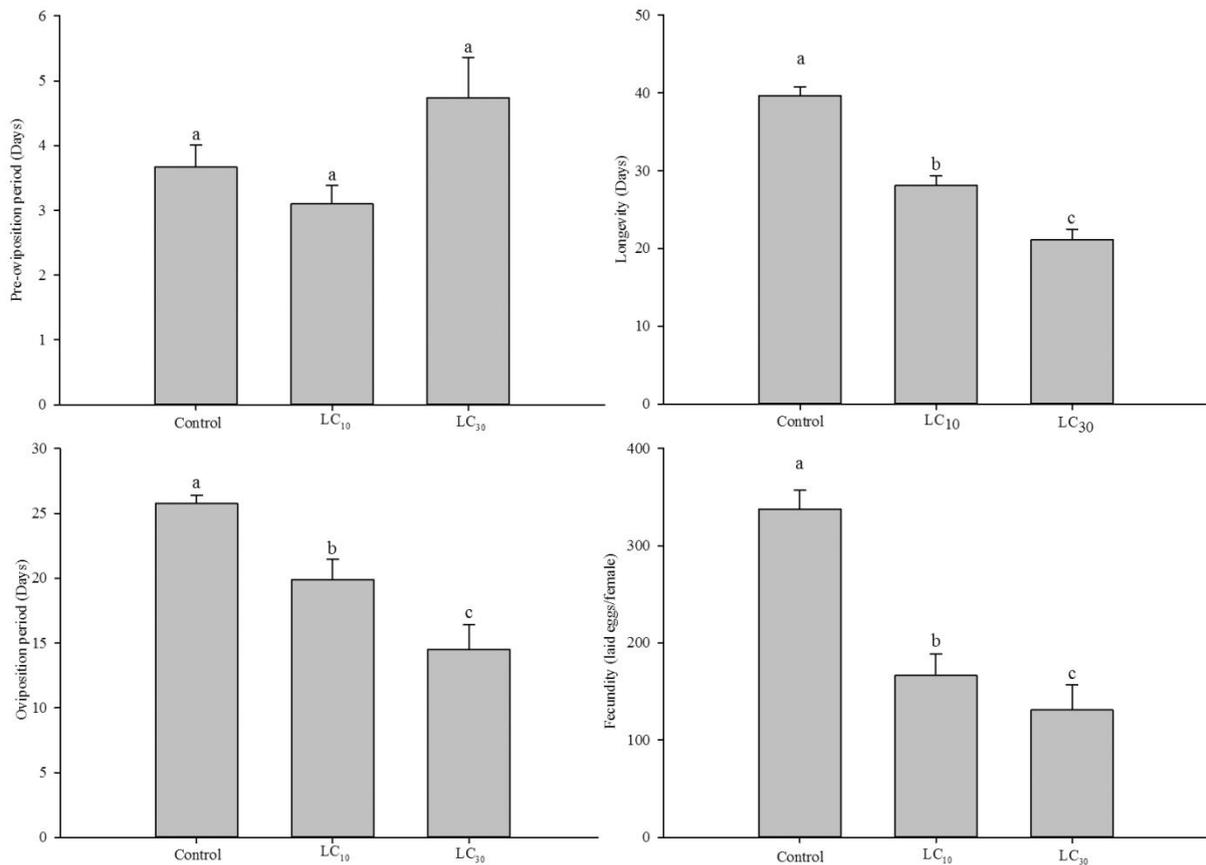


Figure 4.1. Sublethal effects of *B. bassiana* ICMP 8701 on longevity and fecundity of *B. cockerelli* adults. Means followed by the same letter in each bar are not significantly different ($P > 0.05$; Tukey's HSD multiple range test after one-way ANOVA).

4.3.4 Sublethal effects of *B. bassiana* ICMP 8701

The duration of all immature stages of *B. cockerelli* was significantly shorter when adults were treated with LC₃₀ compared with the control except the fifth instar nymph. The same trend in the developmental time was only observed on *B. cockerelli* N3 and N4 in the LC₁₀ treatment (Table 4.3). Compared with the control (6.1 days), the LC₃₀ treatment (7.2 days) significantly prolonged the adult pre-ovipositional period (APOP) of *B. cockerelli* females. But the total preoviposition period of (TPOP) of *B. cockerelli* in control was significantly longer than those in the LC₁₀ and LC₃₀ treatments. Age-stage specific survival rate (S_{xj}) for *B. cockerelli* adults was negatively affected by *B. bassiana*. In the control, S_{xj} reached a maximum of 0.44 for females and 0.43 for males on 30th day, respectively, and continuously decreased in the LC₁₀ and LC₃₀ treatments where these values were 0.33 for females and 0.39 males on 28th day, 0.32 females and 0.37 males on 29th day (Figure 4.2), respectively. In Figure 4.3 the age-specific survival rate (l_x) represents a simplified form of the curves of age-stage survival rate, and this value shows the probability a newly hatched N1 will survive to age x. The curves of l_x in the LC₁₀ and LC₃₀ treatment declined significantly after 34 days (Figure 4.3).

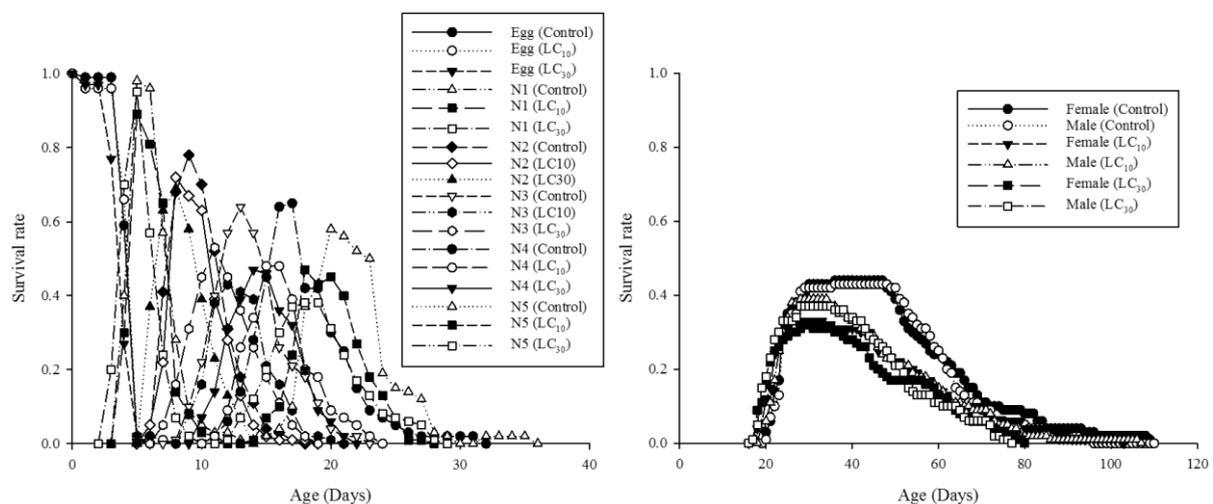


Figure 4.2. Age-stage specific survival rate (S_{xj}) of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Treatment with *B. bassiana* also significantly reduced the longevity of adults, females and males, of *B. cockerelli* in the LC₃₀ (34.1 ± 1.6 , 34.7 ± 2.4 , and 33.5 ± 2.0 , respectively) compared with the control (40.5 ± 1.5 , 41.5 ± 2.4 , and 39.5 ± 1.7 , Table 4), but not in the LC₁₀. Maximum longevities for *B. cockerelli* females and males in the control (109 and 95 days, respectively) were markedly greater than those adults in the LC₃₀ treatment (79 and 76 days, Figure 4.2).

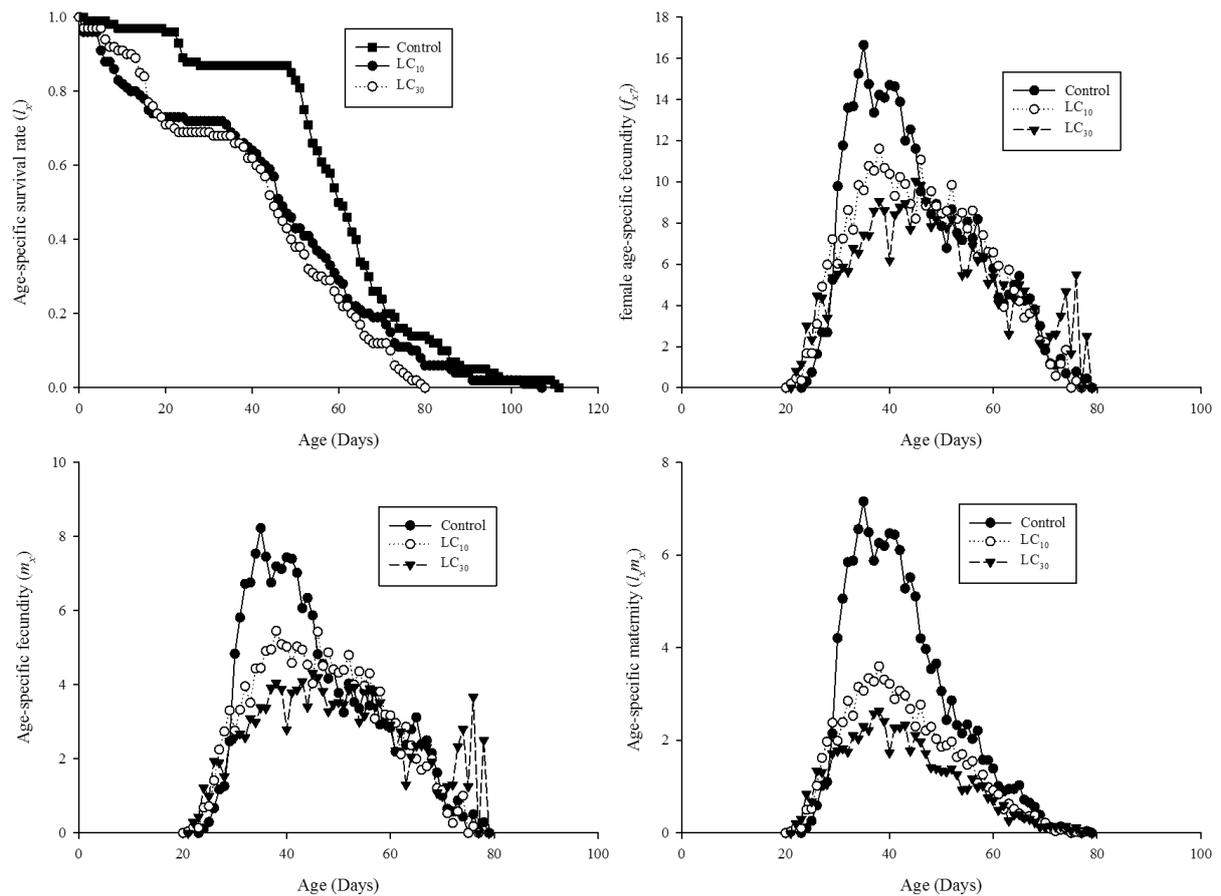


Figure 4.3. Age-specific survival rate (l_x), female age-specific fecundity (f_{x7}), age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Significant reductions in the fecundity of TPP were recorded under the LC₁₀ and LC₃₀ treatment 258.4 ± 22.3 and 199.6 ± 25.9 eggs per female respectively, compared with the control 334.0 ± 22.2 eggs per female. For example, the highest peaks of f_{x7} (i.e. female is the seventh life stage) and m_x in the LC₁₀ (11.6 eggs female⁻¹day⁻¹ and 5.5 eggs individual⁻¹day⁻¹, respectively) and LC₃₀ (10.1 eggs female⁻¹day⁻¹ and 4.3 eggs individual⁻¹day⁻¹, respectively) were lower than those in the control (16.7 eggs female⁻¹day⁻¹ and 8.2 eggs individual⁻¹day⁻¹). Additionally, the peak time of these parameters for the LC₁₀ (38th day) and LC₃₀ (45th day) treatments were significantly delayed, later than for the control (35th day). There was a similar pattern for $l_x m_x$ (which was based on l_x and m_x). However, sublethal concentrations of *B. bassiana* ICMP 8701 did not show significant effect on oviposition period of *B. cockerelli* (Table 4.4).

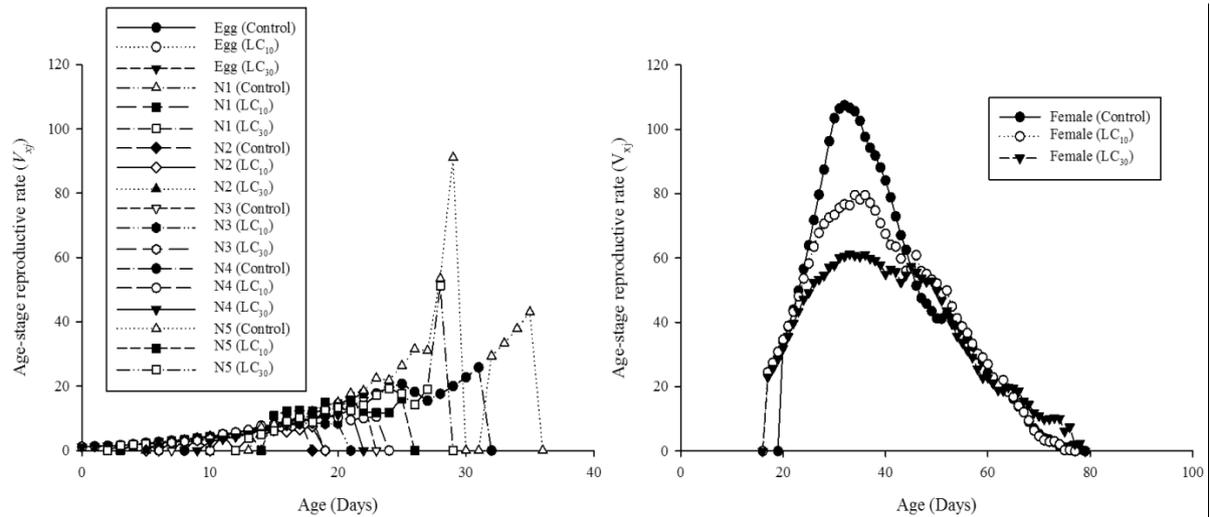


Figure 4.4. Age-stage specific reproductive rates (v_{xj}) of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Table 4.4. Means (\pm SE) developmental time (days) of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Developmental time	Control		LC ₁₀		LC ₃₀	
	N	Mean \pm SE	N	Mean \pm SE	N	Mean \pm SE
Egg	99	4.6 \pm 0.1a	96	4.7 \pm 0.1a	97	4.1 \pm 0.1b
N1	98	3.4 \pm 0.1a	88	3.2 \pm 0.1a	93	2.9 \pm 0.1b
N2	97	3.9 \pm 0.2a	81	3.9 \pm 0.2ab	91	3.4 \pm 0.2b
N3	97	3.9 \pm 0.2a	80	2.9 \pm 0.2b	90	3.2 \pm 0.2b
N4	97	4.3 \pm 0.2a	74	3.5 \pm 0.2b	83	3.4 \pm 0.2b
N5	87	4.3 \pm 0.2a	72	3.9 \pm 0.2a	69	4.1 \pm 0.3a
Pre-adult	87	24.3 \pm 0.3a	72	22.2 \pm 0.3b	69	21.1 \pm 0.4c
APOP	44	6.1 \pm 0.2b	33	6.5 \pm 0.5ab	32	7.2 \pm 0.4a
TPOP	44	30.3 \pm 0.5a	33	27.9 \pm 0.7b	32	28.2 \pm 0.7b
Adult longevity	87	40.5 \pm 1.5a	72	36.5 \pm 1.9ab	69	34.1 \pm 1.6b
Female longevity	44	41.5 \pm 2.4a	33	39.4 \pm 2.8ab	32	34.7 \pm 2.4b
Male longevity	43	39.5 \pm 1.7a	39	34.0 \pm 2.6ab	37	33.5 \pm 2.1b
Fecundity (eggs/female)	44	334.0 \pm 22.2a	33	258.4 \pm 22.3b	32	199.6 \pm 25.9b
Oviposition days	44	27.4 \pm 1.3a	33	27.2 \pm 1.6a	32	23.1 \pm 2.4a

A paired bootstrap test in all treatments was used to detect statistical differences among developmental time and reproduction parameters of *B. cockerelli*. Means in a row followed by the same letter were not significantly different. Standard errors were estimated using 10000 bootstrap resampling.

The age-stage specific reproductive rates (v_{xj})—defined by Fisher (1999) as the contribution of an individual of age x and stage j to the future population—had significantly lower peaks for N5 (16.2 day^{-1}) and females (79.5 day^{-1}) in LC₁₀ and LC₃₀ (51.3 and 61.3, respectively) than the control (91.1 and 107.5, respectively, Figure 4.4).

The life expectancy (e_{xj}) values for *B. cockerelli* immature stages and adults were reduced markedly in the LC₁₀ and LC₃₀ treatments with *B. bassiana*. In the control, the lowest e_{xj} for newly hatched *B. cockerelli* eggs was 58.8 days, which was much higher than those in the LC₁₀ (48.3 days) and LC₃₀ (42.1 days) treatments (Figure 4.5).

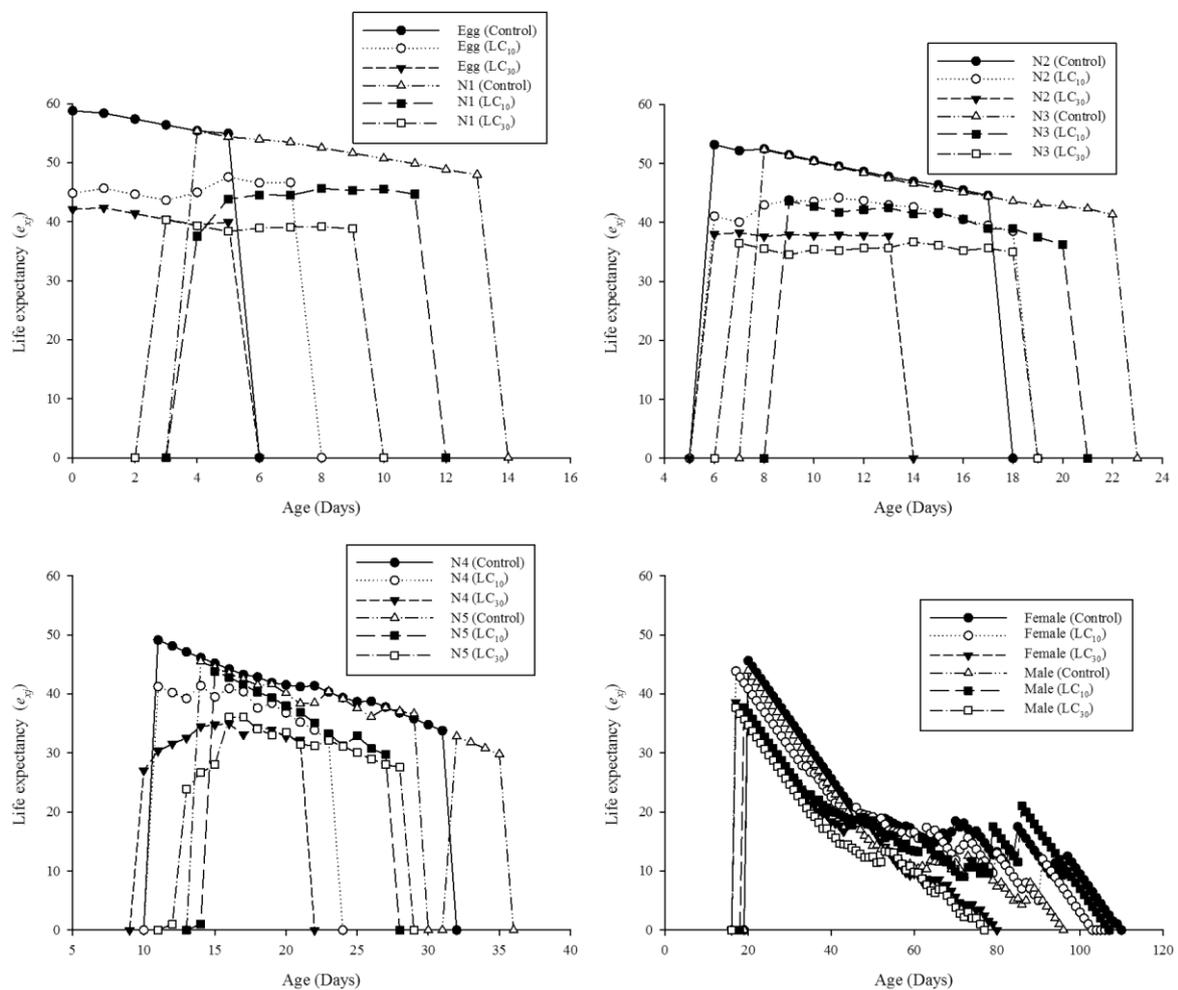


Figure 4.5. Life expectancy (e_{xj}) values of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

4.3.5 Population growth parameters

Treatment with *B. bassiana* (ICMP 8701) markedly decreased the net reproductive rate R_0 in the LC₁₀ and LC₃₀ treatments (85.3±14.1 and 63.9±12.4) compared with the control (147.0±19.3) (Table 5). The intrinsic rate of increase r_m was significantly lower in the LC₃₀ treatment (0.1092±0.0061) than in the control (0.1275±0.0045), which was also the pattern in the finite rate of increase λ (1.1154±0.0068 in the LC₃₀ treatment versus 1.1359±0.0051 in control). However, sublethal concentrations of *B. bassiana* ICMP 8701 had no significant influence on the mean generation (T) or gross reproductive rate (GRR) of *B. cockerelli* (Table 4.5).

Table 4.5. Means (±SE) life table parameters of offspring from females of *B. cockerelli* exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Treatments	r_m day ⁻¹	λ day ⁻¹	GRR(Offspring)	R_0 (Offspring/individual)	T (Day)
Control	0.1275±0.0045a	1.1359±0.0051a	196.1954±25.43a	146.96±19.28a	39.15±0.74a
LC ₁₀	0.1154±0.0050ab	1.1223±0.0056ab	167.8840±26.46a	85.28±14.12b	38.53±0.96a
LC ₃₀	0.1092±0.0061b	1.1154±0.0068b	152.31±29.27a	63.87±12.40b	38.07±1.20a

A paired bootstrap test in all treatments was used to detect statistical differences among developmental time and reproduction parameters of *B. cockerelli*. Standard errors were estimated using 10000 bootstrap resampling.

4.4 Discussion

The measurement of acute lethal effects of EPF on pest populations largely depends on the determination of direct mortality and an acute median lethal concentration. However, limited information suggests that sublethal effects of EPF on pest physiology may also be important, such as reducing survival rates, shortening or prolonging development and longevity, and decreasing fecundity (Jarrahi & Safavi 2016a; Torrado-León *et al.* 2006; Wang *et al.* 2014; Wu *et al.* 2016c; Zhang *et al.* 2015a). This study screened 16 native EPF isolates on *B. cockerelli* first instar nymphs and young adults. In addition, we estimated demographic parameters of *B. cockerelli* for the first time by investigating the lethal and sublethal effects of the most virulent fungal isolate on the life table parameters of *B. cockerelli*. Such information can enable the determination of control efficiency of EPF as part of an integrated pest management (IPM) of *B. cockerelli*. Our results showed that *B. bassiana* ICMP 8701 was the most virulent strain, based on higher mortality and shorter LT₅₀ against *B. cockerelli* first instar nymphs and adults among the 16 NZ fungal isolates of EPF we tested. The

treatment of *B. bassiana* at sublethal concentrations can cause serious disturbances to parental TPP reproduction, and these changes were also expressed in the next generation by accelerating the development of immature and decreasing reproduction of adult females. To the best of our knowledge, this is the first report to evaluate the lethal and sublethal effects of EPF on the life table of *B. cockerelli*.

B. cockerelli first instar nymphs and adults had similar susceptibility to all strains at 1×10^7 conidia mL⁻¹ except *M. novozealandicum* (ICMP 3258). Previous studies found similar results that higher mortality of TPP nymphs and adults caused by *B. bassiana*, such as GHA strain (BotaniGard® ES and BotaniGard® 22WP), UA-21 strain, Bb-CIAD1 strain, BB09 strain, BB42 strain, BB40 strain and BB37 strain (Casique-Valdez *et al.* 2011; Mauchline & Stannard 2013; Rios-velasco *et al.* 2014; Sánchez-Peña *et al.* 2007; Tamayo-Mejía *et al.* 2014; Villegas-Rodriguez *et al.* 2017). In addition, some other entomopathogenic fungi also showed higher mortality of TPP in the laboratory, greenhouse or fields, including, *I. fumosorosea* (Pfr 97, 36 isolate, FE 9901, Apopka 97 and F129), *Metarhizium anisopliae* (UA-12, UA-11, F 52 (USA), DWR 346, F52 (Australia), Ma-CIAD1, MA25 and MA28), *Lecanicillium muscarium* (F421, F425, F426 and eNtocide L™) (Lacey *et al.* 2009, 2010, 2011; Mauchline & Stannard 2013; Rios-velasco *et al.* 2014; Sánchez-Peña *et al.* 2007; Tamayo-Mejía *et al.* 2014; Villegas-Rodriguez *et al.* 2014, 2017). Moreover, our bioassay indicated that *B. pseudobassiana*, *I. cicadae*, *M. anisopliae* and *M. novozealandicum* also had good potential against *B. cockerelli* under optimal conditions. The LT₅₀ of *B. bassiana* ICMP 8701 on TPP first instar nymphs (3.1 days) and adults (2.9 days) was faster than *B. bassiana* (Bb-CIAD1) on TPP nymphs (6.4 days), which means that *B. bassiana* ICMP 8701 may have good potential to decrease Lso transmission (Rios-velasco *et al.* 2014).

The survival rate and longevity of TPP was significantly affected by sublethal concentration of EPF. Additionally, age-stage-specific survival rate (S_{xj}) and age-specific survivorship (l_x) of *B. cockerelli* offspring was negatively influenced by sublethal concentrations of *B. bassiana* (ICMP 8701). Based on the survival curves (S_{xj} and l_x), TPP offspring showed higher mortality during the immature period of their life table. Other studies are consistent with our results; the survival rates of *P. operculella* offspring was significantly decreased when treated with a sublethal concentration (LC₅₀) of *B. bassiana* during their immature stages (Yuan *et al.* 2018). Another EPF species, *M. anisopliae* also significantly reduced the

age-specific survivorship (l_x) of F1 immature stages of *H. armigera* in the LC₂₅ treatment (Jarrahi & Safavi 2016b). Application of *B. bassiana* (ICMP 8701) decreased TPOP and female and male longevity. Interestingly, Yuan *et al.* (2018) and Wang *et al.* (2014) show that sublethal concentrations of *B. bassiana* could decrease the total preoviposition period in *P. operculella* and *B. tabaci*. Some previous studies have also reported that *B. bassiana* can significantly decrease adult longevity in *H. armigera*, *H. hebetor*, *P. operculella* and *T. urticae* (Jarrahi & Safavi 2016a; b; Seyed-Talebi *et al.* 2012; Yuan *et al.* 2018).

Sublethal effects of EPF have been demonstrated to influence the fecundity of insect species. The fecundity of parental and offspring of the plant hopper *Nilaparvata lugens* (Hemiptera: Delphacidae) was significantly decreased when the parental nymphs were treated with a sublethal concentration (5×10^5) of *B. bassiana* (Wang *et al.* 2018). Yuan *et al.* (2018) also observed that the fecundity of the moth *Phthorimaea operculella* in the offspring generation was reduced significantly when the parental larvae were treated with median lethal concentration (LC₅₀) of *B. bassiana*. Similarly, another EPF species, *M. anisopliae*, led to a significant reduction in daily and total fecundity of *Helicoverpa armigera* offspring when parental third instar larvae were exposed to sublethal concentrations (LC₂₀ and LC₂₅). *Leptolegnia chapmanii* also caused a lower fertility and smaller number of gonotrophic cycles of *Aedes aegypti* (L.) females when adults were infected with zoospores (Jarrahi & Safavi 2016a; Pelizza *et al.* 2013). We also found a similar trend where sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 significantly decreased the fecundity of both parental and offspring generations. The LC₃₀ treatment lowered the female age-specific fecundity (fx_7), age-specific maternity ($l_x m_x$) and reproduction (V_{xj}) of *B. cockerelli* offspring compared with the control. The decreasing fecundity of females may be caused by the shorter life of the females or by nutritional deficiency as the fungal infection consumed nutrients (Yuan *et al.* 2018). These results also suggest that the physiological effects of parental generation infected by fungal isolates could be carried over to their offspring (Zhang *et al.* 2015a).

In this study, we show that exposure of TPP adults to low sublethal concentrations of *B. bassiana* had carryover effects to the development of offspring. The LC₃₀ *B. bassiana* treatment was associated with significant shortening of the developmental time of *B. cockerelli* immature stages in the offspring generation. This result agrees with other studies

that also showed the negative effects of *B. bassiana* on the developmental time of *Bemisia tabaci*, *P. operculella* and *F. occidentalis* immature stages when parents were exposed to sublethal concentrations of *B. bassiana* (Wang *et al.* 2014; Yuan *et al.* 2018; Zhang *et al.* 2015a). In contrast, Wang *et al.* (2018) reported that the immature stages of *N. lugens* offspring could be slightly prolonged when parental 3rd nymphs were sprayed with a sublethal concentration (5×10^5) of *B. bassiana*. Similarly, Jarrahi and Safavi (2016b) reported that *M. anisopliae* prolongs immature stages of *H. hebetor* offspring when parental 3rd instar larvae were treated with sublethal concentration (LC₃₀) of *M. anisopliae* at different time intervals (24, 48, and 72h). These different results might be due either to the different virulence potential of the fungi isolates or to the different susceptibility of insects (Farooq & Freed 2016). Nevertheless, it is clear that sublethal exposure to EPF can affect developmental rates in the next generation of insects.

Assessment of life table parameters is an important approach to assist with evaluating the overall sublethal effects of EPF on the performance of insects (Jarrahi & Safavi 2016a; b). Population growth of *Habrobracon hebetor*, *F. occidentalis*, *T. urticae*, and *H. armigera* were all reduced when exposed to sublethal concentrations of entomopathogenic fungi *B. bassiana* or *M. anisopliae* (Jarrahi & Safavi 2016a; b; Seyed-Talebi *et al.* 2012; Zhang *et al.* 2015a). In our study, while the sublethal effects of *B. bassiana* (LC₃₀) on *B. cockerelli* offspring influenced a number of life table parameters, such as reduced intrinsic rate of increase (r_m), finite rate of increase (λ), and net reproductive rate (R_0), it did not influence gross reproductive rate (GRR) and mean generation time (T). Compared with the control, lower r_m and stable T showed relatively lower population densities in a given period. These results suggest that even low sublethal concentrations (LC₃₀) of EPF may have shown long-term deleterious influence on insect physiological change.

There are a number of mechanisms that could lead to the sublethal effects we observed in psyllids. The effects may be related to the large energy consumption of females with fungal infection and secondary metabolites produced by *B. bassiana* (Qu & Wang 2018; Wang *et al.* 2018; Yuan *et al.* 2018). *B. bassiana* infection in an insect's hemocoel could also increase the level of fatty acids, and these accumulations of higher levels of fatty acids are a response of energy-cost immunoreactions in hosts (Xu *et al.* 2015). Moreover, during invasion, EPF could secrete a wide range of bioactive compounds in insect hemocoel (such fungal

secondary metabolites, bassianin, bassiacridin, oosporeins, cyclosporine), which could produce potential insecticidal activity against pests (Chauzat & Faucon 2007; Gindin *et al.* 1994; Gurulingappa *et al.* 2011; Qu & Wang 2018). These enzymes and toxins directly cause physiological changes during the penetration process, such as breaking the physiological system balance, decreasing respiratory efficiency, and reducing the survival rate and fecundity of hosts (Jarrahi & Safavi 2016a; SHARMA *et al.* 1994). As the reproductive system of the host also resides in the hemocoel, it has been hypothesized that small secondary metabolites may play a role in influencing host oogenesis or even enter eggs and further influence the performance of psyllid offspring (Medeiros *et al.* 2009). Further research is needed to elucidate the detailed mechanism of the sublethal effects of *B. bassiana* on psyllids in order to determine which factors are involved in pre- and post-zygotic disruption of the life table of the parents and offspring.

4.5 Conclusion

Our study provides basic information about the acute toxicity and sublethal effects of *B. bassiana* on *B. cockerelli*. Above all, *B. bassiana* ICMP 8701 caused direct lethal effects, with the highest mortality and lowest LT_{50} on *B. cockerelli* first instar nymphs and young adults. Indirect sublethal effects of LC_{30} of *B. bassiana* significantly reduced the longevity and fecundity of parental *B. cockerelli* and shortened the developmental time and reduced longevity and fecundity of offspring *B. cockerelli*. *B. bassiana* ICMP 8701 could be considered as part of an effective component of integrated pest management (IPM) for controlling *B. cockerelli* in bell pepper. This could in turn reduce the dependence on chemical interventions. It would also be interesting to augment the observed efficacy of *B. bassiana* by combining *B. bassiana* ICMP 8701 with other biocontrol agents, such predators, to increase the control efficiency of TPP. Further studies should also test the lethal and sublethal effects of *B. bassiana* on TPP's predators and the application of *B. bassiana* against *B. cockerelli* in the greenhouse or field.

Chapter 5

Influence of pathogenic fungi on the life history and predation rate of mites attacking a psyllid pest

Publication status: Submitted to *Ecotoxicology and Environmental Safety* on 16 April, 2019

5.1. Introduction

Tomato/potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an invasive and highly destructive pest causing severe losses to many solanaceous crops in the United States, Mexico, and New Zealand (Butler & Trumble 2012b; Vereijssen *et al.* 2018; Yang & Liu 2009). *B. cockerelli* can cause direct damage by feeding on host plant phloem. However, the most significant damage caused by *B. cockerelli* to potato is the transmission of “*Candidatus Liberibacter solanacearum*” (Lso) which severely affects plant growth and yield (Butler & Trumble 2012b; Munyaneza 2015). Application of chemical insecticides is currently the most often used management strategy against TPP in the United States, Mexico, and New Zealand. However, insecticide resistance (imidacloprid and spinosad) in *B. cockerelli* has been reported in the US (California) and Mexico (Almeyda-León *et al.* 2008; Liu & Trumble 2007). Currently, biological control has become one of the most promising alternatives for the control of TPP (Butler & Trumble 2012b; Lacey *et al.* 2009; Mauchline & Stannard 2013; Prager & Trumble 2017). Compared with toxic chemical pesticides, predatory mites are safer to the environment and human health and highly selective against TPP (Patel & Zhang 2017a; b; Xu & Zhang 2015). Recently, an integrated control strategy combining multiple biological control agents is used to suppress pest populations (Francisco *et al.* 2018; Maniania *et al.* 2016; Mohammed 2018; Seiedy 2015; Shaef & Lim 2017; Ullah & Lim 2017; Wu *et al.* 2016b, 2017).

In New Zealand three phytoseiid mite species has been found to feed on TPP. Patel and Zhang (2017a; b) observed that *N. cucumeris* consume TPP eggs and first instar nymphs in the laboratory. *A. limonicus* females develop and produce eggs on eggs and I–III instar nymphs of TPP (Davidson *et al.* 2016; Ma *et al.* 2018; Xu & Zhang 2015). In addition, *A. baccarum* develop to adults when fed on TPP nymphs and psyllid sugars, but its long life cycle (two to three generations per year) and diapause habit at high temperature (28 °C) limited the application of *A. baccarum* in protected crops (Geary *et al.* 2016; Wu 1994). Compared with *N. cucumeris*, *A. limonicus* females laid more eggs and consumed more TPP eggs and first instar nymphs (Patel & Zhang, 2017a; b). However, the control efficacy of predatory mites for TPP is somewhat limited due to the biological traits of *A. limonicus* and TPP. A key problem is that *A. limonicus* cannot prevent the feeding damage and plant disease transmission by later stages and adults of TPP, which have a short generation time (25.6 days) and high fecundity (392.2 eggs) (Yang *et al.* 2013; Yang & Liu 2009). This could be

overcome by entomopathogenic fungi (EPF) which can rapidly infect and kill various life stages of psyllids due to their complementary or synergistic insecticidal activity (Lacey *et al.* 2011). Many EPF have been tested for their control efficiency against TPP in the laboratory and field conditions (Ai-Jabr 1999; Lacey *et al.* 2009, 2011; Mauchline & Stannard 2013; Pérez-González & Sánchez-Peña 2017; Rios-Velasco *et al.* 2014; Sánchez-Peña *et al.* 2007a; Tamayo-Mejía *et al.* 2014, 2015; Villegas-Rodriguez *et al.* 2014, 2017). Our previous research has shown that *Beauveria bassiana*, ICMP 8701, had a higher mortality and shorter median lethal time against *B. cockerelli* first instar nymphs and adults than 15 other fungal strains tested (Chapter 4).

An integrated strategy combining the release of predatory mites and entomopathogenic fungi could further promote the bio-control of pests in the greenhouse (Chandler *et al.* 2005; Wu *et al.* 2017). The benefits and risks of simultaneous application of these two types of biological control agents are not fully evaluated. Generalist EPF might debilitate or even kill predatory mites. Some strains of EPF show no pathogenicity or low mortality against predatory mites *Phytoseiulus persimilis*, *Amblyseius swirskii*, *N. cucumeris*, *N. californicus*, *N. barkeri*, *N. womersleyi*, *Stratiolaelaps scimitus*, *Axinoscymnus cardilobus*, and *Ipheseius degenerans* (Castagnoli *et al.* 2005; Jacobson *et al.* 2001a; Lin *et al.* 2017; Ludwig & Oetting 2001; Numa Vergel *et al.* 2011; Saito & Brownbridge 2016, 2018; Seiedy 2015; Seiedy & Moezipour 2017; Shang *et al.* 2018; Sun *et al.* 2018; Wu *et al.* 2014, 2016b; Zhang *et al.* 2015b; Zhou *et al.* 2010). In contrast, other researchers have reported that some strains of EPF caused significantly high mortality (even up to 99.5%) to some predatory mite strains (Dogan *et al.* 2017; Donka *et al.* 2008; Duso *et al.* 2008; Lin *et al.* 2017; Ludwig & Oetting 2001; Pytlak *et al.* 2014; Saito & Brownbridge 2016; Seiedy *et al.* 2015; Seiedy & Moezipour 2017). These compatible or sensitive relationships between EPF and predatory mites do not indicate that the combined application of two agents could significantly improve or reduce control efficiency compared with the single application of two agents in the greenhouse (Automated *et al.* 2018; Midthassel *et al.* 2016; Numa Vergel *et al.* 2011). The life table parameters and predation behavior of some parental (F_0) generation predatory mites could be adversely affected by the sublethal effects of EPF when the mites are directly sprayed or immersed by EPF or fed on the contaminated prey (Castagnoli *et al.* 2005; Midthassel *et al.* 2016; Seiedy 2015; Seiedy *et al.* 2012a; b; Ullah & Lim 2017; Wu *et al.* 2015). These sublethal effects of EPF could further reduce the egg hatching rates of the

offspring (F₁) generation (Castagnoli *et al.* 2005; Pozzebon & Duso 2010). When we evaluate the compatibility of EPF with predators, we should not only simply test the lethal effect on their mortalities, but also study the sublethal effects on life table parameters and predation performance over their entire life span and F₁. In our study, our previous research showed that *B. bassiana*, ICMP 8701, had great potential against *B. cockerelli*. There was no information on the lethal and sublethal effect of *B. bassiana* on the performance of F₀ predatory mites, especially on life table parameters and predatory behavior in F₁ mites.

Here, this study aimed to assess the lethal and sublethal effects of entomopathogenic fungus *B. bassiana* ICMP 8701 on the life table parameters and predation rates of F₀ and F₁ *A. limonicus* fed on TPP first instar nymphs. The various biological traits and population dynamics of *A. limonicus* were assessed by age-stage, two-sex life table theory. This study could provide comprehensive information for the combined application of EPF and predatory mites in TPP integrated pest management (IPM) in the greenhouse.

5.2. Material and methods

5.2.1 Predatory mite and psyllid colonies

A stock colony of *A. limonicus* was maintained as described in section 2.2.1. *T. orientalis* pollen was prepared as described in section 2.2.1. To obtain synchronized developmental stages, more than 100 *A. limonicus* females were transferred from the colony to a new tray with fresh *T. orientalis* pollen and cotton threads for 24 h and then removed from the tray. After 7 d, gravid females were available for use.

A stock colony of *B. cockerelli* was reared as described in section 4.2.2. Psyllid adults were allowed to oviposit for 3 days and then removed from leaves of bell pepper. First instar nymphs were prepared for the experiment after 5 days.

5.2.2 Fungal preparation

The isolate of *B. bassiana*, ICMP 8701, was prepared as described in section 4.2.3. Conidia were scraped from the PDA media surface. They were then suspended in 10 mL 0.05% Tween 80 (Merck KGaA, Darmstadt, Germany) and diluted to the desired concentration: 1 ×

10^4 , 1×10^5 , 1×10^6 , 1×10^7 and 1×10^8 conidia mL^{-1} . Spore germination rates exceeded over 90% in the experiment.

5.2.3 Experimental set-up

The experimental arena consisted of one bell pepper leaf disc (5cm diameter), one Zip plastic bag (7*10 cm) and three transparent plexiglass slides modified from Wu *et al.* (2016). In the first experiment on the lethal effect of *B. bassiana* on *A. limonicus* females, the Munger cell consisted of a leaf disc on filter paper (6 cm diameter) placed under a 2.5-cm diameter hole of a plastic bag, and then a second plexiglass slide (5*5*0.4 cm) was pressed on the hole of the plastic bag. The top plexiglass slide (7*5*0.2 cm, 2.5 cm diameter hole) was sealed with a piece of cling film and punctured with holes made by an insect pin (size 00) to allow gas exchange. In the second experiment on the sublethal effect of *B. bassiana* on F_0 and F_1 *A. limonicus*, the Munger cell was similar to those in experiment 1. The only difference was the size of three slides: the bottom slide was 3.9 cm long, 2.5 cm thick and 0.3 cm thick, the middle slide was 3.8 cm long, 3.8 cm wide and had a 1.6 cm diameter hole in the middle area, the top slide was 2.5 cm wide, 0.3 cm thick, 3.9 cm long with a 0.3 cm diameter hole in the middle area. The experimental arena was formed between the leaf disc and the hole of second transparent plexiglass.

5.2.4 Lethal effect of *B. bassiana* on *A. limonicus* females

To assess the toxicity of fungus on the females of *A. limonicus*, we conducted a concentration-response bioassay of *B. bassiana* (ICMP 8701) ranging from 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , to 1×10^8 conidia mL^{-1} . The bioassays were replicated five times using 20 *A. limonicus* females in each replicate. Each *A. limonicus* female was transferred to a clean leaf disc and immersed in each fungal suspension for 10 s, and then deposited on a leaf disc with the aid of a fine brush in the Munger cell. Control females were treated with sterile distilled water containing 0.05% Tween 80. Test predatory mites were incubated at 25 ± 1 °C and 85 ± 5 % relative humidity (RH) with a photoperiod of 16:8 h (L:D). TPP first instar nymphs were supplied daily to the experimental arenas containing predatory mites. The mortality of *A. limonicus* females was checked daily for 10 days. Dead predatory mites were placed on a fresh and clean bell pepper leaf to allow the development of fungal mycelia from the cadavers in a petri dish. The presence of fungal mycelia on the surface of the dead predatory mite body confirmed death by mycosis.

5.2.5 Sublethal effect of *B. bassiana* on F₀

Based on the mortality data of *A. limonicus* females from the preliminary test, 30 × 2-day-old *A. limonicus* gravid females were immersed individually into sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* (ICMP 8701) for 10 s. In order to record the effect of *B. bassiana* on the predation rates of females *A. limonicus*, 10 TPP first instar nymphs were supplied daily to each experimental arena containing predatory mites. Longevity and oviposition of *A. limonicus* females were recorded daily until all of the mites had died. The numbers of first instar nymphs consumed by females were counted daily. Dead predatory mites from all treatments were transferred to a fresh capsicum leaf in a petri dish to allow the growth of the fungus from cadavers and the presence of fungal mycelia on the surface of the dead mite body confirmed death by mycosis.

5.2.6 Sublethal effect of *B. bassiana* on F₁

To evaluate the carry-over activity of *B. bassiana* on the performance of F₁, a total of 50 eggs laid by treated and untreated females were collected randomly, and life table parameters of F₁ were determined and compared. Each egg (<12h) was transferred to the small Munger cell and monitored until hatching. Each larva, protonymph, and deutonymph of *A. limonicus* was supplied with five first instar nymphs daily in Munger cell. The newly emerged male of *A. limonicus* was supplied with 10 first instar nymphs daily in Munger cell. Newly emerged *A. limonicus* females (up to 12 hours old after emergence) were paired with young males from the colony. The development, survival and reproduction of F₁ were observed daily. Pre-oviposition, oviposition, post-oviposition, and adult longevity were recorded daily. The numbers of first instar nymphs of *B. cockerelli* predated by *A. limonicus* immatures and adults were counted daily until the death of the mite.

Our previous study showed that there was a significant difference between male and female predation rates (Chapter 7). In order to calculate the female daily predation rate of *A. limonicus*, the average male daily predation rate of *A. limonicus* was subtracted from the daily predation rate of the pairs (Farhadi *et al.* 2011; Moghadasi *et al.* 2014).

5.2.7 Data analysis

Mortality data from the toxicity bioassay of *A. limonicus* females were subjected to probit regression analysis by using the software SPSS 17.0 (Chicago, IL, USA). The data obtained

from F₀ *A. limonicus* were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test in SPSS 17.0. The raw data for the development, survival rate, longevity and fecundity of F₁ *A. limonicus* individuals were analyzed according to age-stage, two-sex life table procedure (Chi 1988; Chi & Liu 1985) using the computer program TWOSEX-MSChart (Chi 2017c). Data on daily predation rates of F₁ *A. limonicus* immatures and adults were analyzed using the computer program CONSUME-MSChart (Chi 2017a). The mean values and standard errors of developmental time, survival rates, longevity, fecundity, population parameters and predation rates of *A. limonicus* F1 were estimated by using 100,00 bootstraps. The difference among sublethal concentrations (LC₁₀, LC₃₀) on life table parameters of F₁ was compared with a paired bootstrap test. We used TWOSEX -MSChart to compute bootstraps and paired bootstrap tests (Chi 2017c). SigmaPlot 13.0 software was used to construct the curves for all populations, including the survival rate, fecundity, life expectancy, reproductive values, predation rate, and population growth.

5.3. Results

5.3.1 Toxicity of *B. bassiana* on *A. limonicus* females

The females of *A. limonicus* were susceptible to *Beauveria bassiana* at various concentrations. Concentration-response bioassay showed that the value of LC₅₀ of *A. limonicus* female was 2.2×10^5 conidia mL⁻¹, and the concentrations causing 10 and 30 percent mortality were 2.4×10^3 and 3.5×10^4 conidia mL⁻¹ respectively (Table 5.1).

Table 5.1. Acute toxicity of *B. bassiana* ICMP 8701 against *A. limonicus* females at 10 days post-treatment.

Isolate	N	conidia mL ⁻¹				Slope ±SE	χ ²
		LC ₁₀	LC ₃₀	LC ₅₀	LC ₉₀		
<i>B. bassiana</i> (ICMP 8701)	500	2.4×10^3 (7.7×10^2 – 5.5×10^3)	3.5×10^4 (1.7×10^4 – 6.1×10^4)	2.2×10^5 (1.4×10^5 – 3.5×10^5)	2.1×10^7 (1.1×10^7 – 5.1×10^7)	0.649±0.053	7.548

5.3.2 Sublethal effects of *B. bassiana* on F₀

Sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* significantly reduced the fecundity and longevity of *A. limonicus* females (Figure 5.1). The oviposition period of *A. limonicus* females was significantly decreased by LC₃₀ sublethal concentration, but not by LC₁₀. *A.*

limonicus females consumed fewer *B. cockerelli* first instar nymphs when treated with a sublethal concentration of *B. bassiana* (LC₃₀) (Figure 5.1).

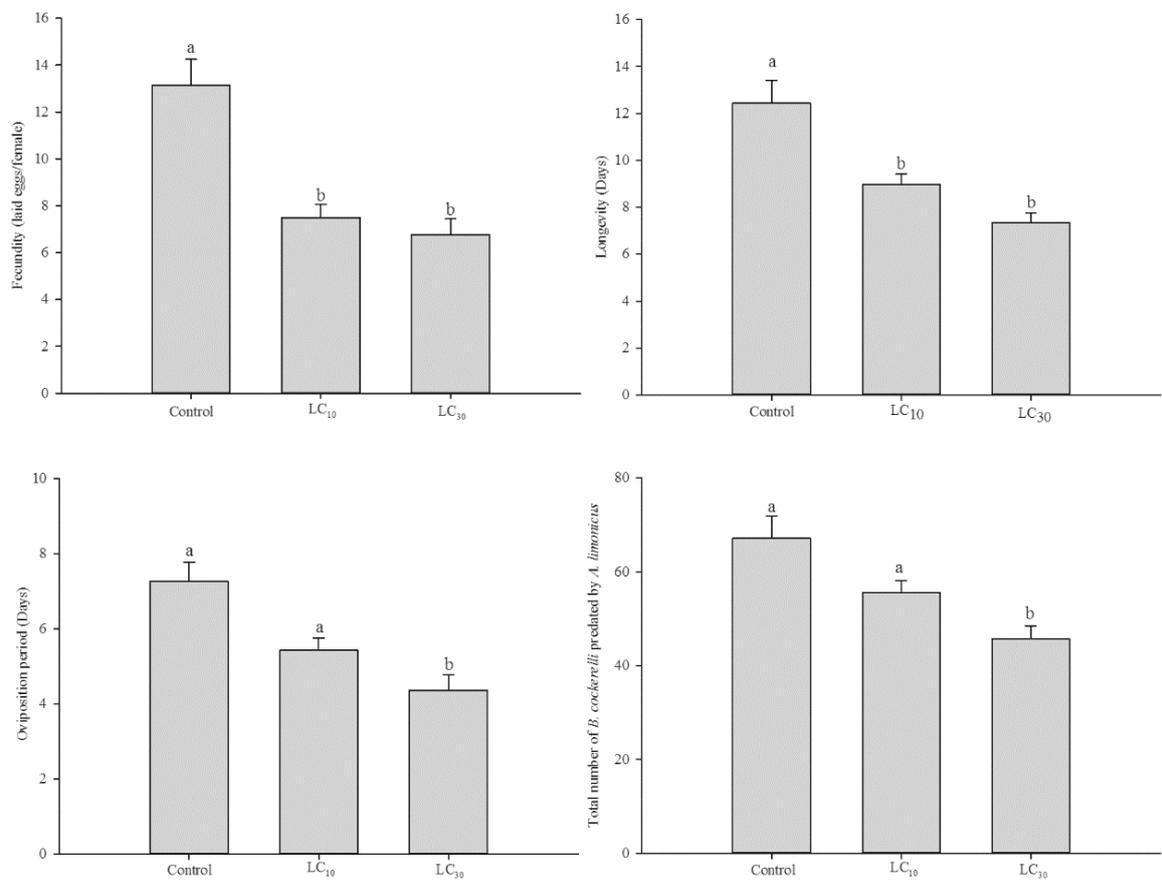


Figure 5.1. Sublethal effects of *B. bassiana* ICMP 8701 on fecundity, longevity, oviposition period and predation rates of *A. limonicus* females. Means followed by the same letter in each bar are not significantly different ($P > 0.05$; Tukey's HSD multiple range test after one-way ANOVA).

5.3.3 Sublethal effects of *B. bassiana* on the survival rates of F₁

Sublethal concentrations (LC₁₀ and LC₃₀) did not influence the survival percentages of *A. limonicus* immatures except the larval stage at LC₃₀ (Figure 5.2, Table 5.2). Of the 50 eggs initially collected from the sublethal (LC₁₀ and LC₃₀) treatments of *A. limonicus* females for the life table study, 42 eggs hatched successfully, and 39 (LC₁₀) and 37 (LC₃₀) developed to adults (Table 5.3). Age-stage specific survival rate (s_{xj}) clearly showed the survival rate, stage differentiation, and the variation in developmental rates among individuals (Figure 5.2). The probability that a newly laid egg survived to the adult female (N_f/N) and male were similar among different treatments (Figure 5.2, Table 5.2).

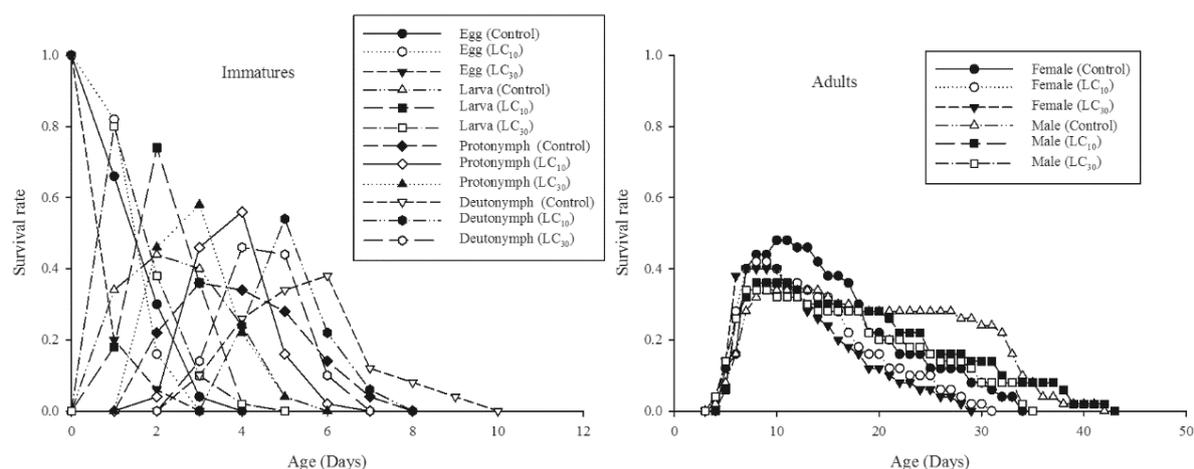


Figure 5.2. Age-stage specific survival rate (s_{ij}) of F_1 immatures and adults from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Table 5.2. Mean (\pm SE) of egg hatching rate, larva, protonymph, deutonymph, preadult survival rate and proportion of female adult N_f/N (%) of F_1 from females of *A. limonicus* exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Parameters	Control	LC_{10}	LC_{30}
	Mean \pm SE	Mean \pm SE	Mean \pm SE
Egg hatching rate (%)	86.0 \pm 4.9a	84.0 \pm 5.1a	84.0 \pm 5.2a
Larva survival (%)	100.0 \pm 0a	96.0 \pm 2.80ab	90.0 \pm 4.3b
Protonymph survival (%)	98.0 \pm 2.0a	98.0 \pm 2.0a	100.0 \pm 0a
Deutonymph survival (%)	100.0 \pm 0a	100.0 \pm 0a	100.0 \pm 0a
Preadult survival (%)	84.0 \pm 5.1a	78.0 \pm 5.9a	74.0 \pm 6.1a
Female adult N_f/N (%)	59.5 \pm 7.6a	53.9 \pm 1.4a	54.1 \pm 8.2a

A paired bootstrap test was used to detect statistical differences with different sublethal concentrations (LC_{10} and LC_{30}). Standard errors were estimated using 10000 bootstrap resampling.

5.3.4 Sublethal effects of *B. bassiana* on developmental time, adult longevity and fecundity of F_1

When F_0 *A. limonicus* females were exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana*, there was no significant difference in the developmental time of F_1 *A. limonicus* (Table 5.3). Sublethal concentration (LC_{30}) significantly reduced the egg duration and total developmental time of F_1 (Table 5.3). The female pre-oviposition period (APOP) of F_1 was not significantly influenced by sublethal concentrations (LC_{10} and LC_{30}) treated with F_0 females. However, the total pre-oviposition period (TPOP) of F_1 was significantly reduced at a higher sublethal concentration (LC_{30}). The TPOP value at LC_{30} sublethal concentration was

significantly shorter than that of individuals at LC₁₀ sublethal concentration and control (Table 5.3).

Table 5.3. Means (\pm SE) developmental time (days) of F₁ from *A. limonicus* females exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Developmental time	Control		LC ₁₀		LC ₃₀	
	N	Mean \pm SE	N	Mean \pm SE	N	Mean \pm SE
Egg	50	2.00 \pm 0.13a	50	1.98 \pm 0.08a	50	1.26 \pm 0.08b
Larva	43	1.53 \pm 0.11a	42	1.36 \pm 0.07a	42	1.36 \pm 0.08a
Protonymph	43	1.60 \pm 0.11a	40	1.5 \pm 0.11a	37	1.62 \pm 0.11a
Deutonymph	42	1.55 \pm 0.10a	39	1.33 \pm 0.08a	37	1.54 \pm 0.11a
Preadult	42	6.69 \pm 0.21a	39	6.23 \pm 0.13a	37	5.68 \pm 0.14b
APOP	25	2.04 \pm 0.20a	21	2.10 \pm 0.33a	20	1.63 \pm 0.37a
TPOP	25	8.88 \pm 0.35a	21	8.33 \pm 0.42a	20	7.26 \pm 0.37b
Adult longevity	42	18.48 \pm 1.39a	39	16.38 \pm 1.39a	37	15.14 \pm 1.22a
Female longevity	25	14.36 \pm 1.43a	21	12.9 \pm 1.34a	20	12.15 \pm 1.26a
Male longevity	17	24.53 \pm 1.98a	18	20.44 \pm 2.26ab	17	18.65 \pm 1.92b
Fecundity (eggs/female)	25	12.84 \pm 1.88a	21	12.86 \pm 1.44a	20	10.00 \pm 1.21a
Oviposition days	25	7.08 \pm 0.89a	21	6.14 \pm 0.60a	20	5.33 \pm 0.55a

APOP: female pre-ovipositional period; TPOP: the total pre-ovipositional period (counted from birth to oviposition). A paired bootstrap test was used to detect statistical differences with different sublethal concentrations (LC₁₀ and LC₃₀). Standard errors were estimated using 10000 bootstrap resampling.

There was no significant difference in the fecundity of F₁ females when the F₀ females were treated with sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* (Table 5.3). Age-specific survival rate (l_x) calculated by pooling the survival rates of all stages together determines the probability which a newly laid egg will survive to age x . The l_x curve declined earlier at LC₃₀ sublethal concentration, and 8.0% of individuals in control and LC₁₀ sublethal concentration survived longer than 35d (Figure 5.3). The maximum peak of female age-specific fecundity ($f_{xj}=1.94$) and maximal age-specific daily fecundity ($m_x=1.03$) of F₁ *A. limonicus* females occurred at the age of 13d at LC₁₀ sublethal concentration (Figure 5.3). However, the age-specific maternity showed a maximal peak ($l_x m_x=0.76$) at 8d at LC₃₀ sublethal concentration. Although no significant differences were observed in the F₁ *A. limonicus* oviposition days, the f_{xj} and m_x curves extended to the age of 30d in control, but those curves at LC₁₀ and LC₃₀ sublethal concentration only stopped at the age of 20 and 19d (Table 5.3, Figure 5.3).

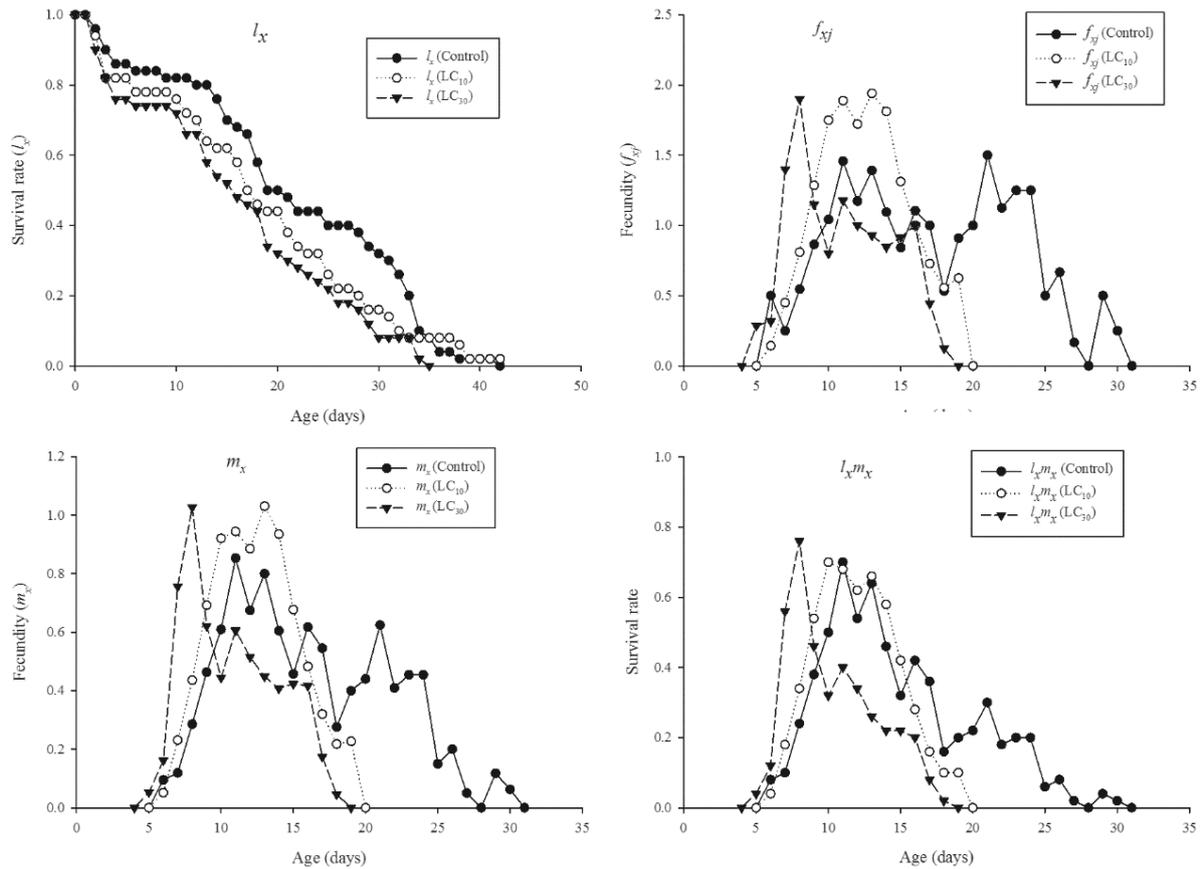


Figure 5.3. Age-specific survival rate (l_x), female age-specific fecundity (f_{xj}), age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) of *F*₁ from *A. limonicus* females exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

The life expectancy of a newly laid *A. limonicus* egg (e_{01}) was 21.68, 18.36, and 16.20 for control, LC₁₀ and LC₃₀ sublethal concentrations, which were similar to the mean adult longevity of all individuals (Table 5.3, Figure 5.4).

The maximum life expectancy of larval stage (e_{xj} =20.72d), protonymph stage (e_{xj} =23.08d) and deutonymph stage (e_{xj} =22.76d) occurred at age 1 d, 2 d and 3 d in the control (Figure 5.4). Compared with the control group, sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* had no significant effect on the female and male longevity of *F*₁ (Table 5.3). Male adults had longer lifespans than females in all treatments (Control, $P<0.0001$; LC₁₀, $P=0.0029$; LC₃₀, $P=0.0048$). The life expectancy of *A. limonicus* males was higher than the females' values in all treatments (Figure 5.4).

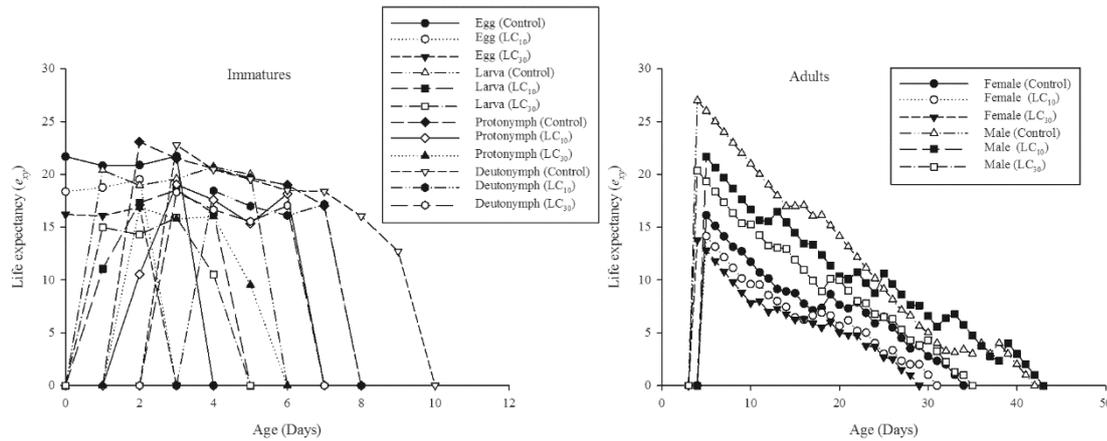


Figure 5.4. Age-specific life expectancy (e_{xy}) of F_1 immatures and adults from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

The maximum peak for age-stage specific reproductive value (v_{xj}) of *A. limonicus* deutonymph ($v_{xj}=5.73$ at 9 d) in the control was later than those values at LC_{10} ($v_{xj}=3.64$ at 6 d) and LC_{30} ($v_{xj}=3.08$ at 5 d) (Figure 5.5). When females emerged, the maximum v_{xj} value of females ($v_{xj}=7.55$) was at 9 d at the LC_{10} sublethal concentration, which was the same day the peak was observed in the control ($v_{xj}=6.59$ at 9 d), but later than the peak observed at the LC_{30} sublethal concentration ($v_{xj}=6.50$ at 7 d). The longest duration of v_{xj} values was 30 d in the control, whereas those values at LC_{10} and LC_{30} sublethal concentrations were only 20 and 19 d respectively (Figure 5.5).

5.3.5 Population parameters of F_1 *A. limonicus* of the treated females exposed to LC_{10} and LC_{30} sublethal concentrations of *B. bassiana*

Sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* did not have any significant effect on the population parameters of F_1 *A. limonicus*, including the intrinsic rate of increase (r_m), finite rate of increase (λ), gross reproduction rate (GRR), and the net reproduction rate (R_0) (Table 5.4). The mean generation time (T), however, was significantly shorter when the treated females were exposed to LC_{10} and LC_{30} sublethal concentrations of *B. bassiana*.

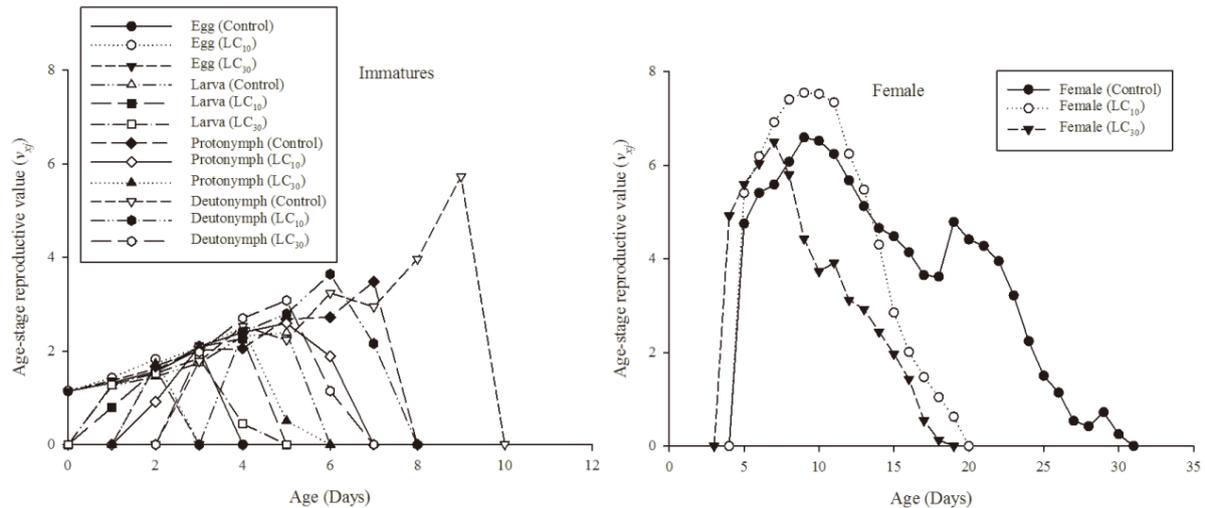


Figure 5.5. Age-stage specific reproductive value (v_{xj}) of F_1 immatures and female from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Table 5.4. Means (\pm SE) population parameters of F_1 from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Treatments	Intrinsic rate of increase (r_m) (day^{-1})	Finite rate of increase (λ) (day^{-1})	Gross reproduction rate (GRR)(Offspring)	Net reproduction rate (R_0)(Offspring/individual)	Mean generation time (T) (Day)
Control	0.1295 \pm 0.0135a	1.1383 \pm 0.0153a	9.77 \pm 2.13a	6.42 \pm 1.29a	14.36 \pm 0.61a
LC_{10}	0.1350 \pm 0.0167a	1.1445 \pm 0.0190a	8.05 \pm 1.54a	5.40 \pm 1.09a	12.50 \pm 0.36b
LC_{30}	0.1281 \pm 0.0204a	1.1368 \pm 0.0230a	6.10 \pm 1.20a	4.00 \pm 0.83a	10.82 \pm 0.30c

A paired bootstrap test was used to detect statistical differences with different sublethal concentrations (LC_{10} and LC_{30}). Standard errors were estimated using 10000 bootstrap resampling.

5.3.6 Predation rate

The age-stage predation rate (c_{xj}) of *A. limonicus* fed on TPP first instar nymphs increased with the increasing age of the predatory mite in all treatments, and the highest daily predation rate was seen in adult females of *A. limonicus* (Figure 5.6). *A. limonicus* females consumed more TPP than males in all treatments (Control, $P=0.0192$; LC_{10} , $P=0.0019$; LC_{30} , $P=0.0009$), which also can be depicted in the c_{xj} curve of adults in Figure 6, while the survival time of *A. limonicus* males was much longer than those for females. Both age-specific predation rate (k_x) and age-specific net predation rate (q_x) curves in the control were higher than those curves at sublethal concentrations (LC_{10} and LC_{30}) after the age of 16 d (Figure 5.7).

When the F_0 females were treated with a sublethal concentration (LC_{30}) of *B. bassiana*, F_1 larvae and deutonymphs of *A. limonicus* consumed significantly more prey, while there were no differences in prey consumption among the immatures of male *A. limonicus*. Sublethal concentrations (LC_{10} and LC_{30}) did not influence the prey predation rates of F_1 females, but

significantly reduced the mean number of TPP fed by F₁ males (Table 5.5). These results showed that there was a significant sexual dimorphism in the predation rates of F₁ *A. limonicus* when the F₀ females were treated with sublethal concentrations.

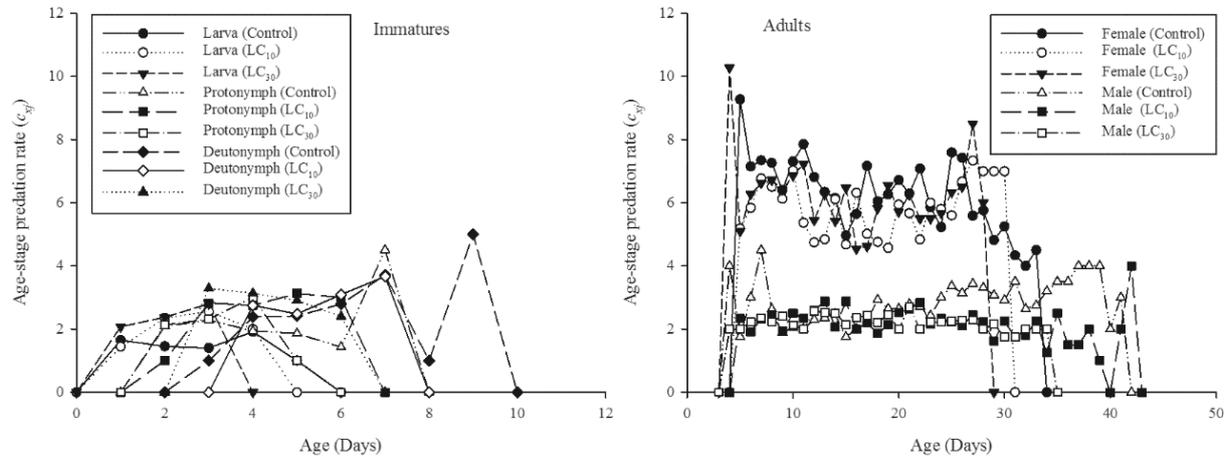


Figure 5.6. Age-stage, two-sex predation rate (c_{xy}) of F₁ immatures and adults from *A. limonicus* females exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

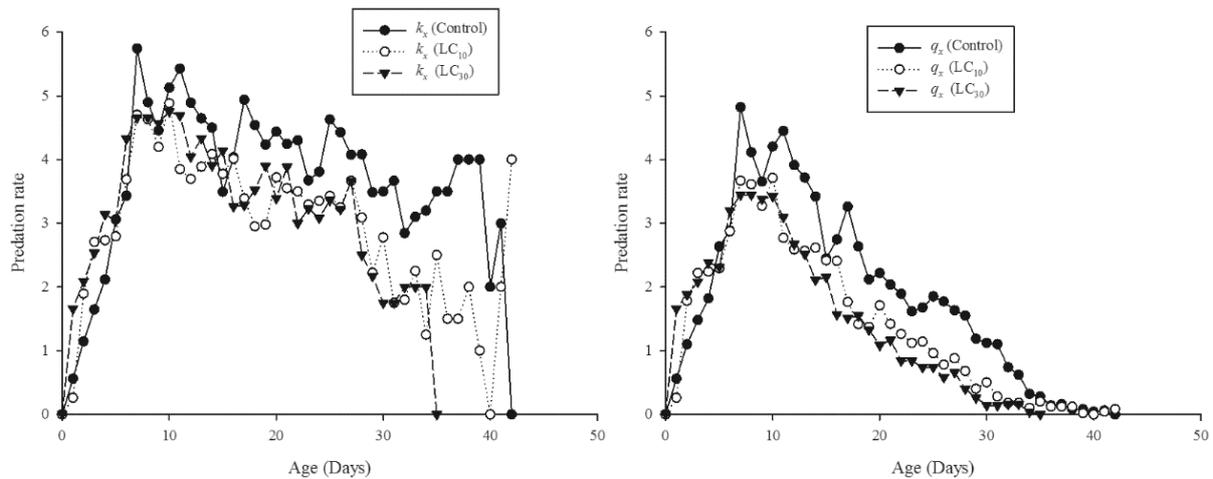


Figure 5.7. Age-specific predation rate (k_x) and age-specific net predation rate (q_x) of F₁ from *A. limonicus* females exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

Taking survival rates and longevities into account, sublethal concentrations significantly reduced the net predation rate (C_0) of F₁ *A. limonicus* (LC₁₀ =58.14 and LC₃₀=53.68) compared with the control group (78.16). However, sublethal concentrations of *B. bassiana* did not affect stable predation rate (ψ) and finite predation rate (λ) (Table 5.5). The finite predation rate (λ) values were 2.86, 2.69 and 3.04 prey/d in control, LC₁₀ and LC₃₀ sublethal concentrations, respectively (Table 5.5). *A. limonicus* in the control needed 12.17 TPP to lay

one offspring (Q_p), which was not significantly different from the number of TPP consumed in the sublethal concentrations ($LC_{10}=10.77$ and $LC_{30}=13.41$) (Table 5.5).

Table 5.5. Predation rates of F_1 from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80) fed on *B. cockerelli*.

Sex/Stages	Control	LC_{10}	LC_{30}
Female			
Larva	2.56±0.25b	3.67±0.41a	3.85±0.45a
Protonymph	3.30±0.33a	4.19±0.55a	3.90±0.42a
Deutonymph	4.01±0.45b	4.29±0.47b	5.35±0.27a
Adult	94.26±10.01a	74.58±6.80a	75.15±8.94a
Male			
Larva	2.82±0.31a	3.50±0.35a	3.12±0.35a
Protonymph	3.00±0.49b	4.44±0.54a	4.29±0.55ab
Deutonymph	3.88±0.52a	3.06±0.36a	3.76±0.50a
Adult	66.47±6.39a	48.67±4.77b	42.18±4.25b
Net predation rate, C_0	78.16±7.33a	58.14±5.67b	53.68±6.19b
Stable predation rate, ψ	2.51±0.17a	2.35±0.13a	2.67±0.17a
Finite predation rate, λ	2.86±0.22a	2.69±0.19a	3.04±0.25a
Transformation rate, Q_p	12.17±2.05a	10.77±1.95a	13.41±2.40a

A paired bootstrap test was used to detect statistical differences with different sublethal concentrations (LC_{10} and LC_{30}). Standard errors were estimated using 10000 bootstrap resampling.

5.3.7 Population and predation projection

In order to evaluate the effect of sublethal concentrations on population size, stage structure and predation potential of F_1 *A. limonicus* on TPP first instar nymphs, we used the data from life tables and predation rates to project the population and consumption growth (Figures 5.8, 5.9, 5.10). From an initial ten eggs, the population growth of immature and adult *A. limonicus* were similar in all treatments and tended to follow a straight line after 27 d, which indicated that the population growth of all stages in all treatments was approaching a stable stage distribution (Figure 5.8). The stage growth rate curves of different stages showed a similar stable trend and approached the intrinsic rate of increase (r_m) 0.1295, 0.1350, and 0.1281 day⁻¹ in control and sublethal concentrations (LC_{10} and LC_{30}), respectively (Figure 5.9). The total population and consumption of F_1 increased in a similar trend in all treatments—demonstrating that sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* did not influence the biocontrol potential of F_1 *A. limonicus* against TPP (Figure 5.10).

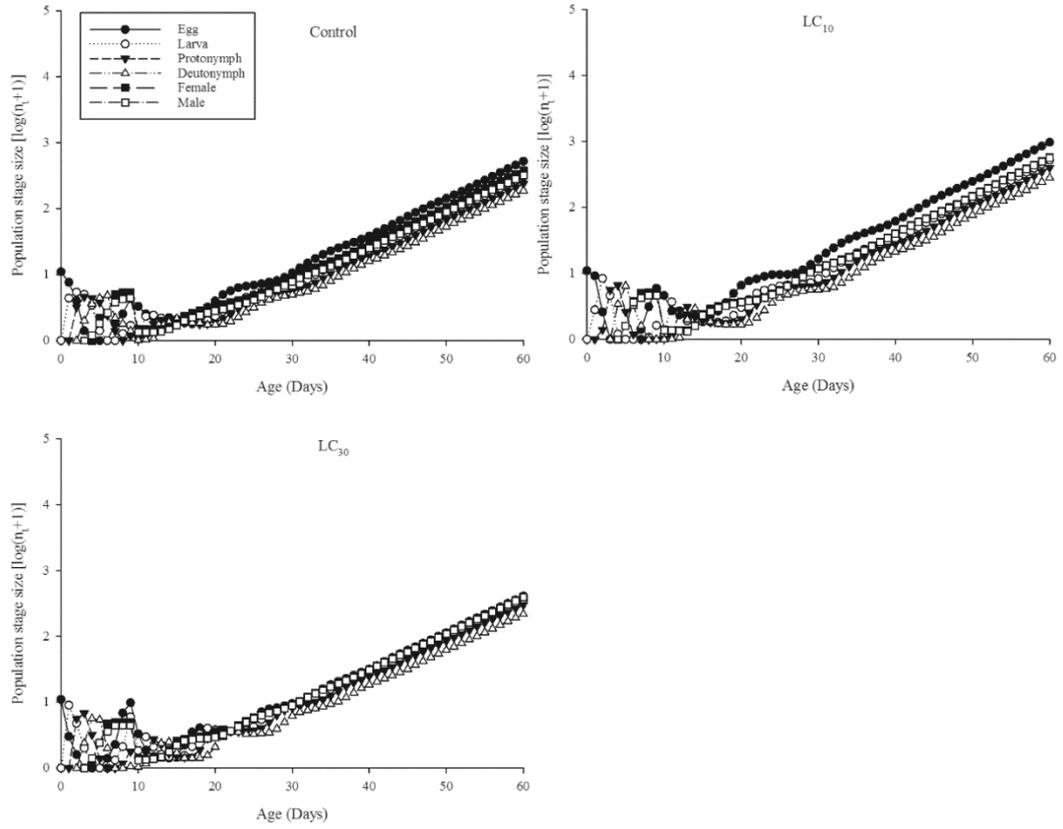


Figure 5.8. Computer simulation of population growth of F_1 from *A. limonicus* females exposed to sublethal concentrations (LC_{10} and LC_{30}) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

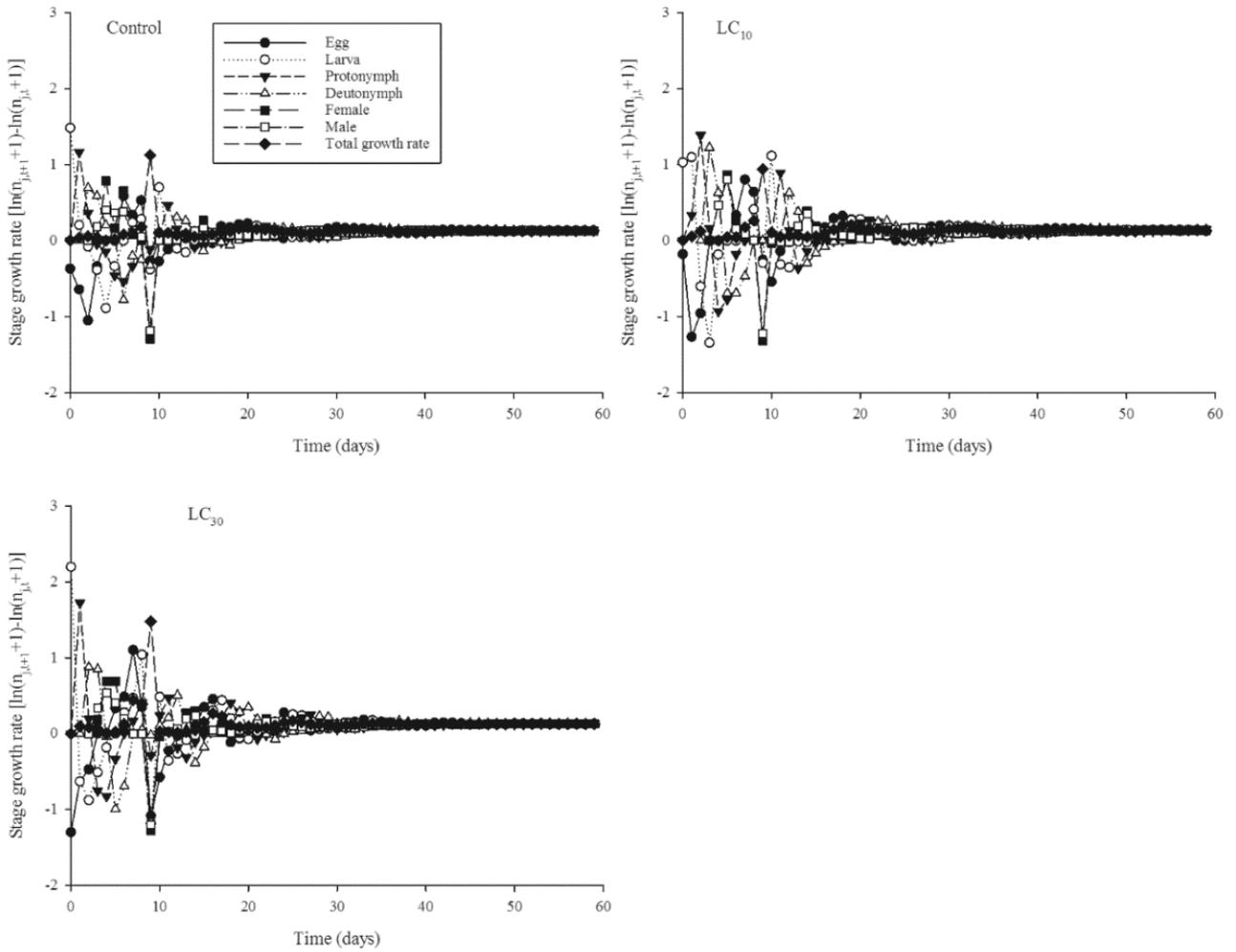


Figure 5.9. Computer simulation of stage growth rate of F₁ from *A. limonicus* females exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

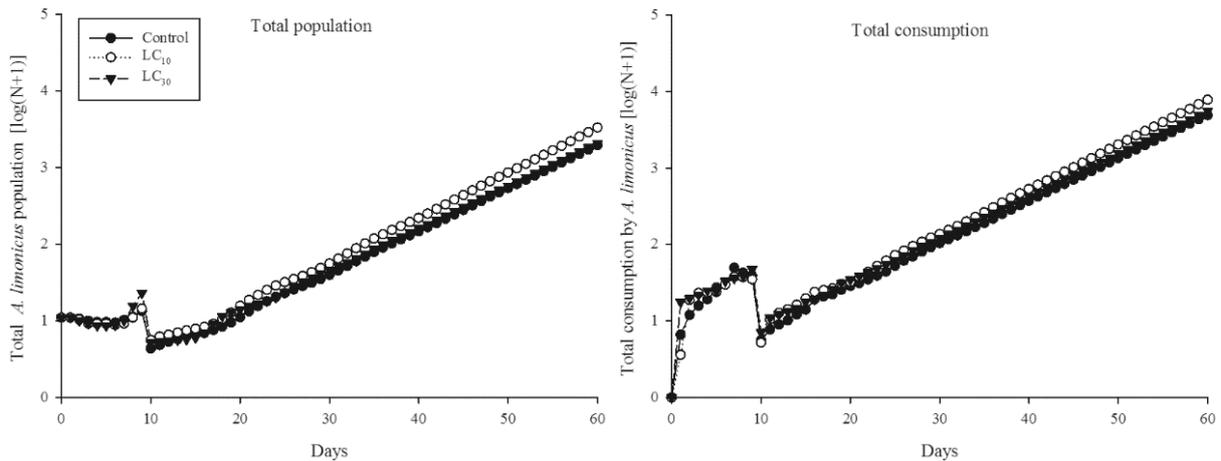


Figure 5.10. Computer simulation of total population and consumption of F₁ from *A. limonicus* females exposed to sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 or water (0.05% Tween 80).

5.4. Discussion

In augmentative biocontrol, the integrated strategy of applying EPF with predatory mites is used to control different stages of the same pest species or different species under higher pest pressures in order to eliminate or greatly reduce the application of broad-spectrum insecticides (Dogan *et al.* 2017; Jacobson *et al.* 2001; Midthassel *et al.* 2016; Saito & Brownbridge, 2018; Shaef & Lim 2017; Ullah & Lim 2017). However, EPF can potentially exert a strong negative effect on biological control agents (Dogan *et al.* 2017; Lacey *et al.* 2015; Seiedy *et al.* 2012b). The compatibility and interactions between EPF and predatory mites are critical to developing IPM programs against pests. Pytlak *et al.* (2014) found that *A. limonicus* are susceptible to *B. bassiana*, *Isaria fumosorosea*, *Lecanicillium lecanii*, and *Paecilomyces lilacinus* at 1×10^5 , 1×10^6 , and 1×10^7 conidia/mL. Our study found similar results: *A. limonicus* females were susceptible to *B. bassiana* ICMP 8701 at various concentrations. In order to understand the sublethal effect of *B. bassiana* on the population parameters and predation performance of *A. limonicus*, we investigated in detail the sublethal and transgenerational effects of *B. bassiana* on demographic fitness and predation potential of *A. limonicus* against TPP. Sublethal concentrations of *B. bassiana* could significantly influence the reproduction parameters of F_0 *A. limonicus*, but there were no significant differences in the life table parameters and predation rates of F_1 . Therefore, the simultaneous application of *B. bassiana* and *A. limonicus* could have the potential to control *B. cockerelli*. To the best of our knowledge, this is the first study to investigate the sublethal and transgenerational effects of *B. bassiana* on life table parameters and predation rates of *A. limonicus* on TPP.

5.4.1 Compatibility

The International Organisation for Biological Control (IOBC) recommends a classification system to evaluate the potential side effect of pesticides on natural enemies in the laboratory condition (Boller *et al.* 2005). The toxicity classes range from 1 (harmless or slightly harmful, mortality < 30%), 2 (moderately harmful, mortality = 30–79%), 3 (harmful, mortality = 80–99%) to 4 (harmful, mortality = 80–99%) (Boller *et al.* 2005). According to the IOBC system, our results suggest that applying *B. bassiana* ICMP 8701 at 1×10^7 and 1×10^8 conidia/mL could be considered as “harmful” to *A. limonicus* females. Pytlak *et al.* (2014) found that *B. bassiana* was also harmful to *A. limonicus* adults (90% mortality at 1×10^5 and 1×10^6 conidia/mL). Similar results were found in other EPF strains or species, *Metarhizium*

anisopliae V275 at 1×10^7 and 1×10^8 (conidia/mL) which were also harmful to females of *P. persimilis* (80.5 and 99.5%) and *N. californicus* (75.0 and 90.5%) (Dogan *et al.* 2017). The susceptibility of predatory mites to entomopathogenic fungi seems to be dependent on the different applications, the different strains, and doses under different environments (Dogan *et al.* 2017). Although *B. bassiana* ICMP 8701 did cause higher mortality to *A. limonicus* under laboratory condition, this did not mean that it was harmful to the mites in the greenhouse and field. *B. bassiana* JW-1 caused 100% infection rate of *P. persimilis* adults at 1.7×10^7 conidia/mL in the laboratory condition but only results in 11.8 and 4.0% infection rates under greenhouse conditions (Ludwig & Oetting 2001). *L. muscarium* V 24 at 2.7×10^7 spores/ml caused lower corrected mortality to *P. persimilis* in *Phaseolus vulgaris* plant (29.8%) than in the petri dish (67.1%) (Donka *et al.* 2009). Compared with the greenhouse condition, the microenvironment in the Munger cell maintained higher humidity, optimal conditions for pathogen growth. To better understand the pathogenicity of EPF on predatory mites, further research is needed to determine the effect of humidity on plants in the greenhouse condition.

5.4.2 Sublethal effect of *B. bassiana* on F₀

The compatibility of predatory mites and EPF is a critical issue for crops in the greenhouse and field conditions (Ghazy *et al.* 2016). When predatory mites are directly applied by EPF conidia or predated on EPF-treated prey, the mites may be exposed to the sublethal effect of EPF (Seiedy *et al.* 2012a). There has been no previous research on the sublethal effects of EPF on predatory mites by using sublethal concentrations. In the present study, we first found that sublethal concentration of *B. bassiana* (LC₃₀) significantly reduced the fecundity, longevity, and oviposition period of F₀ *A. limonicus* females. The fecundity and life table parameters of *P. persimilis* (2.3×10^7 and 1.0×10^8 conidia/mL) (Duso *et al.* 2008; Ullah & Lim 2017) and *A. swirskii* (1.0×10^8 conidia/mL) (Midthassel *et al.* 2016) were significantly reduced by directly applying *B. bassiana* (JW-1 and GHA strain) to the adult stage. Similarly, *M. anisopliae* V275 significantly reduced the fecundity of *P. persimilis* and *N. californicus* at 1×10^6 , 1×10^7 and 1×10^8 (conidia/mL) (Dogan *et al.* 2017). It is reasonable to conclude that applying *B. bassiana* to phytoseiid mite adults could influence their fecundity (Midthassel *et al.* 2016). When predatory mite eggs were immersed in *B. bassiana* at 1×10^8 conidia/mL, the fecundity of *P. persimilis* was also reduced by the fungi (GHA strain) (Ullah & Lim 2017). During infection, the host cuticle is the first and most important barrier against entomopathogenic fungi (Butt *et al.* 2016). Differences in susceptibility of predatory mites to

entomopathogenic fungi might be related to the defense mechanism in the cuticle of predatory mites (Wu *et al.* 2018a). Wu *et al.* (2018a) found that the dorsal cuticle of *N. barkeri* processes significantly greater hardness and stiffness than the cuticles in *T. urticae* when treated with *B. bassiana*, meaning this area could protect *N. barkeri* from *B. bassiana* infection more effectively than could the softer *T. urticae*. Besides, the different host species might also secrete different antifungal glandular secretions with fatty acids to influence the penetration of entomopathogenic fungi (Butt *et al.* 2016).

5.4.3 Sublethal effect of *B. bassiana* on F₁

Although the application of sublethal concentrations of *B. bassiana* significantly influenced the reproduction parameters of F₀ *A. limonicus*, the population parameters of the F₁ generation, such as s_{xj} , f_{xj} , m_x , v_{xj} , r_m , λ , GRR and R_0 , were not affected by the LC₁₀ and LC₃₀ sublethal concentrations. Apart from juvenile survival, there was no previous research on the effects of sublethal concentrations of EPF on F₁ phytoseiid mites (Midthassel *et al.* 2016). Our results are consistent with studies of other mite species, demonstrating that the egg-hatching rates of *P. persimilis* and *A. swirskii* were not significantly influenced by EPF when phytoseiid mite females were sprayed with *B. bassiana* (JW-1 and GHA strain) at 2.3×10^7 , 2.5×10^7 , and 1.0×10^8 conidia/mL (Duso *et al.* 2008; Midthassel *et al.* 2016). This indicates that, although *B. bassiana* compromises the fitness of F₀, the transgenerational effects of entomopathogenic fungi are not carried over to their offspring (Midthassel *et al.* 2016).

Life-table parameters provide useful information for deep understanding of the population dynamics and biocontrol efficiency of predators (Huang *et al.* 2017; Wang *et al.* 2016; Wen *et al.* 2017; Yu *et al.* 2013). The population parameters of *A. limonicus* fed on TPP that we observed are similar to those of our previous study when *A. limonicus* consumed the same prey with or without *Technomyrmex albipes* ant odour (Chapter 7). Although the sublethal concentration of *B. bassiana* reduced the mean generation time (T) of *A. limonicus*, it did not alter the intrinsic rate of increase (r_m). The r_m of natural enemies should be equal to or higher than the r_m of their targeted pest population (Van Lenteren 2000). In our study, the r_m of *A. limonicus* in all treatments (Control=0.1295, LC₁₀=0.1350, LC₃₀=0.1281) were higher than the r_m (0.1275) of *B. cockerelli* on bell pepper (*C. annuum*) (Chapter 7). Even though sublethal concentrations of *B. bassiana* influenced the reproduction parameters of F₀, higher

r_m values of F₁ *A. limonicus* show that predatory mites still have greater reproductive potential than TPP.

5.4.4 Sublethal effect of *B. bassiana* on predation performance of *A. limonicus*

The predation potential of predatory mites is significantly influenced by entomopathogenic fungi when predatory mites are fed on prey treated with EPF (Wu *et al.* 2015; Wu *et al.* 2018c). The predation rates of *N. barkeri* adults were significantly reduced when predatory mites were fed prey treated with *B. bassiana* (Wu *et al.* 2015). *B. bassiana* could significantly reduce the behavioral predation parameters (searching time, feeding time and predation rates) of *A. swirskii* and *P. persimilis* when fed on *B. bassiana*-treated *T. vaporariorum* and *T. urticae* at 0, 24, 48, and 72 hours intervals after inoculation (Seiedy 2015; Seiedy *et al.* 2012b). *P. persimilis* had a higher attack rate and spent longer handling time on *T. urticae* treated with *B. bassiana* than those *T. urticae* treated with Tween 80, but there was no significant effect on the predation rates of *P. persimilis* on *T. urticae* eggs (Wu *et al.* 2018b). However, no previous study focused on the sublethal effects of EPF on the predation rates of predatory mites when treated by EPF. Our study first showed that a sublethal concentration (LC₃₀) significantly decreased the predation performance of *A. limonicus* on *B. cockerelli*, including, the predation rates of F₀ females and F₁ males, and net predation rate (C_0) of offspring. However, population projections based on the life-table parameters and stage-specific predation rates of *A. limonicus*, suggest potential fitness and predation rates for the total consumption of F₁ *A. limonicus* in treatments were similar to that in control. Therefore, we expect that the negative effect on predation performance of predatory mites might be negligible in the long term.

Greenhouse and field studies of the simultaneous applications of EPF with some species of phytoseiid mites conclude that predatory mites are compatible to EPF (Jacobson *et al.* 2001a; Midthassel *et al.* 2016; Numa Vergel *et al.* 2011; Saito & Brownbridge 2018; Wu *et al.* 2016a). However, this compatible relationship in the laboratory and greenhouse does not guarantee a better pest control efficiency by combining applications rather than by a single application. Jacobson *et al.* (2001) reported that the combined application of *B. bassiana* with *N. cucumeris* did not significantly reduce more *F. occidentalis* populations in cucumber (*Cucumis sativus*) plants. Numa Vergel *et al.* (2011) also found similar results with the

combined release of *N. californicus* and *P. fumosoroseus* (DSM 12256) and *B. bassiana* (DSM 15126). Moreover, there were no benefits from combining *P. longipes* with *M. anisopliae* (ICIPE 78) on *T. evansi* in tomato (*Solanum lycopersicum*) crops (Automated *et al.* 2018; Maniania *et al.* 2016). EPF with no pathogenicity or low mortality to predators does not seem to be the only important evaluation criterion in the concomitant use of predatory mites and EPF (Midthassel *et al.* 2016). Although *B. bassiana* SZ-26 is innocuous for *N. barkeri*, the simultaneous application of *N. barkeri* and *B. bassiana* did not significantly reduce *F. occidentalis* densities compared with applying either the mites or EPF singly (Wu *et al.* 2014, 2016a). Their follow-up experiment showed that the population decline of *F. occidentalis* was significantly higher if *N. barkeri* was released 2 weeks after spraying EPF rather than spraying *B. bassiana* at the same time mites were released (Wu *et al.* 2017). In this study, although control may not be synergistic by applying both *A. limonicus* and *B. bassiana*, these two biological control agents may result in the maximum efficiency in the field. Due to the devastating effect of Zebra Chip, insecticide-spraying programmes need to be taken when the action threshold of TPP is over 3TPP/trap (Walker *et al.* 2015). *B. bassiana* could act as the first line of defense to rapidly infect and kill TPP adults so that their density would be lower and fecundity reduced. Lacey *et al.* (2011) have reported that *M. anisopliae* (F 52[®]) and *I. fumosorosea* (Pfr 97[®]) could significantly reduce plant damage and ZC symptoms in the potato crops by four applications with 14–19 day intervals. *A. limonicus* can be used as the second aggressive line of defense against TPP eggs and nymphs (Patel & Zhang 2017a; b; Xu & Zhang 2015).

In conclusion, *A. limonicus* females were susceptible to *B. bassiana*, ICMP 8701, and sublethal concentrations of *B. bassiana* (LC₃₀) significantly adversely influenced F₀ *A. limonicus* females. But this isolate did not affect the life-table parameters and predation potential of F₁ *A. limonicus*, and their mean generation time (*T*) at LC₁₀ and LC₃₀ sublethal concentrations were only 12.50 and 10.82 days. If the simultaneous application of *B. bassiana* and *A. limonicus* was used at appropriate intervals (>14 days) during the crop season, this integrated strategy by both lines targeted at adults and immatures has great potential to control TPP. Further evaluation in the field will confirm the conclusion for this possible viable approach to control TPP.

Chapter 6

Provisioning predatory mites with entomopathogenic fungi or pollen improves biological control of a greenhouse psyllid pest

Publication status: Accepted and published online first

Liu, J.F., Zhang, Z.Q., Beggs, J.R., & Zou, X. (2019) Provisioning predatory mites with entomopathogenic fungi or pollen improves biological control of a greenhouse psyllid pest. *Pest Management Science*, <https://doi.org/10.1002/ps.5438>.

6.1 Introduction

Tomato/potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) is a recently established invasive polyphagous pest in New Zealand. It has resulted in severe losses in the production and economics of many solanaceous crops in the US, Mexico, and New Zealand as immature stages and adults feed directly on the plant and vector the plant pathogenic bacteria “*Candidatus Liberibacter solanacearum*” (Lso) (Mustafa *et al.* 2015; Yang & Liu 2009). Long-term reliance on broad-spectrum chemical insecticides had resulted in insecticidal resistance and destroyed the ability to control pests with an integrated pest management system (IPM) (Almeyda-León *et al.* 2008; Guenther *et al.* 2012; Liu & Trumble 2007; Prager & Trumble 2017; Wright *et al.* 2017). Consequently, the utilization of biological control agents can be considered as alternative control strategy in the management of TPP populations, for example, the combined application of predatory mites with entomopathogenic fungi and a supplementary diet may be a viable IPM strategy to control TPP in greenhouse conditions (Chandler *et al.* 2005).

Several predatory mite species, such as *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae), *Amblydromalus limonicus* (Garman & McGregor) (Acari: Phytoseiidae), *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae), *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae), and *Anystis baccharum* (L.) (Acari: Anystidae) have been identified as potential biological control agents of psyllids (Fang *et al.* 2013; Geary *et al.* 2016; Patel & Zhang 2017a; b; Xu & Zhang 2015; Zhang *et al.* 2015b). The new associations between predatory mites and TPP have only been studied in New Zealand in the last 3 years (Geary *et al.* 2016; Patel & Zhang 2017a;b; Xu & Zhang 2015). Although *A. baccharum* consumed TPP eggs, early instar nymphs and even psyllid sugars, it will likely be difficult to commercially produce and use this species for control because it has a relatively long generation time (99.5 days) and diapause induction occurs when the temperature reaches 28 °C in summer (Geary *et al.* 2016; Wu 1994). Both *A. limonicus* and *N. cucumeris* feed on vulnerable stages of TPP, such as eggs and first instar nymphs and have a shorter generation time than *A. baccharum*. *N. cucumeris* is already commercially produced in New Zealand as a biocontrol agent, but not *A. limonicus* (Patel & Zhang 2017a; b). Both these mite species could significantly reduce TPP populations on bell pepper (*Capsicum annuum*) in greenhouse conditions (Patel & Zhang, unpublished data; Kean *et al.* 2019). However, TPP has a short generation time and higher

fecundity on solanaceous crops, and it can occur throughout cropping seasons, developing three to seven generations per year (Butler & Trumble 2012b; Munyaneza 2012). The various developmental stages of TPP show different vulnerability to predatory mites (Patel & Zhang 2017a; b; Xu & Zhang 2015); predatory mites mainly attack TPP eggs and first instar nymphs but cannot suppress the later stages and adults of TPP when their populations maintain high densities. In addition, it is challenging to sustain predatory mite populations on crops when vulnerable life stages of TPP are scarce, or predatory mites have high rates of cannibalism (Samaras *et al.* 2015).

Due to the special piercing and sucking mouthparts of TPP, entomopathogenic fungi (EPF) may be the predominant pathogens used for biological control of psyllids because EPF conidia can adhere and penetrate the TPP cuticle and do not require an oral route (Lacey *et al.* 2009). EPF can provide an effective strategy to rapidly and substantially reduce pest populations when the pest approaches injurious levels (Lacey *et al.* 2001). Some previous studies showed that *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) has excellent potential to reduce TPP populations in the US, Mexico, and New Zealand in the laboratory and field crops (Ai-Jabr 1999; Lacey *et al.* 2009, 2011; Mauchline & Stannard 2013; Pérez-González & Sánchez-Peña 2017; Rios-velasco *et al.* 2014; Tamayo-Mejía *et al.* 2014, 2015, Villegas-Rodriguez *et al.* 2014, 2017). Moreover, both TPP nymph stages and adults were vulnerable to *B. bassiana* infection (Lacey *et al.* 2009). However, there was limited evaluation of native strains of EPF under greenhouse conditions in New Zealand (Mauchline & Stannard 2013). In our previous study, we screened 16 New Zealand strains of entomopathogenic fungi against TPP nymphs and adults; one strain (*B. bassiana* ICMP 8701) was found to be most virulent as it caused the higher TPP mortality with shorter median lethal times (LT₅₀). Also, the sublethal concentrations (LC₁₀ and LC₃₀) of *B. bassiana* ICMP 8701 significantly reduced the life table parameters of parental and offspring TPP generations (Chapter 4).

Maintaining sufficiently high generalist predator populations in the crop system is very important to control pests throughout the crop period, especially during the absence of vulnerable prey (Khanamani *et al.* 2017a). *A. limonicus* and *N. cucumeris*, type-III generalist predators, can feed on a wide range of food types, such as pollens, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, and artificial diets (Delisle *et al.* 2015a). In order to achieve a

higher predator/prey ratio and maintain a more stable density of predatory mites in times of prey scarcity, the provision of an alternative food source such as pollen can be used to enhance biological control efficiency of the pest in the crop (Nomikou *et al.* 2004). Chapter 2 showed that *A. limonicus* feeding on *Typha orientalis* Presl (Poales: Typhaceae) pollen achieve a significantly shorter developmental time and a higher reproductive rate than when fed *E. kuehniella* eggs or artificial diets. However, there was no further information in the application of this pollen with predatory mites under greenhouse condition except a related study on whitefly control in New Zealand (Lee & Zhang 2018). Hence, it is essential to evaluate the effect of pollen supply on the control efficiency of predatory mites on TPP.

To overcome some of the above limitations of a single application of biological control agents and to effectively reduce pest populations, attention is now focusing on the combined use of multiple biological control agents. The simultaneous application of predators and EPF has been suggested as it could significantly improve the control efficiency of other crop pests, such as *Tetranychus urticae* Koch (Acari: Tetranychidae), and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Chandler *et al.* 2005; Wu *et al.* 2017). We hypothesize that predatory mites can be primarily applied preventatively on vulnerable prey, using pollen as a supplementary diet to enhance predatory mite populations, whereas entomopathogenic fungi are mainly used as a curative treatment when pest densities are already high (Midthassel *et al.* 2016). The combined application of predatory mite species and *B. bassiana* dry conidia was used to test the carryover of *B. bassiana* dry conidia to TPP populations by predatory mites; *T. orientalis* pollen acted as an adjuvant to attract predatory mites and then afforded an opportunity for dry conidia attached on the mite cuticle to reach TPP populations on plants (Zhang *et al.* 2015b). Although *A. limonicus* females were susceptible to EPF and sublethal concentrations of *B. bassiana* significantly influenced the potential of parental *A. limonicus* generation, transgenerational effects of sublethal concentrations cannot be carried over to offspring generation in the laboratory condition (Chaper 5). The risks and benefits of the simultaneous application of EPF, predatory mites, and pollen have not been evaluated in the greenhouse condition. Therefore, evaluating the compatibility of predatory mites either with entomopathogenic fungi or with *T. orientalis* pollen to control TPP is a critical issue to re-establish IPM programmes.

The aims of this research were to evaluate the compatibility of two predatory mite species (*A. limonicus* and *N. cucumeris*) with *B. bassiana* ICMP 8701 (suspension and dry conidia) or supplemented with *T. orientalis* pollen on bell pepper for controlling TPP population in the greenhouse condition. The population dynamics of TPP and predatory mites were observed over 7 weeks. Additionally, we compared their effects on bell pepper plant growth and fruit yields.

6.2 Materials and methods

6.2.1 Plants, insects and biological control agents

The plants used in this experiment were bell pepper (variety 'Giant Bell' Yates, New Zealand). Bell pepper plants were seeded first in a black transplant tray containing a growing medium (Daltons "Big Value Potting Mix", New Zealand) in the greenhouse. Seedlings with two pairs of true leaves were transplanted into a planter bag (PB6.5) filled with a mixture of 1 kg potting mix, 15 g compound fertilizer (Slowenne 212, Valagro S.p.A. Zona Ind. le 66041 Atessa (Chieti), Italy), and 150 mL vermiculite after 10 days. All plants were kept inside a mesh cage (100*100*80 cm) in a greenhouse at Landcare Research, Auckland, New Zealand at 25 ± 2 °C and 60 ± 5 % RH, L16:D 8 h photoperiod and were irrigated twice a week. Plants selected for experiments were young, healthy and free of pests.

A stock colony of *B. cockerelli* was reared as described in section 4.2.2. TPP adults were allowed to oviposit on fresh and healthy bell pepper plants for 3 days, after which all adult TPP were removed. For the experiments, 7-day-old females were collected from the colony by a suction trap (20 mL).

A stock colony of *A. limonicus* was maintained as described in section 2.2.1. *N. cucumeris* adults were obtained from Bioforce Limited, Auckland, New Zealand, in 2017 and were maintained on *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) with bran (see Patel and Zhang (2017a) for more detailed information). *T. orientalis* pollen was collected from the wild in west Auckland in February 2016 and was prepared as described in section 2.2.1.

B. bassiana, ICMP 8701, was prepared as described in section 4.2.3. In order to obtain dry conidia, the fungus was grown in a 1L plastic microwave oven bag (50*50 cm) with rolled

oats (240 g), milk powder (30 g), soybean powder (30 g), and sterile distilled water (300 mL), incubated at 25 °C for a period of 14 days. Conidia were dried at room temperature (20 °C) for 3 days and harvested carefully by dusting dry conidia from the surface of the oat bran. The fungal suspension was adjusted to 1×10^7 conidia mL⁻¹ prepared by dry conidia and then added to 1 L sterile distilled water with 0.05% Tween 80 (Merck KGaA, Darmstadt, Germany) in a 1L trigger spray. In this study, spore germination was verified at over 90% for all spore preparations.

6.2.2 Greenhouse experiments

The experimental treatments were to evaluate the combined effect of predatory mites (*A. limonicus* or *N. cucumeris*) with *B. bassiana* (suspensions and dry conidia) or *T. orientalis* pollen on TPP populations, using a randomized block design with nine treatments and a single control. The study was conducted in an air-conditioned greenhouse at Landcare Research, Auckland, New Zealand, from December 2017 to January 2018. The planter bags were divided into 60 experimental mesh cages (55*55*75 cm, Hua Yu Ke Pu Company, China). Each treatment was replicated six times. In order to prevent predatory mites from escaping, each bag was placed on a plastic tray (5 cm depth × 20.4 cm diameter) filled with 2–3 cm layer of water. The bag was suspended above water by two plastic lips.

Each isolated bag constituted a replicate, and each treatment was replicated six times. Ten TPP females were released to each caged plant on 1 December 2017. Three days later, all infected plants were randomly assigned to one of the following treatments (Table 1) (Patel 2017). For the pollen treatment, 50 mg of *T. orientalis* pollen were dusted on a 5-mm piece of 1000 cotton threads/plant and released uniformly on each plant twice per week. The *B. bassiana* suspension was applied with a hand sprayer at a dilution volume of 40 mL at 1×10^7 conidia mL⁻¹ per plant. For the combination of *T. orientalis* pollen and dry conidia, 50 mg pollen and 0.00114 g dry conidia dusted on the cotton threads were distributed uniformly on plant leaves twice per week. As in the *B. bassiana* treatment, we used 0.016 g dry conidia to adjust to 1×10^7 conidia mL⁻¹ in 40 mL suspension per plant, and thus 0.00114 g dry conidia were used to mix with 50 mg pollen every time. This method may increase the transmission efficiency of conidia on TPP lateral nymphs or even on adults when mites made contact with TPP.

Table 6.1. Description of treatments for predatory mites, *B. bassiana* suspension and dry conidia, and *T. orientalis* pollen in greenhouse experiments.

Treatment	Releasing number of predatory mite females	Suspension/mL×10 ⁷ Dry conidia/mg	Pollen/mg
C—Control, Sterile distilled water	-	-	-
B— <i>B. bassiana</i> suspension	-	1.00	-
A— <i>A. limonicus</i>	40	-	-
N— <i>N. cucumeris</i>	40	-	-
AP— <i>A. limonicus</i> + <i>T. orientalis</i> pollen	40	-	50
NP— <i>N. cucumeris</i> + <i>T. orientalis</i> pollen	40	-	50
AB— <i>A. limonicus</i> + <i>B. bassiana</i> suspension	40	1.00	-
NB— <i>N. cucumeris</i> + <i>B. bassiana</i> suspension	40	1.00	-
ABP— <i>A. limonicus</i> + <i>T. orientalis</i> pollen+ <i>B. bassiana</i> dry conidia	40	1.14	50
NBP— <i>N. cucumeris</i> + <i>T. orientalis</i> pollen+ <i>B. bassiana</i> dry conidia	40	1.14	50

Temperature (24.75 ± 0.14 °C) and relative humidity ($55.92 \pm 0.81\%$) in the greenhouse were recorded at daily intervals by Dataloggers (DS1922L-F5#, Thermochron) and thermohygrometer (Irox, Switzerland). Figure 6.1 presents the detailed temperature and relative humidity fluctuation over the seven week experiment.

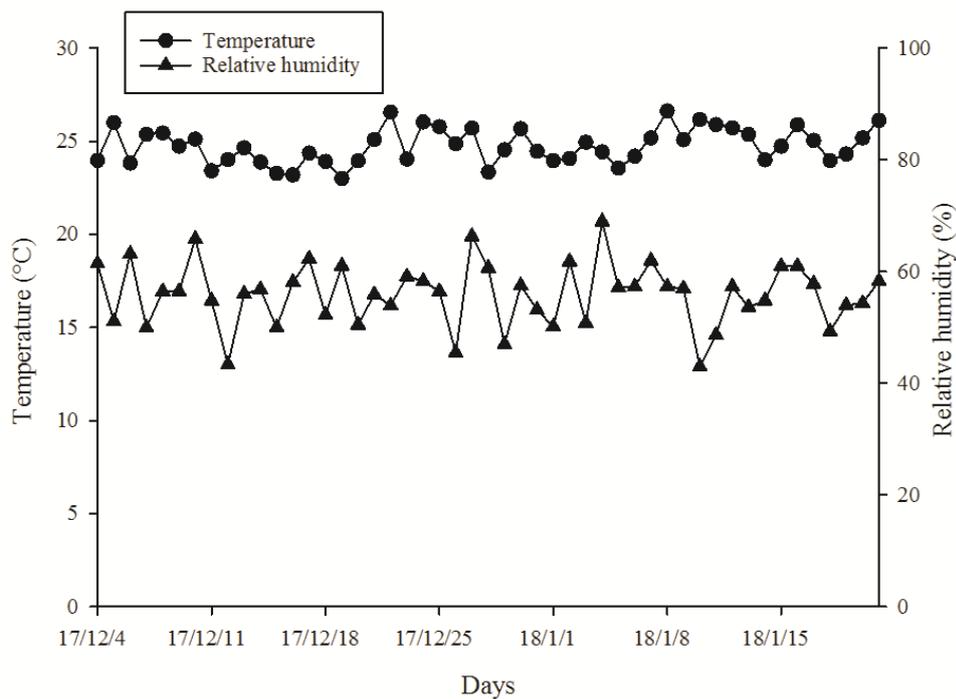


Figure 6.1. Temperature (°C) and relative humidity (%) under the greenhouse at daily interval were recorded during the experiment from early December 2017 to late January 2018.

6.2.3 Sampling of predatory mites and TPP

To monitor the number of predatory mites and TPP, direct visual counts of all life stages of TPP (eggs, nymphs, and adults) and predatory mites (mobile mites and mite eggs) per plant were recorded in the greenhouse with 20x hand lens magnifier. Five leaves (>3 cm) per plant

were randomly examined at 7-day intervals for 7 weeks. The sampling regime was initiated 7 days after the application of *B. bassiana* and predatory mites. Juvenile and adult mites were referred to as “mobile mites”.

6.2.4 Plant parameter assessments

The effects of treatments on bell pepper growth parameters were measured after 7 weeks. Bell peppers were separated into leaves, stems, roots, and fruits. After collection, the fresh weight of leaves and stems was measured by electronic scale ($d=0.01$ g, XS4002S, Mettler Toledo). The roots were carefully washed in water and then weighed. The roots were cut into small segments (1–2 cm) to fully spread and minimize root overlap for measuring the root area (Delory *et al.* 2017). The total area of all bell pepper leaves and roots was measured by Epson Expression 12000XL (ESPON, Japan) and image-processing software (ImageJ). Dry weights for each bell pepper plant were measuring by drying all leaves, stems, and roots in paper bags in a 60 °C oven for 48 h. In addition, we determined the fruit number per plant and total fruit fresh weight per planter bag. A digital vernier caliper was used to measure bell pepper stem diameter from bottom portions and the diameter and height of each fruit.

6.2.5 Data analysis

The effects of two predatory mite species with *B. bassiana* (suspensions and dry conidia) or *T. orientalis* pollen on TPP populations (total, eggs, nymphs, and adults) and predatory mites (mobile mites and mite eggs) per leaf were analysed using Friedman’s test (Week number was considered as an error term) (Medd & Greatrex 2014). The data were assessed for non-normality of data by a robust test to test the distribution of TPP and predatory mites, and pairwise comparisons of each two treatments were tested by *post hoc* function (Hothorn *et al.* 2006, 2008; Medd & Greatrex 2014). The significant correlation between mobile mites and vulnerable TPP numbers in early or later observed weeks was evaluated using Spearman’s rank correlation test (Medd & Greatrex 2014). The effects of different treatments on plant growth parameters were determined by one-way analysis of variance (ANOVA) using Tukey’s HSD multiple range test at $P<0.05$. Before analysis, all data were log-transformed to normalize the means and meet the requirements of analysis. All analyses were conducted using SPSS 22 and R package stats. TPP and predatory mite population curves were constructed using Sigmaplot 13.0 (Systat Software, San Jose, CA, USA).

6.3 Results

6.3.1 Effects of predatory mites on TPP

The mean number of TPP (total, eggs, nymphs and adults) was significantly different between the control and each treatment (Figure 6.2). Both *A. limonicus* or *N. cucumeris* significantly reduced the mean number of total, eggs, nymphs, and adults TPP found on bell pepper leaves throughout the trial (Figure 6.2 and Table A1). The application of *A. limonicus* resulted in significantly lower densities of the total, eggs, nymphs, and adults TPP on bell peppers compared with those treated with *N. cucumeris* (Figure 6.2 and Table A1). The number of mobile *A. limonicus* mites increased with increasing numbers of vulnerable TPP after the fourth week, but the density of mobile *N. cucumeris* mites and eggs decreased with increasing TPP density (Figure 6.3). In the early stages, from weeks 1 to 4, the two predatory mite species showed a weak correlation between mobile mites and vulnerable TPP (eggs and nymphs) when vulnerable TPP were presumed to be unavailable (*A. limonicus*: $r_s=0.014$; $P=0.950$; *N. cucumeris*: $r_s=-0.038$; $P=0.874$). However, while *A. limonicus* showed a significant negative correlation between mobile mites and vulnerable TPP numbers between weeks 5 to 7, there was still a weak negative correlation between *N. cucumeris* numbers and TPP populations in the trial (*A. limonicus*: $r_s=-0.593$, $P=0.010$; *N. cucumeris*: $r_s=-0.479$, $P<0.071$).

6.3.2 Application of *B. bassiana* suspension and predatory mites on TPP

The single application of *B. bassiana* suspension and the combined use of *B. bassiana* suspension with *A. limonicus* and *N. cucumeris* both had significant positive effects on total, eggs, nymphs, and adults TPP per plant sampled compared with the control and the single application of predatory mite species over 7 weeks (Figure 6.2 and Table A1). The combined use of *B. bassiana* suspension and *A. limonicus* significantly reduced the total, eggs, nymphs, and adults TPP, but the combined use of *B. bassiana* suspension and *N. cucumeris* did not cause significant differences in the egg and adult TPP populations compared with the single application of *B. bassiana* suspension (Figure 6.2 and Table A1). *B. bassiana* suspension did not affect the population dynamics of mobile mites *A. limonicus* or *N. cucumeris* (Figure 6.2). When predatory mites were combined with spray of *B. bassiana* suspension during the trial, the densities of *A. limonicus* were kept relatively stable, even though TPP populations were maintained at a much lower level. However, the number of *N. cucumeris* gradually decreased with the increasing number of vulnerable TPP in the trial (Figure 6.2). The combined

application of *A. limonicus* with *B. bassiana* suspension caused the highest reduction in TPP populations in all treatments but did not influence *A. limonicus* mobile mites (Figure 6.3).

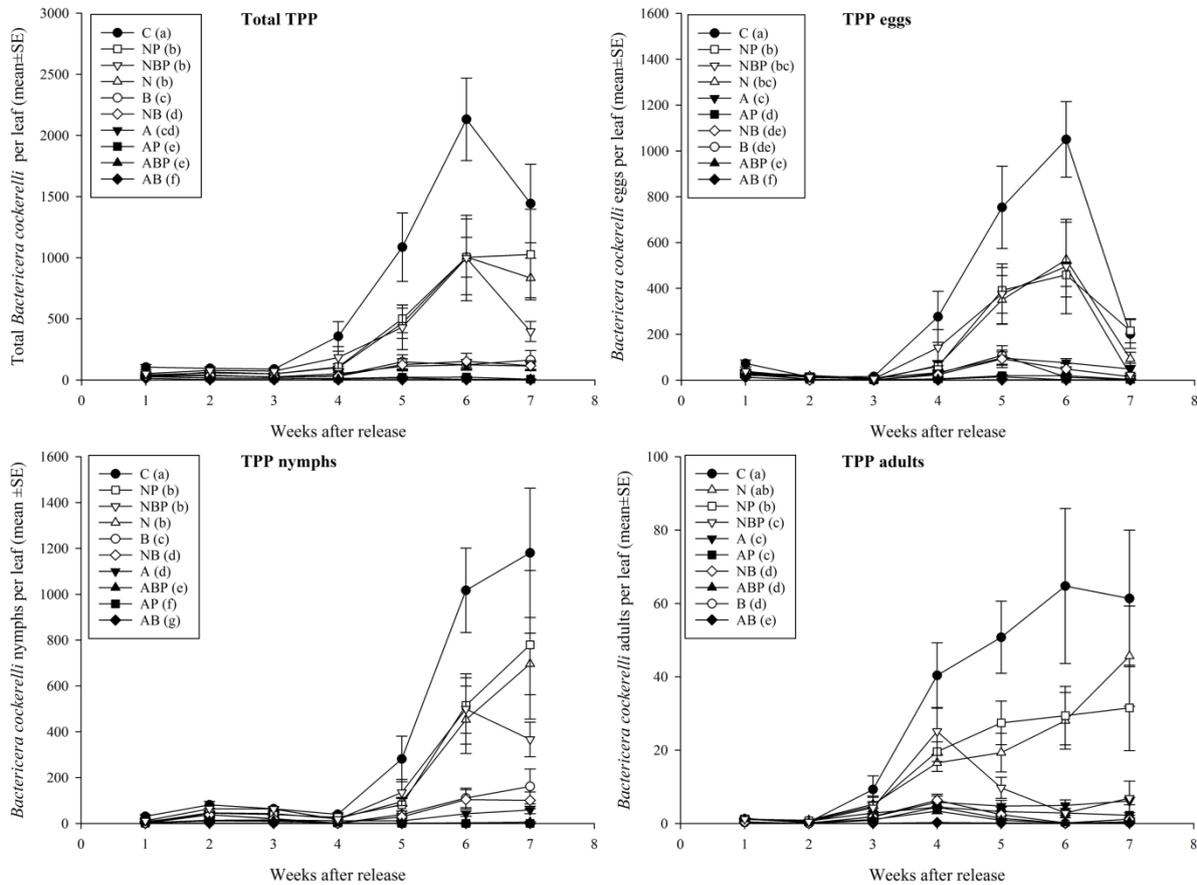


Figure 6.2. The effects of predatory mite species, *B. bassiana* suspension, *T. orientalis* pollen, and *B. bassiana* dry conidia on the mean number (\pm SE) of *B. cockerelli* (total, eggs, nymphs, and adults) per bell pepper leaf over a period of 7 weeks in the greenhouse. Initially 10 TPP females and 40 predatory mite females were released per bell pepper plant. Legend with capital letters indicated the different treatments: C—Control, B—*B. bassiana* suspension, A—*A. limonicus*, N—*N. cucumeris*, AP—*A. limonicus*+*T. orientalis* pollen, NP—*N. cucumeris*+*T. orientalis* pollen, AB—*A. limonicus*+*B. bassiana* suspension, NB—*N. cucumeris*+*B. bassiana* suspension, ABP—*A. limonicus*+*T. orientalis* pollen+*B. bassiana* dry conidia, NBP—*N. cucumeris*+*T. orientalis* pollen+*B. bassiana* dry conidia. Legend with different small letter in brackets indicates significant differences among treatments through time (Friedman, $P < 0.05$).

6.3.3 Combined use of *T. orientalis* pollen and predatory mites on TPP

Supplementing *T. orientalis* pollen maintained the highest number of mobile mites in bell peppers and significantly decreased the density of TPP (Figure 6.2 and Figure 6.3). *A. limonicus* supplemented with *T. orientalis* pollen significantly reduced the abundance of total, eggs, and nymphs TPP on plants, whereas there was no significant difference in the TPP population receiving both *T. orientalis* pollen and *N. cucumeris* (Figure 6.2 and Table A1).

The use of *T. orientalis* pollen resulted in significantly higher mobile mites and eggs on bell pepper plant treated with *A. limonicus*, but no significant difference was reported on mobile mite and mite egg dynamics treated with *N. cucumeris* (Figure 6.3 and Table A1).

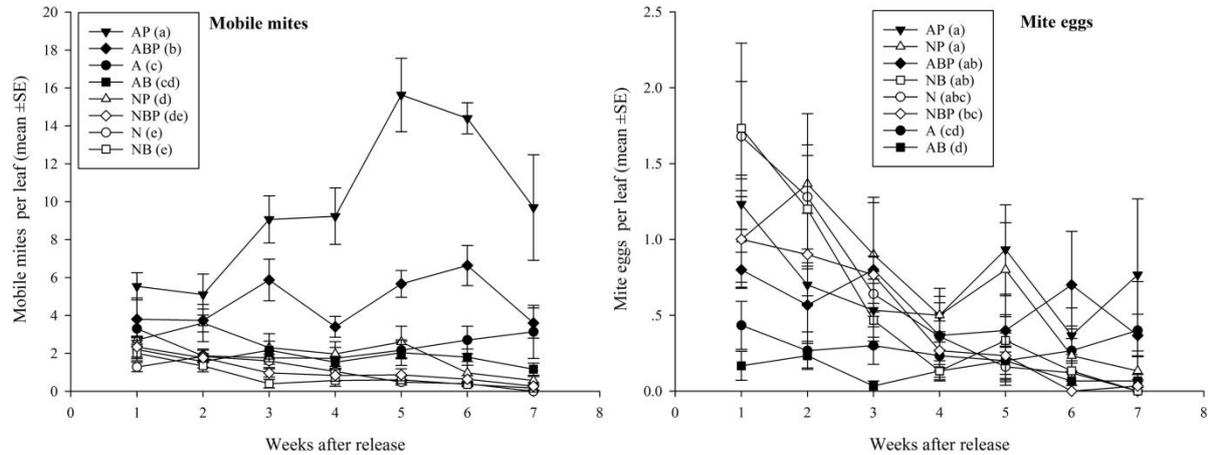


Figure 6.3. The effects of *B. bassiana* suspension, *T. orientalis* pollen, and *B. bassiana* dry conidia on mean number (\pm SE) of mobile mites and mite eggs per bell pepper leaf over a period of 7 weeks in the greenhouse. Initially 10 TPP females and 40 predatory mite females were released per bell pepper plant. Legend with capital letters indicated the different treatments: AP—*A. limonicus*+*T. orientalis* pollen, ABP—*A. limonicus*+*T. orientalis* pollen+*B. bassiana* dry conidia, A—*A. limonicus*, AB—*A. limonicus*+*B. bassiana* suspension, NP—*N. cucumeris*+*T. orientalis* pollen, NBP—*N. cucumeris*+*T. orientalis* pollen+*B. bassiana* dry conidia, N—*N. cucumeris*, NB—*N. cucumeris*+*B. bassiana* suspension. Legend with different small letters in brackets indicates significant differences among treatments through time (Friedman, $P < 0.05$).

6.3.4 Combined use of dry conidia and predatory mites on TPP

Dry conidia could not significantly decrease the mean number of total TPP (Figure 6.2). Although *T. orientalis* pollen could increase the numbers of *A. limonicus*, feeding on *T. orientalis* pollen mixed with *B. bassiana* dry conidia significantly reduced the densities of mobile mites and eggs of *A. limonicus* (Figure 6.3). This reduction in mobile mites and eggs of *N. cucumeris* was not found in the combined use of dry conidia and *N. cucumeris* (Figure 6.3 and Table A1).

Table 6.2. Effects of different mite treatments on bell pepper growth parameters (Means \pm SE) as assessed after 7 weeks under greenhouse conditions.

Treatments	Leaf		Root		Stem		Fruit	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Number	Total weight (g)
C	27.41 \pm 3.75c*	4.98 \pm 0.62a	13.38 \pm 6.25a	3.83 \pm 0.76b	37.95 \pm 5.22b	6.62 \pm 1.05b	0.33 \pm 0.21b	26.75 \pm 17.53b
B	61.52 \pm 3.35ab	10.21 \pm 0.38a	13.15 \pm 2.60a	6.02 \pm 0.42ab	53.97 \pm 3.61ab	10.49 \pm 0.80a	1.67 \pm 0.49ab	76.24 \pm 24.20ab
A	52.83 \pm 7.14ab	8.89 \pm 0.93a	20.74 \pm 6.51a	5.56 \pm 0.38ab	56.86 \pm 5.65ab	10.49 \pm 0.75a	1.80 \pm 0.58ab	209.52 \pm 53.25ab
N	37.70 \pm 2.67bc	6.60 \pm 0.76a	17.78 \pm 5.58a	4.90 \pm 0.49ab	50.07 \pm 1.05ab	9.23 \pm 0.37ab	2.33 \pm 0.71ab	138.75 \pm 47.58ab
AP	68.69 \pm 9.71ab	10.17 \pm 2.16a	22.91 \pm 4.92a	7.21 \pm 0.91a	62.64 \pm 4.49a	11.29 \pm 0.48a	3.17 \pm 0.60a	254.57 \pm 35.55a
NP	41.47 \pm 5.84abc	6.98 \pm 0.67a	15.87 \pm 3.18a	4.58 \pm 0.35ab	50.88 \pm 6.42ab	8.50 \pm 0.83ab	1.17 \pm 0.48ab	76.70 \pm 32.79ab
AB	76.23 \pm 12.98a	11.20 \pm 1.35a	21.80 \pm 5.66a	8.38 \pm 1.14a	60.58 \pm 6.30ab	11.36 \pm 0.96a	4.00 \pm 0.52a	284.93 \pm 47.14a
NB	62.51 \pm 7.31ab	10.56 \pm 1.21a	18.97 \pm 3.42a	6.29 \pm 1.04ab	50.75 \pm 6.72ab	9.49 \pm 1.24ab	1.50 \pm 1.12ab	112.69 \pm 88.27ab
ABP	71.21 \pm 8.90a	11.66 \pm 1.44a	22.97 \pm 4.23a	6.82 \pm 0.55a	57.95 \pm 4.28ab	10.86 \pm 0.72a	3.17 \pm 0.70a	203.91 \pm 52.43ab
NBP	51.54 \pm 5.54ab	9.06 \pm 0.93a	21.95 \pm 6.16a	5.83 \pm 0.70ab	48.18 \pm 4.01ab	8.85 \pm 0.54ab	1.50 \pm 0.96ab	92.40 \pm 58.16ab

*Means within a row followed by the same lower-case do not differ significantly according to Tukey's HSD multiple range test at $P > 0.05$. C—Control, B—*B. bassiana* suspension, A—*A. limonicus*, N—*N. cucumeris*, AP—*A. limonicus*+*T. orientalis* pollen, NP—*N. cucumeris*+*T. orientalis* pollen, AB—*A. limonicus*+*B. bassiana* suspension, NB—*N. cucumeris*+*B. bassiana* suspension, ABP—*A. limonicus*+*T. orientalis* pollen+*B. bassiana* dry conidia, NBP—*N. cucumeris*+*T. orientalis* pollen+*B. bassiana* dry conidia.

6.3.5 Efficacy of predatory mites, *B. bassiana* suspension, *T. orientalis* pollen, and *B. bassiana* dry conidia for plant growth under greenhouse conditions

The combined application of *A. limonicus* with either *B. bassiana* or *T. orientalis* pollen resulted in significantly higher plant and fruit growth compared with the control; namely leaf area, the weight of roots and stem plant⁻¹, diameter of stem plant⁻¹, number of fruits plant⁻¹,

and the total weight of fruit (g plant^{-1})—all increased when the mite and either a pathogenic fungus or a supplementary food was provided (Table 6.2 and A2).

6.4. Discussion

Previous studies reported the individual potential of predatory mites or entomopathogenic fungi for the control of TPP (Ai-Jabr 1999; Kean *et al.* 2019; Lacey *et al.* 2011; Mauchline & Stannard 2013; Sánchez-Peña *et al.* 2007; Tamayo-Mejía *et al.* 2014; Villegas-Rodriguez *et al.* 2014); however, this is the first study to evaluate the compatibility of predatory mites with entomopathogenic fungi or *T. orientalis* pollen against TPP populations under greenhouse conditions. Although all treatments suppressed the expansion of growing TPP populations, only the combined use of *A. limonicus* with *B. bassiana* suspension or *T. orientalis* pollen maintained significantly lower TPP populations and harvested significantly higher plant growth and fruit yields compared with other treatments and control throughout our test. Moreover, the results revealed that *B. bassiana* suspension did not negatively affect the non-target arthropod potential by reducing the *A. limonicus* population. These results confirm our previous laboratory studies demonstrating the compatibility between predatory mites and entomopathogenic fungi. For example, the combined use of predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and EPF *Neozygites floridana* (Weiser & Muma) (Zygomycetes: Entomophthorales) significantly decreased the numbers of two-spotted spider mite *T. urticae* to zero on bean (*Phaseolus vulgaris*) plants (Trandem *et al.* 2016); the combined releases of *Typhlodromalus aripo* De Leon (Acari: Phytoseiidae) and *Neozygites tanajoae* Delalibera, Hajek & Humber (Zygomycetes: Entomophthorales) significantly reduced the *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) population but did not negatively influence *T. aripo* populations (Onzo *et al.* 2013); and the combined application of *B. bassiana* 432.99 (Naturalis-L) with *P. persimilis* mites successfully controlled the *T. urticae* population on tomatoes but also significantly reduced predatory mites (Chandler *et al.* 2005). However, other studies reported that the simultaneous application of some predatory mites (*Neoseiulus californicus* sensu Athias-Henriot (Acari: Phytoseiidae), *N. barkeri*, *Phytoseiulus longipes* Evans (Acari: Phytoseiidae), *T. aripo*) and EPF (*B. bassiana*, *Metarhizium brunneum* Petch (Ascomycota; Hypocreales), *N. tanajoae*, *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Deuteromycota: Hyphomycetes) did not enhance the control efficiency of biocontrol agents against pests compared with the single

application of biological control agents (Agboton *et al.* 2013; Azandémè Hounmalon *et al.* 2018; Maniania *et al.* 2016; Numa Vergel *et al.* 2011; Wu *et al.* 2016a), indicating that each combination of a fungus, mite, and arthropod pest needs to be assessed for efficacy under different environments and host plants before large-scale regular applications.

6.4.1 Effect of entomopathogenic fungi

Entomopathogenic fungi generally have a competitive advantage over predators due to their ability to rapidly infect and kill pests (Furlong & Pell 2005). The application of BotaniGard®ES (*B. bassiana* GHA strain) resulted in the most significant reduction of TPP nymphs and adults on capsicums and tomatoes (*Solanum lycopersicum*) in New Zealand (Mauchline & Stannard 2013). In this study, the native strain *B. bassiana* ICMP 8701 suspension quickly and effectively reduced TPP densities on bell pepper. This confirms the finding of our previous study that *B. bassiana* ICMP 8701 suspension had the higher mortality and shorter median lethal times (LT₅₀) against TPP (Chapter 4). Moreover, sublethal effects of EPF could reduce the longevity and fecundity of pests and influence the performance of their offspring (Jarrahi & Safavi 2016b; Pelizza *et al.* 2013; Wang *et al.* 2014; Yuan *et al.* 2018). Although significant reductions of TPP eggs, nymphs, and adults by EPF delayed the establishment of psyllid populations on crops, TPP still decreased the development of bell pepper plant and fruit, because eggs produced by infected females still developed into the next generation and continued damaging plant leaves. Regarding the remnants of TPP on leaf surface, the best-integrated strategy is the simultaneous release of predatory mites that efficiently consume vulnerable TPP (eggs and first instar nymphs) in a relatively short time, considering the developmental time of TPP eggs and first instar nymphs was only 8 days on bell peppers (Kean *et al.* 2019; Patel & Zhang 2017a; b; Xu & Zhang 2015).

6.4.2 Effect of predatory mites

A single application of *A. limonicus* or *N. cucumeris* significantly reduced TPP populations, although *A. limonicus* provided more efficient control than *N. cucumeris* throughout the trial. These results were consistent with the laboratory studies where *A. limonicus* females consumed more TPP eggs and first instar nymphs and had a much higher reproductive rate than *N. cucumeris* (Patel & Zhang 2017b). Moreover, *A. limonicus* showed a stronger functional and numerical response to TPP eggs and first instar nymphs than did *N. cucumeris*,

such as a higher attack rate and a lower handling time for both prey stages of TPP (Patel & Zhang 2017a). In this study, the number of *N. cucumeris* mobile mites and eggs gradually decreased with the increasing number of TPP throughout the trial. Lower rates of reproduction and predation of *N. cucumeris* on vulnerable TPP suggested that the nutritional value of TPP might not be very high for *N. cucumeris*. Earlier greenhouse experiments also confirmed that *A. limonicus* was the best predatory mite of western flower thrips *F. occidentalis* (Van Houten 1996; Messelink *et al.* 2006). *A. limonicus* reached the highest population levels and caused a much lower number of *F. occidentalis* on greenhouse cucumber (*Cucumis sativus*) than *N. cucumeris* (Messelink *et al.* 2006). Overall, *A. limonicus* was a highly effective predator of TPP on bell peppers (Patel & Zhang 2017a; b). However, it is essential to solve the problem of the commercialized production of *A. limonicus* in New Zealand. Importing *A. limonicus* from Europe might be problematic, because the importation of biological control agents needs to be registered and a permit needs to be applied for in New Zealand (Glare & O’Callaghan 2017), and the mass rearing food source, *Carpoglyphus lactis* (L.) (Acari: Carpo-glyphidae), present a high risk to New Zealand biosecurity because the food source might mix with some other unknown organisms (*e.g.* storage mites, pathogens) (Vangansbeke *et al.* 2014a).

6.4.3 Effect of *T. orientalis* pollen

Supplemental food for predatory mites could influence the performance of predators on pests in crops (Duarte *et al.* 2015; Kutuk 2017; Nomikou *et al.* 2010). The addition of pollen does not always improve the outcome of predatory mite on target prey. The increasing *A. limonicus* population on strawberries supplemented by *T. latifolia* pollen does not improved the control efficiency on *F. occidentalis* and whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (Hoogerbrugge *et al.* 2011). Besides, the significant high number of *A. swirskii* population by provision of *Pinus brutia* pollen and *T. latifolia* pollen did not significantly reduce thrips *F. occidentalis* populations on eggplants cultivar “Cukurova topagi” (Kutuk 2017). However, in our study the provisioning of *T. orientalis* pollen resulted in a significantly higher abundance of the resident *A. limonicus* after the second week. The combined use of *A. limonicus* and *T. orientalis* pollen achieved a lower density of TPP egg and nymph populations after the fourthweek on bell peppers. *A. limonicus* feeding on *Typha* pollen achieved a shorter developmental time and a higher r_m value under laboratory conditions (Lee & Zhang 2016; Nguyen *et al.* 2015b; Samaras *et al.* 2015; Vangansbeke *et al.*

2014a). Additionally, the addition of *Typha* pollen had a positive effect on *A. limonicus* population on strawberries (*Fragaria x ananassa*) and cucumber (*C. sativus*) plants (Hoogerbrugge *et al.* 2011; Van Rijn *et al.* 1999), and sharply reduced the density of western flower thrips (*F. occidentalis*) on cucumber (*C. sativus*) (Van Rijn *et al.* 1999) and greenhouse whitefly (*T. vaporariorum*) on bell peppers in greenhouses (Lee & Zhang 2018). Similarly, other studies demonstrated that combining *A. swirskii* and *T. latifolia* pollen significantly reduced whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and thrips *Echinothrips americanus* Morgan (Thysanoptera: Thripidae) under greenhouse trails (Ghasemzadeh *et al.* 2017; Nomikou *et al.* 2010). The addition of alternative food on plant might decrease the strength of cannibalism (Calabuig *et al.* 2018). We therefore conclude that the combining application of supplemental food and phytoseiid mites to bell pepper could result in increased bio-control efficiency of TPP.

Typha pollen is a suitable food source for *N. cucumeris* to develop and reproduce in the lab (Delisle *et al.* 2015a; Van Rijn & Tanigoshi 1999), but providing pollen in the greenhouse did not increase the density of *N. cucumeris* or reduce TPP populations in this study. The combined use of *N. cucumeris* with apple pollen also did not enhance the establishment of a *N. cucumeris* population, nor did it decrease the levels of *F. occidentalis* larvae on potted *Chrysanthemum* sp. (Delisle *et al.* 2015b). We suggest that variation in *T. orientalis* pollen suitability as a supplementary diet for *N. cucumeris* compared with other studies was attributed to the pollen-related traits, such as nutrient content, or special morphological, or chemical traits (Samaras *et al.* 2015). In addition, bell pepper pollen on the plant might be a food source for the population growth of predatory mites. Oviposition of *A. limonicus* and *N. cucumeris* on sweet pepper pollen on cucumber leaf discs were 1.5 and 2.1 eggs per days (van Houten *et al.* 1995). The existence of pepper pollen might improve the density of predatory mites. However, in our study we did not test the treatments with or without pepper flowers, so this effect of plant flower pollen for predatory mite populations needs to be tested in further research.

6.4.4 Combination effect of predatory mites with entomopathogenic fungi and *T. orientalis* pollen

The successful application of the combined use of predatory mites and entomopathogenic fungi mainly depended on the simultaneous action of biological control agents into integrated pest management programmes. EPF could be used as the first defensive line to infect later nymphs and adults of TPP; predatory mites acted as a fallback to effectively consume any remaining prey produced by infected females on crops during the first week. Although EPF significantly reduce the reproduction parameters and predation potential of parental *A. limonicus* generation in the laboratory (Chapter 5; Pytlak *et al.* 2014), *B. bassiana* ICMP 8701 suspension did not cause significant reduction of *A. limonicus* mobile mites in this study, indicating that *B. bassiana* suspension did not readily infect *A. limonicus* in the greenhouse condition. However, the combined application of *B. bassiana* dry conidia with *A. limonicus* significantly reduced the density of *A. limonicus* mobile mites. Moreover, this treatment did not significantly improve the control efficiency of TPP and crop yield. The population growth of *A. limonicus* may be affected by dry conidia when the mites fed on the mixture of dry conidia and *T. orientalis* pollen. *B. bassiana* SZ-26 conidia had no direct pathogenicity to the predatory mite *N. barkeri*—the conidia were not able to penetrate the cuticle of *N. barkeri* and could be ingested by *N. barkeri*, although the fungi had indirect harmful effects on the life table parameters and body size of *N. barkeri* when feeding on *B. bassiana*-treated *F. occidentalis* (Wu *et al.* 2014, 2015, 2016d). Similarly, feeding on *N. tanajoae*-treated *M. tanajoa* reduced the survival rates and oviposition of the predatory mite *T. aripo* (Agboton *et al.* 2013). Also, *B. bassiana* dry residue significantly reduced the survival rates and fecundity of *A. swirskii* under laboratory conditions (Midthassel *et al.* 2016). These negative effects of dry conidia on phytoseiid mites may be caused by secondary metabolites and toxins produced by *B. bassiana* during fungal infection, which might negatively influence the performance of predatory mites (Qu & Wang 2018). Therefore, the synergistic application of *B. bassiana* dry conidia and predatory mites would not be recommended for the biological control of TPP. The detailed mechanism of the effects of the mixture of dry conidia and pollen on predatory mites requires further research.

The combined use of *A. limonicus* with *B. bassiana* suspensions or with *T. orientalis* pollen achieved a significantly higher plant growth and fruit yield compared with the control—a result that can be explained by the combined effects on the reductions of TPP populations.

High densities of hemipteran pests caused heavy damage to sweet pepper fruits (Shipp *et al.* 2000). Reduction in plant growth and fruit yield in the control may be caused by two mechanisms: the massive consumption by higher densities of TPP adults and nymphs directly piercing and sucking on leaves, and the growth of sooty mold covering leaves, which reduced photosynthesis (Palumbo *et al.* 2000).

6.5. Conclusion

Our study revealed that the combined use of the predatory mite, *A. limonicus*, with an entomopathogenic fungus *B. bassiana* suspension or with *T. orientalis* pollen could be the most effective IPM measures for the control of TPP. Compared with the combined use of *B. bassiana* suspension with *A. limonicus*, the application of *T. orientalis* pollen cost a lot in labor. Therefore, the combined use of *A. limonicus* and *B. bassiana* suspension might be a suitable strategy for growers to apply in the greenhouse. Environmental conditions in greenhouses, such as temperature and humidity, can be maintained at levels favorable to the growth of fungi and predatory mites. However, control efficiency may be reduced by more unpredictable environmental factors in the field. Therefore, field research is required to test the combined effects on controlling TPP and predatory mite populations in field crops.

TPP host plant species may be another factor that influences the integration of *A. limonicus* with *B. bassiana* suspension or with *T. orientalis* pollen as a management tool. Kean *et al.* (2019) confirmed the potential of *A. limonicus* in suppressing TPP populations on bell peppers but not on tomato. The microenvironment of host leaf morphology such as domatia and trichomes can influence the dispersal response and population establishment of predatory mites (Kean *et al.* 2019; Walter & O'Dowd 1992). Further studies should focus on the interactions among host plant species, *A. limonicus*, *B. bassiana*, and *T. orientalis* pollen as an effective combination for the re-establishment of an IPM programme, at least in the greenhouse.

Chapter 7

Tri-partite complexity: Odour from a psyllid's mutualist ant increased predation by a predatory mite on the psyllid

Publication status: Published

Liu, J.F., Zhang, Z.Q. & Beggs, J.R. (2019) Tri-partite complexity: Odour from a psyllid's ant mutualist increased predation on the psyllid by a predatory mite. *Pest Management Science*, 2019, 75, 1317–1327.

7.1 Introduction

Predator-prey interactions have been traditionally considered as direct consumption of preys by predators. These density-mediated effects of predators are critical to understanding their impact in population structure, community dynamics and top-down control (Bucher *et al.* 2015; Hermann & Landis 2017; Hermann & Thaler 2014). However, the impact of predators other than directly killing prey, known as trait-mediated effects, can be equal to or stronger than direct consumption (Preisser *et al.* 2005). The non-consumptive effects of predators can induce plastic responses by prey to predation risk, including survival, development, behaviour, morphology and physiology traits (Hermann & Thaler 2014; Roux *et al.* 2015; Werner & Peacor 2003).

Theoretical and empirical work on predation risk in aquatic system has been well studied for semiochemicals from predator pheromones, prey chemical alarm cues, and disturbance cues (Ferrari *et al.* 2010). However, relatively little similar information is known in terrestrial insect systems (Ache & Young 2005). Semiochemicals can be cues for detecting enemies and they have beneficial effects for prey because they can reveal the presence of natural enemies by a variety of predator cues such as footprints of previously patrolled or foraging predators and isolated semiochemicals (Cembrowski *et al.* 2014; Gonthier 2012; Hermann & Landis 2017; Ninkovic *et al.* 2013; Sidhu & Wilson Rankin 2016). For example, bird cherry-oat aphids, *Rhopalosiphum padi* (L.), avoid leaves with seven spot ladybird (*Coccinella septempunctata* L.) footprints. Although this anti-predator behavioural adaption in prey obtains immediate fitness benefits, it imposes substantial fitness cost to prey (Barnes *et al.* 2002). Exposure to predator cues by stink bug predator (*Podisus maculiventris*) reduces Colorado potato beetle (*Leptinotarsa decemlineata*) feeding behaviour (Hermann & Thaler 2014). Preys might exhibit plastic responses to variation in risk by differential allocation of feeding and antipredator-efforts—preys are expected to allocate more antipredator efforts when the predation risk is high, but feed more in low-risk situations (Lima *et al.* 1999).

It is well established that aphids can reduce their predation/parasitism by natural enemies when they are attended by mutualistic ants in agricultural ecosystems (Stadler & Dixon 2005; Stewart-Jones *et al.* 2008). Mutualistic ants protect aphids mainly by reducing the survival and abundance of natural enemies, changing their spatial distribution on plants or even killing natural enemies (Herbert & Horn 2008; Styrsky & Eubanks 2007). For example, mutualistic

ants *Lasius niger* attack ladybirds *Adalia bipunctata* immatures and adults which also show avoidance response to ants and reduce oviposition in response to ant semiochemicals (Oliver *et al.* 2008). However, there was little information on defence interactions in psyllids provided by mutualistic ants (Stadler & Dixon 2005). Mutualistic ants protect psyllids eggs and nymphs from their natural enemies in different strategies, such as build shelter-like structure to protect psyllids eggs (Alén *et al.* 2011), reducing the parasitism of nymphs (Navarrete *et al.* 2013; Novak 1994). In this study, we observed that an invasive psyllid *Bactericera cockerelli* (Šulc) was tended by the ant *Technomyrmex albipes* (Fr. Smith) in Auckland (Liu *et al.* unpublished data). This mutualistic ant could directly kill predator adults and reduced the predation rates of predatory mites, *Amblydromalus limonicus* Garman & McGregor, on *B. cockerelli* (Liu *et al.* unpublished data; Teulon *et al.* 2009). *B. cockerelli*, an invasive pest in New Zealand, is a phloem-feeding hemipteran that causes serious damage by direct feeding on plants or vectoring Zebra Chip (ZC), a major solanaceous crops disease caused by the bacterial pathogen ‘*Candidatus Liberibacter solanacearum*’ (Lso) (Liefting *et al.* 2008; Munyaneza 2012). *A. limonicus* is a good potential predatory mite with higher predation rates and reproductive rates on tomato/potato psyllid, *B. cockerelli* in the lab and greenhouse (Patel & Zhang 2017a; b; Xu & Zhang 2015). In this tri-partite system among *T. albipes*, *A. limonicus*, and *B. cockerelli*, ants are mutualistic to psyllids and considered as a predator of *A. limonicus* and could potentially disturb the performance of predatory mites against its prey, *B. cockerelli*. It is crucial to understand the non-consumptive effects of mutualistic ants on the biocontrol efficiency of *A. limonicus* against *B. cockerelli*. Therefore, in this study we propose that *A. limonicus* may also modify their life-history traits and feeding behaviours on psyllids in response to odour from mutualistic ants.

In order to understand the indirect effect of ant odour on the effectiveness of *A. limonicus* on TPP, it is necessary to compare the difference in parameters, such as survival rate, developmental time, stage differentiation, fecundity, longevity, and the predation rates of females and males (Xu *et al.* 2018). Life table analysis is an important tool for providing a more comprehensive analysis to predict population dynamics (Chi & Su 2006). The life table analysis and population parameters of *A. limonicus* on thrips, whiteflies, artificial and natural diets have been studied using the traditional female age-specific life table theory (Birch 1948; Leslie 1945; Lewis 1942). However, this approach ignores the male populations and variable developmental rates among individuals. Because there were some differences between

females and males, such as, longevity, survival rates, and predation rates, Chi and Liu (1985) developed two-sex life table theory through utilizing data for both females and males and taking their variable developmental rates into consideration at different age-stages. This age-stage, two-sex life table is economically important to determine population characteristics (Chi & Liu 1985; Huang & Chi 2012), as well as the population growth and predation capacity of predator populations without sex-bias or avoiding stage differentiation (Yu *et al.* 2005). In this study, we aim to understand the effects of mutualistic ant odour on different population characteristics of *A. limonicus* females and males, especially in developmental time, survival rates, and predation rates. Specifically, we examined the novel hypothesis that the odour from mutualistic ants could influence the development and reproduction of the predatory mite feeding on TPP, and also reduce the predation rates of *A. limonicus* on TPP. The age-stage, two-sex life table theory was used to compare the demographic characteristics and predation rates of *A. limonicus*. Based on the life table and predation rate data, we used computer simulation to project population growth and predation capacity of *A. limonicus* on TPP.

7.2 Material and Method

7.2.1 Mites and insect colonies

A stock colony of *A. limonicus* was maintained as described in section 2.2.1. To maintain the genetic diversity of predatory mite, *A. limonicus* adults were collected from the field few months and mixed with the lab populations.

A stock colony of *B. cockerelli* was reared as described in section 4.2.2. The first nymph *B. cockerelli* were obtained by transferring TPP adults to clean bell pepper plants for oviposition. Psyllid adults were removed after 2 days. The first instar nymphs of TPP were collected for experimental use after 7 days.

Ants *Technomyrmex albipes* (Fr. Smith) 1861 were present in our greenhouse complex for many years and invaded the *B. cockerelli* colonies on their own. The ants were composed of numerous workers, brood, and queens and reared on the soil of plastic bag (PB6.5) that supported bell pepper with *B. cockerelli* colony. The plastic bag was placed on a plastic tray (5cm depth* 20 cm diameter) with 2–3cm layer of water to prevent the ants from escaping in

the greenhouse. Numerous psyllid sugars on bell pepper were used as diets for ants. Worker ants were used in the following experiment.

7.2.2 Experimental set-up

Munger cell mainly consisted of one bell pepper leaf disc (3 cm diameter), one Zip plastic bag (7*10 cm) in single slide with 1 cm diameter middle hole and three transparent plexiglass slides as described by Patel and Zhang (2017b). The bottom and top slides were 2.5 cm wide, 3.9 cm long, and 0.3 cm thick and the top slide had a 0.3 cm diameter middle hole. The middle slide was 3.8 *3.8 cm and had a 1.6 cm diameter middle hole. Leaf disc was placed under the punctured hole of the plastic bag and upside down on filter paper (4*4 cm). The middle hole of the middle slide was tightly placed on punctured hole of the plastic bag and bottom slide was attached on the opposite slide of plastic bag. The top slide was placed with a piece of cling film which was punctured 10 holes by an insect pin (size 00) to allow ventilation. Four metal clips were used to firmly fix three plexiglass slides from four directions in order to provide a relative closed experimental arena for predatory mite and keep ant odour stable. Each plastic bag contained 5 mL distilled water to keep the filter paper moist and leaf disc fresh. It was very important to control the volume of distilled water (5mL) because too much water always submerged the experimental arena.

Before starting the experiments, only one ant worker (*T. albipes*) was transferred to Munger cell for 24h without any other psyllids or predatory mites in the treatment but no ants in Munger cell of the control. In order to keep ant odour stable and prevent ant escape from the middle of top slide by biting the cling film, the top slide would exchange with the bottom slide (without hole) at this stage. Ant normally climbed around the Munger cell and left the mixture of volatiles in this closed arena. Each Munger cell was used for the experiment only for one day. All experiments were conducted at 25 ± 1 °C, $85 \pm 5\%$ relative humidity (RH) 16:8 h (L:D) at Landcare Research, Auckland, New Zealand (McMurtry & Scriven 1965).

7.2.3 Life table analyses

To evaluate the influence of ant odour on life history parameters and predation rate of *A. limonicus* on TPP, a total of 50 eggs laid by *A. limonicus* females were maintained in a new black plastic plate with *T. orientalis* pollen and pieces of black sewing thread (1cm); eggs (<12h) were collected randomly and transferred individually to the treated Munger cell with

ant odour. The control Munger cell had no ant odour. Based on preliminary tests on the consumption rates of the first instar nymph TPP by *A. limonicus*, each larva, protonymph and deutonymph of *A. limonicus* was provided with five first instar nymph TPP daily. The newly emerged males of *A. limonicus* were provided 10 first instar nymph as prey per day in Munger cells. The newly emerged females (<12h old) were provided with young male adults from the culture and provided with 20 first instar nymphs. The developmental time and survival rate of immature stages of *A. limonicus* were recorded under a stereomicroscope every 24 h. The duration of preoviposition, oviposition, post-oviposition, and longevity of *A. limonicus* were also recorded. The number of first instar nymph TPP consumed by *A. limonicus* immature, male and pairs, as well as the number of eggs laid by female *A. limonicus*, was recorded daily until the death of the mite. To calculate the female daily predation rate of *A. limonicus* from the pairs, the average male daily predation rate was subtracted from the daily predation rate of the pairs (Farhadi *et al.* 2011; Moghadasi *et al.* 2014). If the male died first, the total daily predation rate of the pairs was attributed to the female. If the females died, we terminated the observation. The demographic raw data of developmental time, survival rate, longevity, and female daily fecundity of *A. limonicus* were analysed by the age-stage, two-sex life table (Chi 1988; Chi & Liu 1985) using the computer program TWSEX-MSChart (Chi 2017c). The age-stage specific survival rate (s_{xj}), age-specific survival rate (l_x), age-stage specific fecundity (f_{xj}), age-specific fecundity (m_x), intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T) were calculated using the method developed by Chi and Liu (1985).

Age-stage specific survival rate (s_{xj}) is the probability of a newly laid individual surviving to age x and developing to stage j . It was calculated as (Özdoğan *et al.* 2018):

$$s_{xj} = \frac{n_{xj}}{n_{01}} \quad (1)$$

Where n_{01} is the total number of predatory mite eggs used at the start of life table study and n_{xj} is the number of predatory mites that survived to age x and stage j .

The age-specific survival rate (l_x) is the probability that a newly hatched egg survives to age x . It was calculated as follows:

$$l_x = \sum_{j=1}^{\beta} s_{xj} \quad (\beta = \text{the number of stages}) \quad (2)$$

f_{xj} is the average number of offspring produced by an individual female of age x . It was calculated as (Özdoğan *et al.* 2018):

$$f_{xj} = \frac{E_{xj}}{n_{xj}} \quad (3)$$

Where E_{xj} is the total *A. limonicus* eggs produced by n_{xj} . The f_{xj} value for immature stages was zero because only *A. limonicus* females laid eggs.

The age-specific fecundity (m_x) is the mean number of eggs produced per individual at age x . It was calculated as follows:

$$m_x = \frac{\sum_{j=1}^{\beta} s_{xj} f_{xj}}{\sum_{j=1}^{\beta} s_{xj}} \quad (4)$$

The net reproductive rate (R_0) is calculated as the total mean number of offspring produced by an individual during its lifespan.

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (5)$$

The intrinsic rate of increase (r) and finite rate of increase (λ) are the most important population parameters for comparing different populations (Huang and Chi 2012). The intrinsic rate of increase (r) was estimated by using the iterative bisection method according to Euler-Lotka formula with age indexed from 0 as follow (Goodman 1982):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \quad (6)$$

The finite rate of increase was calculated as follows:

$$\lambda = e^r \quad (7)$$

The mean generation time (T) is defined as the time required for a population that needs to increase R_0 -fold of its size at a stable age-stage distribution. It was calculated as follows:

$$T = \frac{\ln R_0}{r} \quad (8)$$

According to the method of Chi and Su (2006), the age-stage life expectancy (e_{xj}) is defined as the time length that an individual of age x and stage y is expected to live, and was calculated as:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^m s'_{iy} \quad (9)$$

Where s'_{xj} represents the probability that an individual of age x and stage y will live to age i and stage j , and is calculated by assuming $s'_{xj}=1$ (Chi & Su 2006)

The reproductive value (v_{xj}) is defined as the contribution of individuals at age x and stage y for the future generation (Huang & Chi 2011). The reproductive value (v_{01}) for a newly oviposited egg is equal to the value of the finite rate of increase (λ). When individuals begin to produce viable eggs, the v_{xj} value increases substantially and reaches the peak value at some time which means that the female contributes the most to the v_{xj} of the population. And then the v_{xj} decreases to zero when the old female no longer lays eggs. It was calculated as follow:

$$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^k s'_{iy} f_{iy} \quad (10)$$

7.2.4 Predation rate calculation and analysis

Data on daily predation rates of *A. limonicus* immatures and adults were analysed according to Chi and Yang (2003) and Yu *et al.* (2005) using the computer program CONSUME-MSChart(Chi 2017a). Age-stage-specific consumption (c_{xj}) is defined as the daily mean predation of an individual of age x and stage j . The age-specific predation rate (k_x) represents the mean number of TPP nymph I consumed by a predator *A. limonicus* at age x and was calculated as follows:

$$k_x = \frac{\sum_{j=1}^{\beta} s_{xj} c_{xj}}{\sum_{j=1}^{\beta} s_{xj}} \quad (11)$$

The age-specific net predation rate (q_x) reveals the weighted consumption of first instar nymph TPP predated by an individual surviving *A. limonicus* at age x . It was calculated as follow:

$$q_x = l_x k_x = \sum_{j=1}^{\beta} s_{xj} c_{xj} \quad (12)$$

Where β represents the number of life stage of *A. limonicus*. The net predation rate (C_0) is defined as the mean number of first instar nymph TPP predated by an average individual *A. limonicus* during its lifespan. It was calculated as follows:

$$C_0 = \sum_{x=0}^{\infty} \sum_{j=0}^{\beta} s_{xj} c_{xj} = \sum_{j=1}^{\beta} k_x l_x \quad (13)$$

The transformation rate from prey population to predator offspring (Q_p) is the ratio of the net predation rate (C_0) to the net reproductive rate (R_0). Q_p is the mean number of first instar nymph TPP that an *A. limonicus* needs in order to produce an offspring, and was calculated as:

$$Q_p = \frac{C_0}{R_0} \quad (14)$$

Yu *et al.* (2013) defined the finite predation rate (ω) to compare the predation skill of an individual predator consumed different preys, which was calculated as follows:

$$\omega = \lambda\psi = \lambda \sum_{x=0}^{\infty} \sum_{j=1}^m a_{xj} c_{xj} \quad (15)$$

where λ is the finite rate of *A. limonicus* population. ψ is defined as the stable predation rate ($\psi = \sum \sum a_{xj} c_{xj}$); a_{xj} is the percentage that individuals belong to age x and stage j , and c_{xj} is the age stage-specific predation rate.

7.2.5 Population dynamics

The program TIMING-MSChart was used to simulate the population growth of *A. limonicus* with an initial population of 10 eggs at 1d and its predicted consumption capacity over a period of 60-day period with or without ant odour (Chi 2017b). The data file for TIMING-MSChart was based on the two-sex life table and age-stage-specific consumption (c_{xj}). The program TIMING is also available at: <http://140.120.197.173/Ecology/prod02.htm>.

According to the mean daily predation rate of each stage, the stage-specific weighting coefficients (w_j) was used to consider the variable predation potential among stages on population level, and was calculated as:

$$w_j = \frac{C_j}{C_{highest}} \quad (16)$$

The weighted population size at time t , i.e. $n_w(t)$, was calculated as:

$$n_w(t) = \sum_{j=1}^m \left(\sum_{x=0}^{\infty} n_{xj}(t) \cdot w_j \right) \quad (17)$$

where $C_{highest}$ is the highest mean daily predation of individual, C_j is the daily mean predation rate of a j th instar, and $n_{xj}(t)$ was the number of individuals at age x and stage j at time t .

The increase rate of stage j from time t to $t+1$ ($\emptyset_{j,t}$) during population growth was calculated using common logarithm.

$$\emptyset_{j,t} = \log\left(\frac{n_{t+1} + 1}{n_t + 1}\right) \quad (18)$$

We also used the natural logarithm to calculate the increase rate of stage j from time t to $t+1$ as:

$$r_{j,t} = \ln\left(\frac{n_{j,t+1}+1}{n_{j,t}+1}\right) = \ln(n_{j,t+1} + 1) - \ln(n_{j,t} + 1) \quad (19)$$

where $n_{j,t}$ is the number of individuals at stage j at time t during population growth. As logarithmic transformation is impossible, the individual number of a stage is zero ($n_{j,t}=0$ or $n_{j,t+1}=0$). So we used $n_{j,t}+1$ and $n_{j,t+1}+1$ in the calculation of $\emptyset_{j,t}$ and $r_{j,t}$.

7.2.6 Statistical analysis

The mean and standard errors of the developmental time, fecundity, adult longevity, r , R_0 , λ , T , and consumption were estimated by the bootstrap technique using 10000 bootstraps. The difference between ant odour and no ant odour treatments was compared using the paired bootstrap test which was based on the confidence interval of differences (Akköprü *et al.* 2015; Efron & Tibshirani 1993).

7.3 Results

7.3.1 Immature development

Developmental time of preimaginal stages (from egg to adult) of *A. limonicus* averaged 5 to 6 days when feeding on first instar nymphs of *B. cockerelli* and was not significantly affected by ant odour (Table 7.1).

7.3.2 Survival, life expectancy, and longevity

Out of the cohorts of 50 eggs of *A. limonicus* collected at the beginning of the life table study, 47 (94%) hatched successfully at 25 °C and 85% RH. The odour from ant did not affect immature survival of *A. limonicus* (Figure 7.1a). The life expectancies of first-day egg, larva, protonymph, and duetonymph without ant odour (20.36d, 19.19d, 19.55d and 19.40d) were systematically higher than those mites with ant odour (17.64d, 17.01d, 15.16d and 17.83d) (Figure 7.3a). When an egg survived to the adult age, the life expectancy of females and males peaked. The life expectancy of females showed a similar trend in the absence and presence of ant odour, but ant odour influenced the life expectancy of males, and the similar trend was also found that ant odour induce a sexually dimorphic response in longevity (Table

7.1, Figure 7.3b). Males *A. limonicus* had a significant longer lifespan than females in the absence of ant odour ($P=0.003$), but not in the presence of ant odour ($P=0.2313$). This is clearly reflected in age-stage specific survival curves (s_{xj}): the curve for males was consistently above that of females in the absence of ant odour, whereas the two curves crossed near day 25 in the presence of ant odour (Figure 7.1b).

Table 7.1. Developmental time, longevity, and fecundity (eggs) (\pm SE) of *A. limonicus* fed on TPP without or with ant odour.

Developmental time	No ant odour		Ant odour	
	N	Mean \pm SE	N	Mean \pm SE
Egg	50	1.54 \pm 0.08a	50	1.54 \pm 0.07a
Larva	47	1.11 \pm 0.04a	47	1.00 \pm 0a
Protonymph	44	1.39 \pm 0.07a	42	1.25 \pm 0.07a
Deutonymph	40	1.30 \pm 0.07a	38	1.62 \pm 0.09a
Preadult	40	5.25 \pm 0.09a	38	5.45 \pm 0.10a
Longevity (Male)	22	21.82 \pm 1.46a	15	17.88 \pm 2.04a
Longevity (Female)	18	15.83 \pm 1.35a	23	15.30 \pm 0.93a
APOP	18	1.78 \pm 0.25a	23	1.65 \pm 0.32a
TPOP	18	7.11 \pm 0.30a	23	7.09 \pm 0.33a
Oviposition days	18	7.78 \pm 0.77a	23	7.91 \pm 0.68a
Mean fecundity	18	13.61 \pm 1.54a	23	11.65 \pm 1.15a

APOP: female pre-ovipositional period; TPOP: the total pre-ovipositional period (counted from birth to oviposition). A paired bootstrap test was used to detect statistical differences with or without ant odour. Standard errors were estimated using 10000 bootstrap resampling.

7.3.3 Reproduction

Ant odour had no significant effect on adult pre-oviposition period, oviposition period, or fecundity (Table 7.1). In the absence and presence of ant odour, mated female predatory mites laid 13.61 \pm 1.54 and 11.65 \pm 1.15 eggs respectively during 7.78 \pm 0.77 and 7.91 \pm 0.68 days (Table 7.1). The highest peaks in the f_{x5} , m_x , $l_x m_x$ curves without ant odour were higher than those mites with ant odour, while the occurrence of those maximum values without ant odour were later than those mites with ant odour (Figure 7.2a, 7.2b). The age-stage reproductive value (v_{xj}) increased when *A. limonicus* began to produce viable eggs (Figure 7.4b). The newly laid eggs of *A. limonicus* had a similar v_{xj} of 1.15 in the control and treatment, which is the finite rate itself (Table 7.2, Figure 7.4a). The peak reproductive value (v_{xj}) with or without ant odour was also estimated to occur at a similar age (6 and 7 days), the highest v_{xj} value without ant odour ($v_6=7.95 \text{ day}^{-1}$) was higher than that for females with ant odour ($v_7=5.90$

day⁻¹), and then declined gradually to zero at a similar age (30 and 29 day⁻¹) (Figure 7.4b). There was no v_{xj} curve for males in the Figure 4b because two-sex life table could not define the contribution of males to future population.

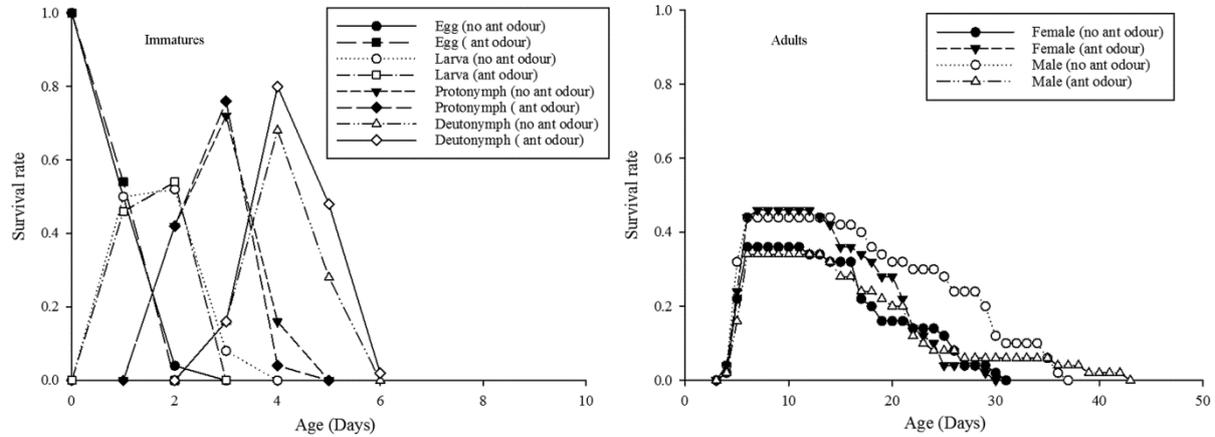


Figure 7.1. Age-stage specific survival rate (s_{xj}) of the predatory mite *A. limonicus* immatures and adults fed on TPP first instar nymph without or with ant odour.

7.3.4 Life table parameters

The intrinsic rate of natural increase (r_m) without or with ant odour was 0.1381 and 0.1442 day⁻¹, respectively, whereas the net reproductive rate (R_0) without or with ant odour was estimated to be 4.9 and 5.4 eggs/female (Table 7.2). There was no statistical difference in the rates of natural increase (r_m and λ) or the rates of reproduction (R_0) and mean generation time (T) due to the presence of ant odour (Table 7.2).

Table 7.2. The mean (\pm SE) two-sex life table parameters of *A. limonicus* fed on TPP without or with ant odour.

Parameters	No ant odour	Ant odour
r_m day ⁻¹	0.1381 \pm 0.0209a	0.1442 \pm 0.0165a
λ day ⁻¹	1.1483 \pm 0.0239a	1.1564 \pm 0.0190a
GRR (Offspring)	6.65 \pm 1.445a	8.38 \pm 1.64a
R_0 (Offspring/individual)	4.8838 \pm 1.0692a	5.3627 \pm 0.9752a
T (Day)	11.317 \pm 0.349a	11.54 \pm 0.586a

Standard errors were estimated using 10000 bootstrap resampling. A paired bootstrap test was used to detect statistical differences between the ant odour treatments. r_m —intrinsic rate of population increase, λ finite rate of population increase, GRR—Gross reproduction rate, R_0 —net reproductive rate, T —mean generation time.

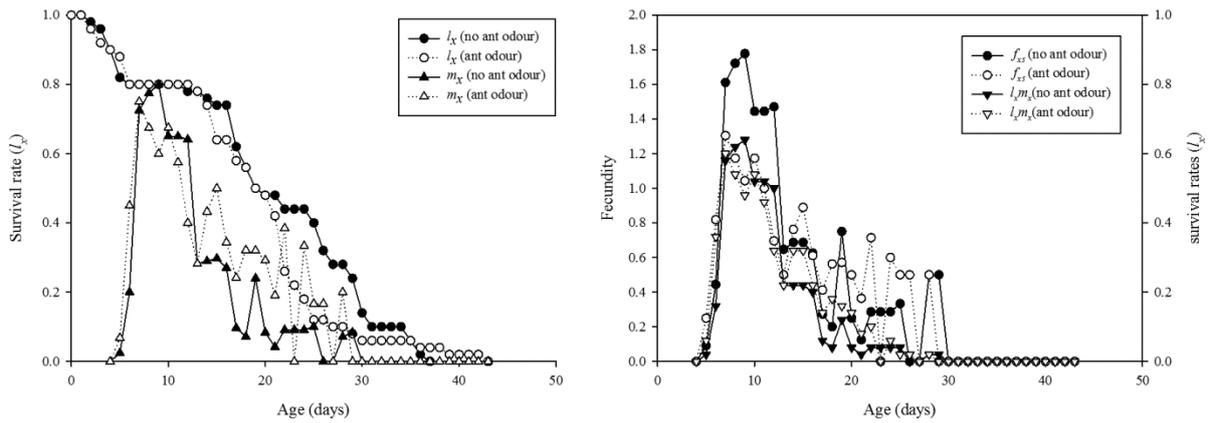


Figure 7.2. Age-specific survival rate (l_x), female age-specific fecundity (f_{x5}), and age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) of the predatory mite *A. limonicus* fed on TPP first instar nymph without or with ant odour.

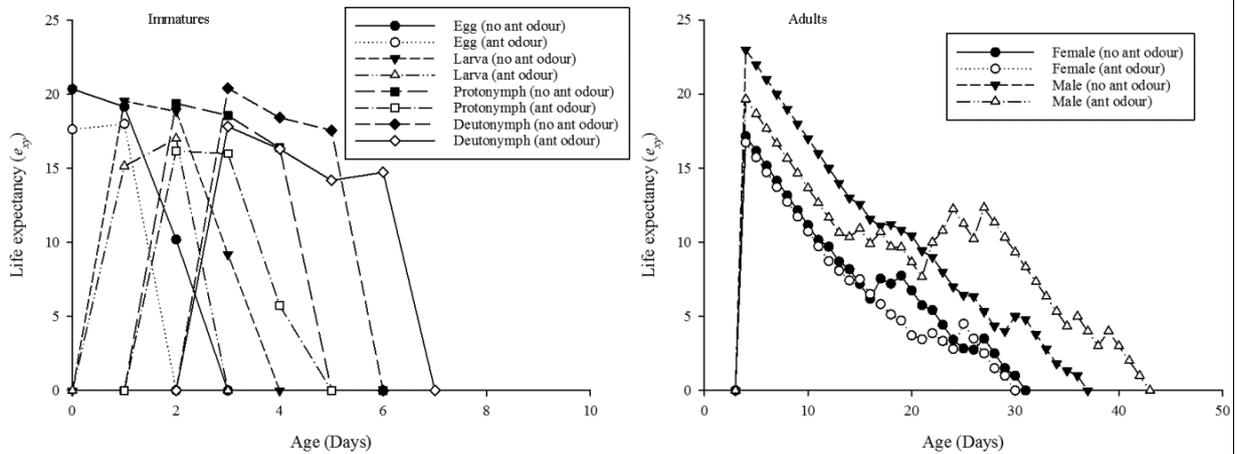


Figure 7.3. Age-specific life expectancy (e_{xy}) of immatures and adults *A. limonicus* fed on TPP first instar nymph without or with ant odour.

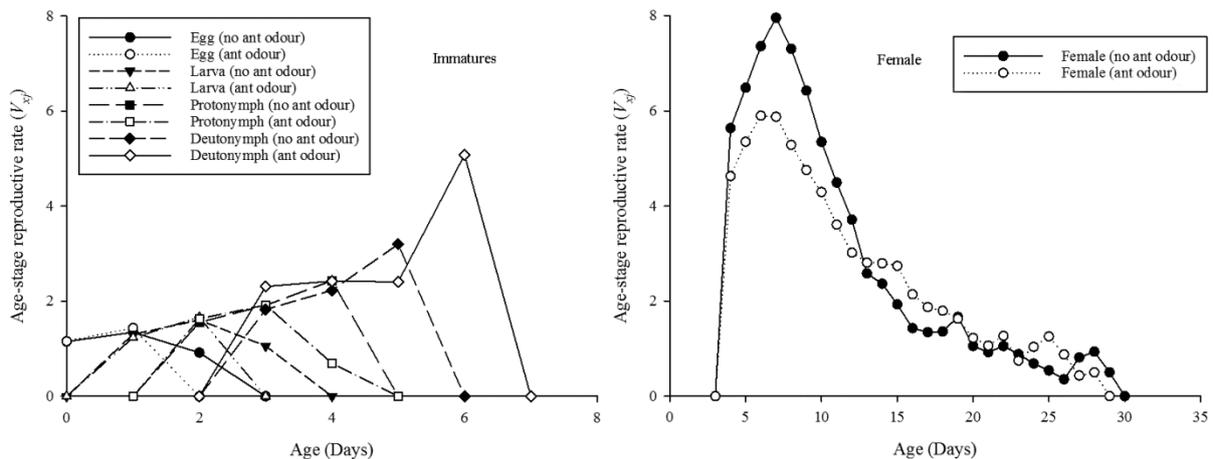


Figure 7.4. Age-stage specific reproductive value (v_{xj}) of *A. limonicus* immatures and female fed on *B. cockerelli* first instar nymph without or with ant odour.

7.3.5 Consumption rate

The age-stage consumption rates (c_{xj}) of *A. limonicus* increased with the age of the individual, with *A. limonicus* adults consuming considerably more TPP than the immatures (Figure 7.5a, 7.5b, Table 7.3). Remarkably, *A. limonicus* larvae were observed predated on TPP first instar nymph.

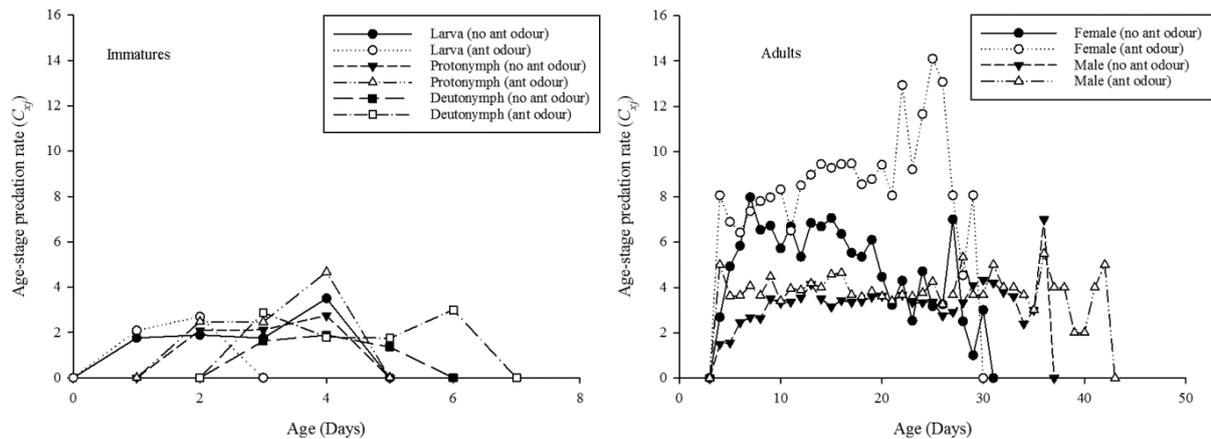


Figure 7.5. Age-stage, two-sex predation rate (c_{xj}) of *A. limonicus* immatures and adults fed on TPP first instar nymph without or with ant odour.

Table 7.3. Predation rates of *A. limonicus* fed on *B. cockerelli* without or with ant odour.

Sex/Stages	No ant odour	Ant odour	<i>P</i> value
Female			
Larva	2.11±0.25b	2.78±0.22a	0.043
Protonymph	3.06±0.40b	4.57±0.62a	0.039
Deutonymph	3.50±0.35a	4.26±0.64a	0.302
Adult	92.14±6.84b	129.39±11.03a	0.005
Male			
Larva	2.05±0.26a	2.29±0.21a	0.449
Protonymph	3.32±0.34a	3.12±0.27a	0.652
Deutonymph	1.82±0.41a	2.41±0.49a	0.349
Adult	71.44±4.88a	70.00±7.75a	0.869
Net predation rate, C_0	71.42±5.86a	91.84±9.36a	0.064
Stable predation rate, ψ	2.84±0.20b	3.73±0.27a	0.009
Finite predation rate, λ	2.47±0.13b	3.22±0.19a	0.001
Transformation rate, Q_p	15.19±3.20a	17.14±2.24a	0.514

Standard errors were estimated using 10000 bootstrap resampling. A paired bootstrap test was used to detect difference different with or with ant odour.

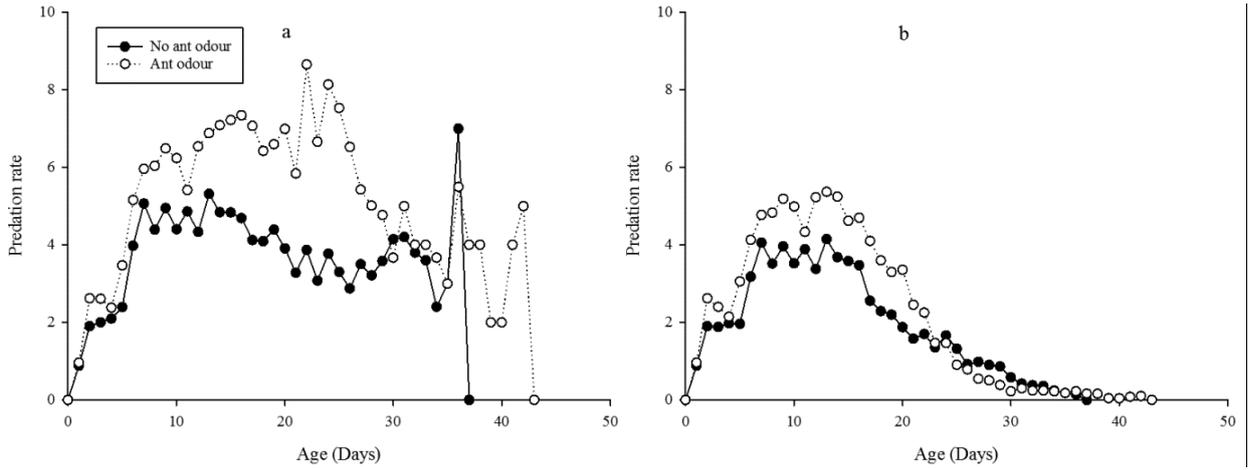


Figure 7.6. Age-specific predation rate (k_x , a) and age-specific net predation rate (q_x , b) of *A. limonicus* fed on *B. cockerelli* first instar nymph without or with ant odour.

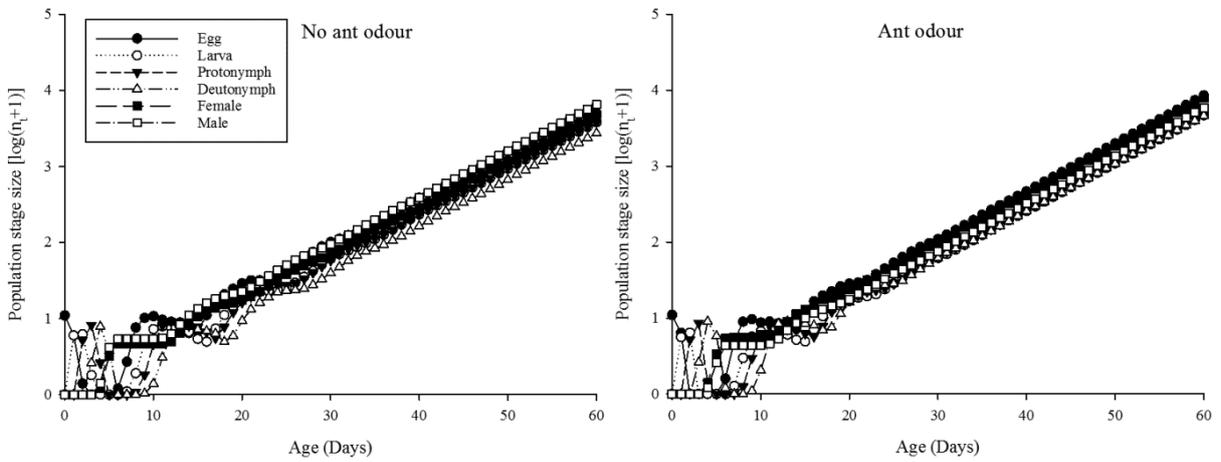


Figure 7.7. Computer simulation of population growth of *A. limonicus* fed on *B. cockerelli* first instar nymph without or with ant odour.

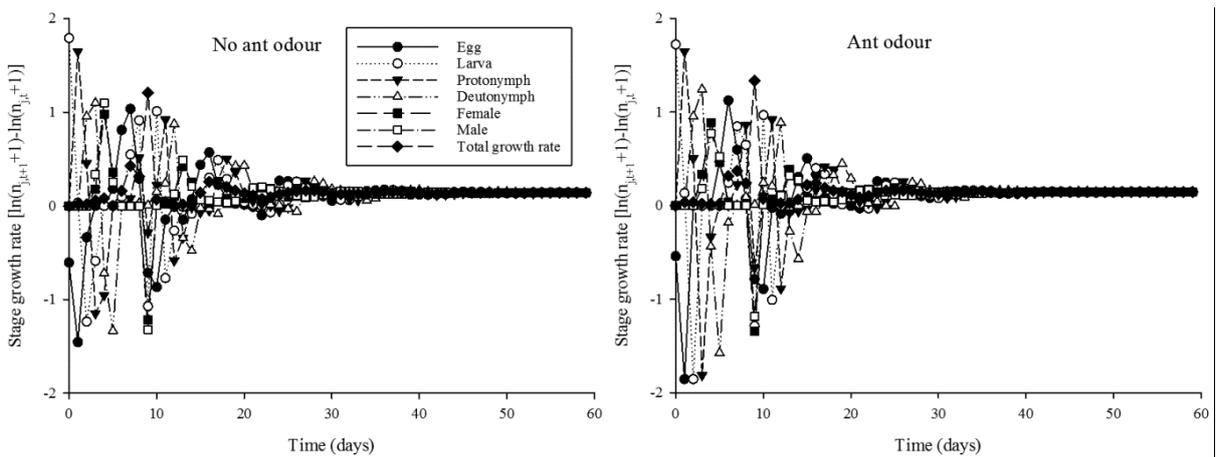


Figure 7.8. Computer simulation of stage growth rate of *A. limonicus* fed on *B. cockerelli* first instar nymph without or with ant odour.

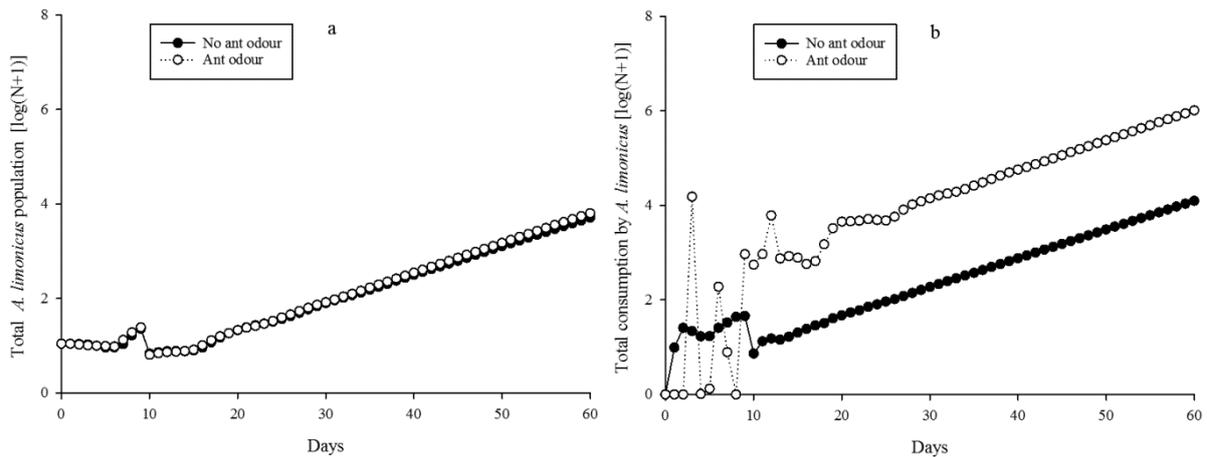


Figure 7.9. Computer simulation of a) total population and b) consumption of *A. limonicus* with or without ant odour.

Although ant odour did not influence the mean number of TPP by an average individual predatory mite during its entire lifespan (C_0), ant odour significantly increased the prey consumption rates of both immature and female *A. limonicus*, but not in males (Table 7.3). The results indicate that there was significant sexual dimorphism in predation rates in the presence of ant odour.

The age-stage and age-specific consumption rates (c_{xj} , k_x) of females in all ages with ant odour were higher than those for females without ant odour (Figures 7.5b and 7.6a). The highest age-stage predation rate of TPP consumed by females with or without ant odour was 14 and 8 prey per day at the ages of 25 and 7 d, respectively, whereas it was 6 and 7 prey per day at the ages of 36 and 36 d for males, respectively (Figure 7.5b). Incorporating the survival rate (l_x), age-specific net predation rate (q_x), and longevity of *A. limonicus* fed on TPP into the net predation rate (C_0), was not significantly different in the presence or absence of ant odour due to the significant sexual dimorphism (Figures 7.2a, 7.6b, Table 7.3). The transformation rate (Q_p) of *A. limonicus* required the similar numbers of first instar nymphs TPP with ant odour or without ant odour (17 TPP and 15 TPP, respectively) to produce a single predator offspring (Table 7.3). The stable predation rate (ψ) with ant odour was significantly higher than those without ant odour (3.73 and 2.84 prey per predator, $P=0.009$). When taken into consideration with the population growth rate and the predation rate, the finite predation rate was significantly higher ($P=0.001$) in the present of ant odour ($\lambda = 3.22$) than in the absence of ant odour ($\lambda = 2.47$).

7.3.6 Population projection

From an initial 10 eggs, the curves of different stage sizes (Figure 7.7) gradually became linear after 20 d with or without ant odour, which meant that the *A. limonicus* population was tending to a stable stage distribution at that time. A similar stable trend can also be observed in the stage growth rate curves (Figure 7.8) where all immatures and adults approached the intrinsic rate 0.1442 and 0.1381 by about 35 d with and without ant odour.

The population projection showed that total population size of *A. limonicus* at 60 days with ant odour was predicted to exceed 6371 individuals, which was higher than the predicted population size without ant odour (5174 individuals) (Figure 7.9a). Ant odour could increase the total consumption potential of *A. limonicus* faster than those without ant odour (Figure 7.9b). Total consumption increased from 13 individuals at 13d to 12,534 individuals at 60d without ant odour, but increased from 659 individuals at 17d to 1,025,723 individuals at 60d which demonstrated that *A. limonicus* would be more effective against *B. cockerelli* with ant odour (Figure 7.9).

7.4 Discussion

We found that ant odour did not affect the overall development and reproduction of *A. limonicus*, providing no support for our hypothesis that the odour of a mutualist ant influences the development and reproduction of a predatory mite when feeding on TPP first instar nymph. However, the presence of ant odour did increase the predation rates of *A. limonicus* females on TPP first instar nymph, based on the life table and predation rate. The presence of ant odour also induced a sexually dimorphic response in the longevity of mites, with males *A. limonicus* surviving longer than females in the absence of ant odour, while there was no difference in the presence of ant odour.

In a previous study significant reduction in the longevity of prey was sexually dimorphic when exposed to cues from predator (Sitvarin *et al.* 2015). When spider *Pardosa milvina* (Hentz 1844) was exposed to predators cues from larger wolf spider, *Tigrosa helluo* (Walckenaer 1837), *P. milvina* females had significantly shorter lifespans than those spiders without predator cues (Sitvarin *et al.* 2015). However, in our study a sexually dimorphic response was only observed in the longevity of male *A. limonicus* exposed to ant odour,

despite a lack of impact on the development and survival rates. Although previous studies show that predator cues can decrease or increase prey development and growth rate (Eckarsky *et al.* 2002; Janssens & Stoks 2013a; b; Roux *et al.* 2015; Xiong *et al.* 2015), we did not find evidence in our research. Ant odour did not reduce the consumption rates of *A. limonicus* males which meant that energy intake by males was not likely the cause for the reduction in longevity (Sitvarin *et al.* 2015). The precise mechanism of sexual dimorphism underlying differences in longevity through exposure to predator cues is still unknown. Stress response to the presence of predators has been commonly known to affect diverse taxa in a variety of ways, such as increasing immune response (Hawlena & Schmitz 2010; Slos & Stoks 2008). The costs of an up-regulated immune response to predator cues may alter the behavioural and physiological plasticity of predatory mites, which may lead to energetic consumption and physiological damage that decreases male longevity.

Chemical cues or byproducts from predators could induce prey plasticity in food utilization efficiency (Thaler *et al.* 2012). A significant increase in predation rates of *A. limonicus* females on *B. cockerelli* first instar nymph was found when the ant odour was present. Similarly, Bucher *et al.* (2014) also found the presence of nursery web spider cues (*Pisaura mirabilis*) increases the consumption of wood crickets (*Nemobius sylvestris*) on woodland strawberry plants *Fragaria vesca* L. It is interesting that under ant odour predation rates of *A. limonicus* increased differentially with sexual dimorphism. This differs from other studies showing reduced consumption rates of preys when preys were under predation risk (Hermann & Landis 2017). According to the predation risk allocation hypothesis (Thaler *et al.* 2012), an animal is expected to reduce their feeding behaviour and allocate more antipredator effort in high-risk situations. For example, Colorado potato beetle *L. decemlineata* larvae significantly reduced feeding by 24% on *S. tuberosum* leaves with cues isolated from stink bug predator *P. maculiventris* (Hermann & Thaler 2014). Similarly, wolf spider *P. milvina* consuming significantly fewer fruit fly (*Drosophila melanogaster*) under the predation risk of the larger wolf spider, *Hogna helluo* (Walckenaer 1837) (Persons *et al.* 2001). In our experiment ant odour only caused an increase in predation rates of *A. limonicus* female on TPP. There are several possible explanations for increasing feeding. In an acarine system, cues from *Phytoseiulus persimilis* Athias-Henriot on leaf discs caused a significant increase in plant damage by female *Tetranychus urticae* Koch; this may be caused by the higher energetic demand of increased walking activity of mites (Škaloudová *et al.* 2007). In nature, female-

biased predation risk shows that large jumping spider (*Jacksonoides queenslandicus*) females are more likely exposed to predators (primitive jumping spider, *Portia fimbriata* (Doleschall)) than small males (Su & Li 2006). The length and width of the idiosoma of *A. limonicus* female (270 and 158um) were larger than *A. limonicus* male (231 and 158um) (Ma *et al.* 2018). Thus, larger females exposed to ant odour may be stimulated to increase their activity level to escape or avoid the higher predation risk, leading to increased energy demand, and thus to increasing consumption.

Predator cues normally influence the oviposition behaviour of mites, such as delayed or reduced oviposition (Dias *et al.* 2016). The spider mite *T. evansi* females produced fewer eggs on leaf disc in the presence of dangerous predatory mite (*P. macropilis*) cues than on clean disc (Dias *et al.* 2016). However, in our study ant odour did not affect the fecundity of *A. limonicus*. The potential explanation is that constant exposure to ant odour changes the other antipredator responses of individuals to odour, such as life expectancy and age-stage predation rates (Bucher *et al.* 2014), in this case causing the female mites to increase their predation rates in response to ant odour. Further experiments should be done to clarify whether the increased predation rate was caused by the increasing activity, or by altered sensitivity to ant odour.

Ant tending might incur metabolic costs to hemipterans due to the different quantity and quality of honeydew sugar produced by the hemipteran in the presence of ants (Fischer & Shingleton 2001). These costs might further influence the fitness of hemipterans, especially in the nutritional value for predators. In this study, *A. limonicus* females might have to consume more psyllids with ant odour (17 TPP) to lay an egg than without ant odour (15 TPP). Therefore, we need to further study tri-partite interactions among ant odour, psyllids and natural enemy.

The present study showed that ant odour had no effects on the population dynamic of *A. limonicus* on TPP. The development of *A. limonicus* on TPP in this study was shorter than values reported by in the Chapter 2 on *T. orientalis* pollen and for *Ephestia kuehniella* Zeller eggs, and Vangansbeke *et al.* (2014a) on *Frankliniella occidentalis* (Pergande), *Trialeurodes vaporariorum* (Westwood), *T. urticae*, and *T. angustifolia* pollen. Moreover, the longevity of adults was longer than those mites on *T. orientalis* pollen and *E. kuehniella* eggs (Chapter 2).

This variation of development and reproduction of *A. limonicus* may be related to genetic differences, the quality of different diets, and/or different rearing techniques (Chapter 2).

Based on the basic data (s_{xj} and f_{xj}) of age-stage, two-sex life table, population projection provided a comprehensive understanding of the dynamics of stage structure and predation fluctuations of *A. limonicus* related to the age-stage structure without or with ant odour during its growth. The total population size of *A. limonicus* without or with ant odour was similar but the total consumption of *B. cockerelli* predated by *A. limonicus* with ant odour was higher than those without ant odour after 10d. Predicting the population size, stage structure, and predation rates of *A. limonicus* without or with ant odour is critical to understand tri-partite interaction involving a pest, its predator and a mutualist of the pest in order to achieve maximum TPP control efficiency⁶⁷.

In conclusion, this research indicates the potential importance of ant odour in the non-consumptive effects on predator-prey systems. Previous research found that hemipteran-tending ants could decrease the control efficiency of natural enemies on preys (Navarrete *et al.* 2013; Novak 1994), but this is the first report to indicate that natural enemies may increase their consumption rates when exposed to ant odour. This result may provide a novel method to improve biocontrol potential by combining of natural enemy and ant odour. Thus, we hope this study stimulates future research on this topic to analyse the components of ant odour and select effective components for combining with natural enemies to control TPP in the greenhouse.

Chapter 8

General discussion

Tomato/potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an invasive pest of solanaceous crops which was first detected in New Zealand in May 2006. Although long-term use of chemical pesticides has resulted in insecticidal resistance, no alternative control strategies are available against TPP. In this study, I sought to discover new ways to control TPP, focussing on understanding how predatory mites (potential biocontrol agents) respond to diet, habitat dispersion, entomopathogenic fungi and the invasive pest's mutualistic ant odour, and evaluate the combined use of the predatory mites with entomopathogenic fungi or with pollen provided as a supplementary food for TPP. My findings will fill some knowledge gaps regarding the development of commercially available biological control agents in New Zealand. The simultaneous application of two agents provide a useful foundation for developing an IPM program for the control of TPP in particular and invasive alien pests in general and reduce unnecessary insecticide application,

8.1 Effect of diets and habitat dispersion on the performance of *A. limonicus*

T. orientalis pollen could be an important food source in solving the problem of mass-rearing of predatory mites in New Zealand. *A. limonicus* had faster developmental time and higher survival rate and fecundity on *T. orientalis* pollen than on punctured *Ephestia kuehniella* eggs and artificial diet (Chapter 2). The developmental time of *A. limonicus* on *T. orientalis* pollen was similar with those mites reared on *T. latifolia* pollen (Nguyen *et al.* 2015; Samaras *et al.* 2015) and *T. angustifolia* pollen (Vangansbeke *et al.* 2014a). It developed faster on *T. orientalis* pollen than those mites fed on *T. latifolia* pollen (Vangansbeke *et al.* 2014b). Besides, the oviposition rate of *A. limonicus* on *T. orientalis* pollen was higher than those observed on other pollens, including *C. annuum* pollen (Van Houten 1996) and *T. latifolia* pollen (Nguyen *et al.* 2015; Vangansbeke *et al.* 2014b). *T. orientalis* pollen also resulted in higher total number of *A. limonicus* in 7 days than *E. kuehniella* eggs (Chapter 3). Moreover, *T. orientalis* pollen had maintained *A. limonicus* population dynamics stable for more than 3 years in my laboratory culture. Therefore, my finding could achieve the mass rearing of *A. limonicus* and provide an effective natural enemy for augmentative biological control purposes against TPP in New Zealand.

Only two % of *A. limonicus* could develop to adulthood under 50% ambient RH when fed on Mediterranean flour moth *E. kuehniella* eggs with no-puncture leaf disc. The punctured *E.*

kuehniella eggs could support the development of *A. limonicus* but could not support its reproduction (Chapter 2). However, *E. kuehniella* eggs showed high nutritional value in protein and amino acid and could satisfy the nutritional needs for the population growth of phytoseiid mites (Delisle *et al.* 2015; Hassan *et al.* 2017; Kishimoto 2015; Momen & El-Laithy 2007; Navarro-Campos *et al.* 2016; Nguyen *et al.* 2014; Vangansbeke *et al.* 2014b; Vantornhout *et al.* 2004; Xie *et al.* 2018). When provided with water or higher ambient relative humidity (90%), *A. limonicus* had a high survival rate and short development time on *E. kuehniella* eggs (Chapter 2). A similar result was found in Chapter 3 that beginning with one *A. limonicus* female on *E. kuehniella* eggs with water produced a high total number of *A. limonicus* (10.87). Besides, McMurtry & Scriven (1965) found that *A. limonicus* required a higher humidity for hatching because none of eggs hatched at 50% RH or lower (McMurtry & Scriven 1965). Therefore, *A. limonicus* may be sensitive to low relative humidity or lack of water, indicating that *E. kuehniella* eggs may be a limited food source for the mass rearing of *A. limonicus* in New Zealand.

Predatory mite *A. limonicus* was a more effective biocontrol agent than *N. cucumeris*. The application of *A. limonicus* significantly reduced total, eggs and nymphs TPP than *N. cucumeris* in bell pepper in the greenhouse after seven weeks (Chapter 6). Similar results are reported by Patel and Zhang (2017a, b) in the laboratory conditions that *A. limonicus* has a stronger functional response (higher attack rate and lower handling time) and higher reproductive rate on TPP eggs and first instar nymphs than *N. cucumeris*. So *A. limonicus* can be a more effective biological control agent of TPP on bell pepper in New Zealand. The biological control potential of *A. limonicus* may play a limited role in crop yields because TPP not only caused direct feeding damage through their nymphs and adults, but also indirectly vectored the bacterium *Candidatus Liberibacter solanacearum* to important crops, such as potato, tomato, bell pepper, eggplant and tamarillo (Liefting *et al.* 2008, 2009). *A. limonicus* mainly suppresses TPP eggs and first instar nymphs and is not able to prevent the feeding damage and plant disease transmission by the later stages and adults of TPP (Davidson *et al.* 2016; Xu & Zhang 2015). Therefore, it was critical to establish and maintain higher levels of predatory mites to suppress TPP population.

The provision of *T. orientalis* pollen enhanced the establishment and conservation of *A. limonicus* and promoted TPP control in bell pepper. Provisioning alternative food for

predatory mites can improve biological control of plant pests through reducing the strength of competitive interactions, such as cannibalism, intraguild predation (IGP), and hyperpredation, in arthropod food webs in times of prey scarcity (Calabuig *et al.* 2018; Duarte *et al.* 2015; Ghasemzadeh *et al.* 2017; Kumar *et al.* 2015; Nomikou *et al.* 2010; Onzo *et al.* 2005; Pozzebon *et al.* 2015; Van Rijn & Sabelis 1990; Shakya *et al.* 2009; Vangansbeke *et al.* 2016). I found that there were no potential risks of *T. orientalis* pollen on the predation potential of *A. limonicus* in greenhouse experiment (Chapter 6). Due to the presence of alternative food, predatory mites may be distracted from consuming prey and finally decreased control efficiency. *A. limonicus* supplemented with *T. orientalis* pollen significantly maintained higher mobiles and mite eggs of predatory mites and reduced the mean number of total, eggs, and nymphs TPP on plants over 7 weeks (Chapter 6). Moreover, adding pollen to *A. limonicus* populations harvested significantly higher number of bell pepper fruits compared with the control (Chapter 6). This high yield confirmed the significant control efficiency of TPP by the combination of predators supplemented with pollen. Therefore, the addition of *T. orientalis* pollen to crop was a viable strategy to result in higher predatory mite densities, increase biocontrol of TPP and final raise crop yield. *T. orientalis* pollen is an excellent alternative diet for the mass production or a supplemental food source to sustain *A. limonicus* population. Based on the tested result, *T. orientalis* pollen might be registered as a commercial pollen product for predatory mites.

8.2 Effects of entomopathogenic fungi on *B. cockerelli* and *A. limonicus*

Entomopathogenic fungi (EPF) are the predominant pathogens of Hemiptera (Lacey *et al.* 2009). Some previous studies showed that fungal entomopathogens have high potential for the reduction of TPP in the laboratory, greenhouse and field conditions (Ai-Jabr 1999; Lacey *et al.* 2009, 2011; Mauchline & Stannard 2013; Pérez-González & Sánchez-Peña 2017; Rios-velasco *et al.* 2014; Sánchez-Peña *et al.* 2007a; Tamayo-Mejía *et al.* 2014, 2015; Villegas-Rodríguez *et al.* 2014, 2017). I also found a similar result, that all native fungal strains caused high mortality levels to TPP first instar nymphs and adults (Chapter 4). Sublethal concentrations of the most virulent strain, *B. bassiana* ICMP 8701, significantly reduced the longevity, fecundity, and oviposition periods of the parental and offspring generations of TPP (Chapter 4). I found that *A. limonicus* females were susceptible to *B. bassiana* and sublethal concentration of *B. bassiana* significantly reduced the reproduction parameters and predation

rates of parental *A. limonicus* generation, but it did not affect the life table parameters and predation potential of offspring generation from females of *A. limonicus* exposed to sublethal concentrations (Chapter 5). Moreover, *B. bassiana* suspension significantly reduced total number of TPP in the bell pepper over 7 weeks in the greenhouse (Chapter 6). Based on the results of my study, native *B. bassiana* ICMP 8701 can be used as an excellent candidate in the control of TPP, thereby reducing the use of chemical pesticides in the season. So local strains of *B. bassiana* ICMP 8701 has the potential to develop as a biopesticide against TPP in New Zealand.

Previous greenhouse studies showed that the simultaneous applications of two agents did not enhance the control efficiency on prey than the single application, although two agents are compatible (Automated *et al.* 2018; Jacobson *et al.* 2001; Midthassel *et al.* 2016; Numa Vergel *et al.* 2011). *N. barkeri* exhibited feeding behavior on EPF conidia, and the majority of conidia had been dissolved and lost their viability within 24h post ingestion (Wu *et al.* 2016b). There were no potential negative effects of *B. bassiana* suspensions on predatory mites. EPF suspensions did not significantly reduce *A. limonicus* densities and the combined use of *A. limonicus* with *B. bassiana* suspensions could significantly reduce total eggs, nymphs, and adults TPP but increase plant and fruit growth (Chapter 6). Similar results were found that the combined releases of predatory mites and EPF could significantly control *Mononychellus tanajoa* and *T. urticae* populations compared with the single application of agents (Chandler *et al.* 2005; Onzo *et al.* 2013). Only when *N. barkeri* was released two weeks on greenhouse cucumber at appropriate intervals after spraying *B. bassiana*, there was a better control efficiency on the thrips *F. occidentalis* density than the single application of *B. bassiana* (Wu *et al.* 2016a, 2017). Therefore, the combined use of EPF and *A. limonicus* could provide a more effective IPM strategy for controlling TPP in bell pepper in New Zealand.

8.3 How hemipteran-tending ant odour influenced the performance of *A. limonicus*

Predator-prey interactions contained direct consumption of prey by predators and trait-mediated effects by predation risk (Bucher *et al.* 2015; Hermann & Landis 2017; Hermann & Thaler 2014; Roux *et al.* 2015). There was a predator-prey interaction between the mutualistic ant *Technomyrmex albipes* and *A. limonicus*, because ants could negatively

influence the predation of *A. limonicus* on TPP. Non-consumptive effects of the predators through semiochemicals could induce prey plasticity in their life table parameters and feeding behaviour (Sitvarin *et al.* 2015; Thaler *et al.* 2012). For example, the colorado potato beetle (*Leptinotarsa decemlineata*) in the presence of stink bug predator (*Podisus maculiventris*) cues could significantly reduce the feeding behavior on potato foliage compared to control without predator cues (Hermann & Thaler 2014). Mutualistic ants protected hemipterans (e.g. aphids, psyllids) by reducing the survival rate, distribution and control efficiency of natural enemies on hemipterans (Alene *et al.* 2011; Herbert & Horn 2008; Navarrete *et al.* 2013; Novak 1994; Oliver *et al.* 2008; Styrsky & Eubanks 2007). Predator cues from the predatory mite (*P. macropilis*) significantly reduced the fecundity of *T. evansi* on leaf discs (Dias *et al.* 2016). In my study, the presence of a mutualistic ant odour did not significantly influence the developmental time, survival rate and fecundity of *A. limonicus* (Chapter 7). This result indicated that ant odour might not affect the performance of *A. limonicus* on crops.

The presence of ant odour could induce *A. limonicus* females consuming more TPP but not in males: i.e. sexual dimorphism in predation rates (Chapter 7). A similar result was found that the presence of nursery web spider *Pisaura mirabilis* cues improved the consumption of wood crickets (*Nemobius sylvestris*) on woodland strawberry plants *Fragaria vesca* L. (Bucher *et al.* 2014). *T. urticae* females also improved their damage on plants in the presence of *Phytoseiulus persimilis* cues (Škaloudová *et al.* 2007). Compared with small male, a high predation risk may stimulate the walking activity of large females, and further increase their energy consumption. My finding may provide a novel method to improve biocontrol potential by combining of natural enemy and ant odour.

8.4 Future research priorities

8.4.1 Effects of diet and habitat dispersion on predatory mites

A. limonicus is a type-III generalist predatory mite, implying that it can feed on alternative foods, including, pollens of various plant species, facultious prey, honeydew and artificial diets (Goleva *et al.* 2015; Leman & Messelink 2015; McMurtry & Croft 1997; McMurtry & Scriven 1965; Vangansbeke *et al.* 2014a; b; Xu & Zhang 2015). The storage mite *Carpoglyphus lactis* is used as a primary food source for mass-producing *A. limonicus* in Europe in January 2012 (Knapp *et al.* 2013). However, using storage mites in commercial

production might cause allergy problems to production workers in the factory and farmers in the fields. The introduction of *A. limonicus* from Europe might be difficult because there is no mass production in other countries, so there may be production issues that needed to be resolved for this species. In order to protect New Zealand biosecurity, importation of biological material needs to be registered before the application in New Zealand, which can be both time consuming and expensive. The mass rearing food source of storage mite *C. lactis* is also a patent-protected material applied by Koppert B.V (Bolckmans *et al.* 2017). Based on my results, *T. orientalis* pollen is an alternative diet that could be used for the mass rearing of *A. limonicus* in New Zealand. Moreover, *T. orientalis* plants mainly grow in wetlands, and it is very easy to collect huge quantities of pollen before pollen is shed. After collecting male flowers, it is important that the flowers should be dried in an oven at 35 °C as soon as possible (Kar *et al.* 2015). *T. orientalis* pollen can maintain *A. limonicus* populations in small-scale for the requirement of laboratory condition. *A. limonicus* preferred to survive in a higher humidity environment, but under higher humidity pollen becomes mouldy easily. Further investigation is required to find out the appropriate humidity for the production of *A. limonicus* under factory conditions. Agricultural communities in the greenhouse or field are usually more complex with multiple pests than my greenhouse cage trial with a single pest. It is warranted to investigate whether the combination method by applying *T. orientalis* pollen on cotton fiber exclusively supports predatory mite populations and not those of other pests, such as pollen-feeder *F. occidentalis*.

8.4.2 The interactions among *B. cockerelli*, *B. bassiana* and *A. limonicus*

There are few biological control agents that can be applied in Solanaceae crop plants in New Zealand (Walker *et al.* 2011). To identify more biological control agents, more investigations associated with TPP in different Solanaceae crop and non-crop host plants are needed to select potential native natural enemies and determine their impacts on TPP (Castillo Carrillo *et al.* 2016). The control potential of predatory mites could be affected by high trichome density of host plants (Buitenhuis *et al.* 2014; Van Haren *et al.* 1987; Kean *et al.* 2019; Kütük *et al.* 2016). The release strategies for predatory mites need to be further investigated based on the different host plant species targeted. My greenhouse experiment only tested the ability of predatory mites to enter low-density TPP (eggs and adults) colonies on bell pepper, but did indicate that the mites could be an effective control agent under these conditions. Understanding the potential of predatory mites to control at high TPP densities will have

serious implications in IPM. I suggest that additional work is carried out to test the control efficiency of predatory mites at high TPP densities in the field. To better understand the interactions between *B. bassiana* and *A. limonicus*, further behavioral and physiological responses on their compatibilities are needed for the management of TPP. In addition, the pathogen Lso significantly reduced the survival rates, developmental time, fecundity and longevity of TPP but not on their population parameters (Thinakaran *et al.* 2015). As my experimental materials are all free of Lso, further investigations are needed to test whether Lso will influence the performance of predatory mites on Lso-infected TPP, the infection rates of EPF and the combined effects of two agents on TPP. In the greenhouse, conditions are relatively stable in terms of temperature and humidity. Future investigations are also required to assess the control efficiency of simultaneous application of two agents for the management of TPP and ZC symptom in the fields.

8.4.3 Non-consumptive effects of ant odour on *A. limonicus*

Chapter 7 gave new insights into understanding tri-partite interaction involving a pest, its predator and a mutualist of the pest. To comprehensively understand this tripartite complexity, more behavioral responses of predatory mites on TPP to the non-consumptive effects of mutualist ants should be studied in the laboratory, fields and landscape levels (Hermann & Landis 2017). Considering the increased consumption of *A. limonicus*, there is the potential to utilize ant odour together with predatory mites for the control of TPP. A full analysis of ant odour and functional response of effective components should be evaluated on predatory mites. More research on the mechanisms that increase the consumption of predatory mites in the presence of ant odour is needed before the simultaneous application of predatory mite and ant odour in the greenhouse.

8.5 Concluding remarks

My study evaluated the effects of entomopathogenic fungi, diets, habitat dispersion and odour from ants on the performance of *A. limonicus*, and their interactions with TPP. Compared with *E. kuehniella* eggs and artificial diet, *T. orientalis* pollen was a better alternative diet in the mass-rearing of *A. limonicus* and could serve as a supplemental food to maintain predatory mite populations in host plants in New Zealand. Dispersed patches of cotton threads provided a spatial refuge for *A. limonicus* to significantly enhance their population density than general and aggregated patches. The most virulent strain screened from 16 NZ

native EPF, *Beauveria bassiana* ICMP 8701, could significantly reduce fecundity and other life table parameters of parental and offspring TPP generations. Also, *A. limonicus* females were susceptible to *B. bassiana* and sublethal concentration of EPF significantly reduced reproduction parameters and predation of parental *A. limonicus* generation which produced normal offspring. In the greenhouse experiment, provisioning the predatory mite *A. limonicus* either with entomopathogenic fungi or *T. orientalis* pollen could cause significant reductions in total TPP density among the treatments, and application of *B. bassiana* suspensions did not affect *A. limonicus* densities over 7 weeks. Mutualist ant odour did not affect life table parameters of *A. limonicus*, but significantly enhanced the control potential of *A. limonicus* females on TPP. My findings will fill some knowledge gaps regarding the development of commercially available biological control agents in New Zealand. *T. orientalis* pollen and local strains of *B. bassiana* may have potential to register as commercial products for the control of TPP in New Zealand. The simultaneous application of two agents provides a useful foundation for developing an IPM program for the control of TPP in particular and invasive alien pests in general. *B. bassiana* ICMP 8701 is compatible with *A. limonicus*. The combined application of two agents could overcome the shortcoming of individual agent and make crop protection more sustainable. Ant odour may be developed as supplementary product for increasing the control efficiency of predators.

Appendix A

Provisioning predatory mites with entomopathogenic fungi or pollen improves biological control of a greenhouse psyllid pest

Table A1. The effects of predatory mite species, *B. bassiana* suspension, *T. orientalis* pollen, and *B. bassiana* dry conidia on the mean total, eggs, nymphs, adults numbers (\pm SE) of *B. cockerelli* and mean mobile mites and mite eggs (\pm SE) of predatory mites per bell pepper leaf were analysed using Friedman's test over a period of 7 weeks in the greenhouse.

Treatments	Total psyllids			TPP eggs			TPP nymphs			TPP adults			Mobile mites			Mite eggs		
	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
C VS B	42	1	<0.001	34.38	1	<0.001	38.1	1	<0.001	39	1	<0.001	-	-	-	-	-	-
C VS A	42	1	<0.001	21.43	1	<0.001	38.1	1	<0.001	21.78	1	<0.001	-	-	-	-	-	-
C VS N	8.26	1	0.004	4.24	1	0.04	6.43	1	0.011	0.47	1	0.493	-	-	-	-	-	-
C VS AP	42	1	<0.001	42	1	<0.001	42	1	<0.001	20.51	1	<0.001	-	-	-	-	-	-
C VS NP	34.38	1	<0.001	12.9	1	<0.001	23.44	1	<0.001	6.4	1	0.011	-	-	-	-	-	-
C VS AB	42	1	<0.001	42	1	<0.001	42	1	<0.001	39	1	<0.001	-	-	-	-	-	-
C VS NB	42	1	<0.001	34.38	1	<0.001	34.38	1	<0.001	31.41	1	<0.001	-	-	-	-	-	-
C VS ABP	42	1	<0.001	42	1	<0.001	42	1	<0.001	17.79	1	<0.001	-	-	-	-	-	-
C VS NBP	21.43	1	<0.001	18.67	1	<0.001	18.67	1	<0.001	12.9	1	<0.001	-	-	-	-	-	-
B VS A	0.381	1	0.537	12.9	1	<0.001	4.12	1	0.042	22.73	1	<0.001	-	-	-	-	-	-
B VS N	17.86	1	<0.001	8.76	1	0.003	6.43	1	0.011	31.11	1	<0.001	-	-	-	-	-	-
B VS AP	13.71	1	<0.001	1	1	0.317	16.9	1	<0.001	14.4	1	<0.001	-	-	-	-	-	-
B VS NP	17.78	1	<0.001	24.38	1	<0.001	5.49	1	0.019	30.86	1	<0.001	-	-	-	-	-	-
B VS AB	34.38	1	<0.001	16.13	1	<0.001	38	1	<0.001	9.78	1	0.002	-	-	-	-	-	-
B VS NB	4.667	1	0.031	0.273	1	0.602	4.9	1	0.027	0.53	1	0.465	-	-	-	-	-	-
B VS ABP	16.1	1	<0.001	2.94	1	0.086	11.52	1	0.001	3.67	1	0.056	-	-	-	-	-	-
B VS NBP	24.38	1	<0.001	33.39	1	<0.001	16.1	1	<0.001	36.1	1	<0.001	-	-	-	-	-	-
A VS N	17.86	1	<0.001	2.31	1	0.128	12.6	1	<0.001	13.36	1	<0.001	17	1	<0.001	2.13	1	0.144
A VS AP	24.38	1	<0.001	23.44	1	<0.001	16.1	1	<0.001	1.6	1	0.206	34	1	<0.001	11.77	1	0.001
A VS NP	27.52	1	<0.001	5.49	1	0.019	24.38	1	<0.001	17.78	1	<0.001	5	1	0.027	7.11	1	0.008
A VS AB	42	1	<0.001	41	1	<0.001	37.1	1	<0.001	37	1	<0.001	4	1	0.41	0.947	1	0.33
A VS NB	0	1	1	8.805	1	0.003	0.024	1	0.876	9.757	1	0.002	24	1	<0.001	3.571	1	0.06
A VS ABP	24.38	1	<0.001	26.56	1	<0.001	7.71	1	0.005	11.31	1	0.001	13	1	<0.001	10.31	1	0.001
A VS NBP	11.52	1	0.001	0.381	1	0.537	18.67	1	<0.001	0.86	1	0.36	12	1	0.001	0.034	1	0.853
N VS AP	27.46	1	<0.001	12.6	1	<0.001	23.06	1	<0.001	18	1	<0.001	35	1	<0.001	3.12	1	0.077
N VS NP	2.31	1	0.128	0.714	1	0.398	3.457	1	0.063	2.941	1	0.086	9	1	0.003	3	1	0.083
N VS AB	31.11	1	<0.001	22.09	1	<0.001	32	1	<0.001	35	1	<0.001	13	1	<0.001	6.545	1	0.01
N VS NB	20.83	1	<0.001	16.03	1	<0.001	24.5	1	<0.001	27.46	1	<0.001	0	1	0.715	0.667	1	0.41
N VS ABP	24.03	1	<0.001	15.11	1	<0.001	19.88	1	<0.001	24.03	1	<0.001	31	1	<0.001	2.13	1	0.144
N VS NBP	0.03	1	0.866	0.26	1	0.612	0.26	1	0.612	8.76	1	0.003	2	1	0.144	0.17	1	0.683
AP VS NP	37.1	1	<0.001	27.52	1	<0.001	38.1	1	<0.001	19.88	1	<0.001	34	1	<0.001	0.273	1	0.602
AP VS AB	37.1	1	<0.001	26.47	1	<0.001	18.62	1	<0.001	37.1	1	<0.001	31	1	<0.001	21.78	1	<0.001
AP VS NB	6.1	1	0.014	0.234	1	0.622	9.76	1	0.002	9.52	1	0.002	37	1	<0.001	2	1	0.157
AP VS ABP	0.381	1	0.537	4.83	1	0.028	4.235	1	0.04	10.53	1	0.001	21	1	<0.001	0.95	1	0.33
AP VS NBP	34.38	1	<0.001	24.38	1	<0.001	42	1	<0.001	2.63	1	0.105	37	1	<0.001	7.53	1	0.006
NP VS AB	38.1	1	<0.001	38.1	1	<0.001	42	1	<0.001	42	1	<0.001	2	1	0.206	14.24	1	<0.001
NP VS NB	24.38	1	<0.001	30.86	1	<0.001	21.43	1	<0.001	42	1	<0.001	6	1	0.011	0.257	1	0.612
NP VS ABP	30.86	1	<0.001	34.38	1	<0.001	33.39	1	<0.001	26.95	1	<0.001	33	1	<0.001	0.47	1	0.493
NP VS NBP	0.1	1	0.758	1.976	1	0.16	0.095	1	0.758	5.16	1	0.023	1	1	0.398	5.143	1	0.023
AB VS NB	37.1	1	<0.001	22.09	1	<0.001	34	1	<0.001	12.45	1	<0.001	23	1	<0.001	8.33	1	0.004
AB VS ABP	31.41	1	<0.001	17.07	1	<0.001	25.49	1	<0.001	25.49	1	<0.001	28	1	<0.001	24.03	1	<0.001
AB VS NBP	42	1	<0.001	41	1	<0.001	42	1	<0.001	36.1	1	<0.001	5	1	0.023	4.84	1	0.028
NB VS ABP	11.52	1	0.001	0.95	1	0.33	5.49	1	0.019	0.421	1	0.516	38	1	<0.001	3.27	1	0.071
AB VS NBP	21.43	1	<0.001	15.24	1	<0.001	30.86	1	<0.001	21.56	1	<0.001	4	1	0.046	1.69	1	0.19
ABP VS NBP	24.38	1	<0.001	21.43	1	<0.001	30.86	1	<0.001	19.7	1	<0.001	35	1	<0.001	3.13	1	0.077

"-" indicated that there were no effects on predatory mite density because no predatory mites were released in those treatments. C—Control, B—*B. bassiana* suspension, A—*A. limonicus*, N—*N. cucumeris*, AP—*A. limonicus*+*T. orientalis* pollen, NP—*N. cucumeris*+*T. orientalis* pollen, AB—*A. limonicus*+*B. bassiana* suspension, NB—*N. cucumeris*+*B. bassiana* suspension, ABP—*A. limonicus*+*T. orientalis* pollen+*B. bassiana* dry conidia, NBP—*N. cucumeris*+*T. orientalis* pollen+*B. bassiana* dry conidia.

Table A2. Effects of different mite treatments on bell pepper growth parameters (Means \pm SE) as assessed after 7 weeks under greenhouse conditions.

Treatments	Leaf	Root	Stem	Fruit	
	Area(m ²)	Area (m ²)	Stem diameter (cm)	Height (cm)	Diameter (cm)
C	0.2269 \pm 0.0360c	0.1267 \pm 0.0388a	9.47 \pm 0.46c	21.06 \pm 13.42a	21.11 \pm 13.45a
B	0.4903 \pm 0.0294ab	0.2038 \pm 0.0282a	10.95 \pm 0.38abc	46.79 \pm 9.50a	45.80 \pm 9.28a
A	0.4530 \pm 0.0616ab	0.2263 \pm 0.0366a	10.43 \pm 0.52abc	55.82 \pm 11.60a	53.05 \pm 10.64a
N	0.3062 \pm 0.0131bc	0.1430 \pm 0.0283a	10.36 \pm 0.16abc	48.83 \pm 12.27a	49.56 \pm 12.46a
AP	0.5487 \pm 0.0597a	0.2337 \pm 0.0307a	10.95 \pm 0.40abc	58.99 \pm 2.26a	69.18 \pm 2.30a
NP	0.3111 \pm 0.0292bc	0.1454 \pm 0.0327a	10.62 \pm 0.16abc	37.89 \pm 12.01a	40.50 \pm 12.92a
AB	0.5965 \pm 0.0694a	0.3037 \pm 0.0576a	11.39 \pm 0.33ab	62.99 \pm 2.32a	61.57 \pm 1.86a
NB	0.5140 \pm 0.0634ab	0.2039 \pm 0.0470a	10.64 \pm 0.38abc	28.07 \pm 12.76a	31.6 \pm 14.30a
ABP	0.5161 \pm 0.0712ab	0.2251 \pm 0.0347a	12.06 \pm 0.37a	59.02 \pm 1.27a	61.25 \pm 2.50a
NBP	0.3930 \pm 0.0342abc	0.2271 \pm 0.0502a	10.03 \pm 0.28bc	29.40 \pm 13.18a	30.00 \pm 13.42a

*Means within a row followed by the same lower-case do not differ significantly according to Tukey's HSD multiple range test at $P > 0.05$. C—Control, B—*B. bassiana* suspension, A—*A. limonicus*, N—*N. cucumeris*, AP—*A. limonicus*+*T. orientalis* pollen, NP—*N. cucumeris*+*T. orientalis* pollen, AB—*A. limonicus*+*B. bassiana* suspension, NB—*N. cucumeris*+*B. bassiana* suspension, ABP—*A. limonicus*+*T. orientalis* pollen+*B. bassiana* dry conidia, NBP—*N. cucumeris*+*T. orientalis* pollen+*B. bassiana* dry conidia.

References

- Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265–267.
- Abou-Awad, B.A., Reda, A.S. & Elsayi, S.A. (1992) Effects of artificial and natural diets on the development and reproduction of two phytoseiid mites *Amblyseius gossipi* and *Amblyseius swirskii* (Acari: Phytoseiidae). *International Journal of Tropical Insect Science*, 13, 441–445.
- Ache, B.W. & Young, J.M. (2005) Olfaction: Diverse species, conserved principles. *Neuron*, 48, 417–430.
- Adar, E., Inbar, M., Gal, S., Gan-Mor, S. & Palevsky, E. (2014) Pollen on-twine for food provisioning and oviposition of predatory mites in protected crops. *BioControl*, 59, 307–317.
- Agboton, B.V., Hanna, R., Onzo, A., Vidal, S. & von Tiedemann, A. (2013) Interactions between the predatory mite *Typhlodromalus aripo* and the entomopathogenic fungus *Neozygites tanajoae* and consequences for the suppression of their shared prey/host *Mononychellus tanajoa*. *Experimental and Applied Acarology*, 60, 205–217.
- Agrawal, A.A. & Karban, R. (1997) Domatia mediate plant-arthropod mutualism. *Nature*, 387, 562–563.
- Akköprü, E.P., Atlıhan, R., Okut, H. & Chi, H. (2015) Demographic assessment of plant cultivar resistance to insect pests: a case study of the dusky-veined walnut aphid (Hemiptera: Callaphididae) on five walnut cultivars. *Journal of Economic Entomology*, 108, 378–387.
- Al-Jabr, A.M. (1999) Integrated pest management of tomato/potato psyllid, *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae) with emphasis on its importance in greenhouse grown tomatoes (Ph.D. thesis). Colorado State University, Fort Collins, USA, 186 pp.
- Alatawi, F.J., Basahih, J. & Kamran, M. (2018) Suitability of date palm pollen as an alternative food source for the predatory mite *Cydnozeius negevi* (Swirski and Amitai) (Acari: Phytoseiidae) at a low relative humidity. *Acarologia*, 58, 357–365.
- Alén, D.C., Djiéto-Lordon, C. & Burckhardt, D. (2011) Unusual behaviour-unusual morphology: Mutualistic relationships between ants (Hymenoptera: Formicidae) and *Diaphorina enderleini* (Hemiptera: Psylloidea), associated with *Vernonia amygdalina* (Asteraceae). *African Invertebrates*, 52, 353–361.

- Ali, O. & Brennan, P. (1997) Development, feeding and reproduction of the predatory mite, *Hypoaspis miles* (Acari: Mesostigmata: Laelapidae) on different types of prey on different types of prey. *Systematic & Applied Acarology*, 2, 81–88.
- Almeyda-León, I.H., Sánchez-Salas, J.A. & Garzón-Tiznado, J.A. (2008) Vectors causal of potato purple top in coahuila and nuevo leon, mexico. *Agricultura Técnica en México*, 34, 141–150.
- Amiri-Jami, A., Sadeghi-Namaghi, H. & Gilbert, F. (2017) Performance of a predatory hoverfly feeding on *Myzus persicae* (Hem. Aphididae) reared on two brassicaceous plants varies with ant attendance. *Biological Control*, 105, 49–55.
- Azandén Hounmalon, G.Y., Maniania, N.K., Niassy, S., Fellous, S., Kreiter, S., Delétré E., Fiaboe, K.K.M. & Martin, T. (2018) Performance of *Metarhizium anisopliae*-treated foam in combination with *Phytoseiulus longipes* Evans against *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae). *Pest Management Science*, 74, 2835–2841.
- Babendreier, D. (2007) Pros and cons of biological control. *Biological Invasions*, 193, 403–418.
- Bakker, F.M., Klein, M.E., Mesa, N.C. & Braun, A.R. (1993) Saturation deficit tolerance spectra of phytophagous mites and their phytoseiid predators on cassava. *Experimental and Applied Acarology*, 17, 97–113.
- Baldini, A. (2008) Efecto de *Cinara cupressi* (Hemiptera: Aphididae) sobre el ciprés de la cordillera (*Austrocedrus chilensis*) después de aplicar control químico. *Ciencia e investigación agraria*, 35, 341–350.
- Barbosa, M.F.C. & de Moraes, G.J. (2015) Evaluation of astigmatid mites as factitious food for rearing four predaceous phytoseiid mites (Acari: Astigmatina; Phytoseiidae). *Biological Control*, 91, 22–26.
- Barbosa, M.F.C. & de Moraes, G.J. (2016) Potential of astigmatid mites (Acari: Astigmatina) as prey for rearing edaphic predatory mites of the families Laelapidae and Rhodacaridae (Acari: Mesostigmata). *Experimental and Applied Acarology*, 69, 289–296.
- Barnes, H. (2017) New biocontrol agent released. *NZGrower*, 72, 18–20.
- Barnes, M.C., Persons, M.H. & Rypstra, A.L. (2002) The effect of predator chemical cue age on antipredator behavior in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). *Journal of Insect Behavior*, 15, 269–281.

- Barratt, B.I.P., Moran, V.C., Bigler, F. & van Lenteren, J.C. (2018) The status of biological control and recommendations for improving uptake for the future. *BioControl*, 63, 155–167.
- De Barro, P.J., Liu, S.-S., Boykin, L.M. & Dinsdale, A.B. (2011) *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology*, 56, 1–19.
- Bartlett, A.J. (1987) Filial cannibalism in burying beetles. *Behavioral Ecology and Sociobiology*, 21, 179–183.
- Bass, C., Denholm, I., Williamson, M.S. & Nauen, R. (2015) The global status of insect resistance to neonicotinoid insecticides. *Pesticide Biochemistry and Physiology*, 121, 78–87.
- Baverstock, J., Alderson, P.G. & Pell, J.K. (2005) Influence of the aphid pathogen *Pandora neoaphidis* on the foraging behaviour of the aphid parasitoid *Aphidius ervi*. *Ecological Entomology*, 30, 665–672.
- Beggs, J.R., Brockerhoff, E.G., Corley, J.C., Kenis, M., Masciocchi, M., Muller, F., Rome, Q. & Villemant, C. (2011) Ecological effects and management of invasive alien Vespidae. *BioControl*, 56, 505–526.
- Berndt, L.A., Withers, T.M. & Gresham, B.A. (2010) Southern ladybird (*Cleobora mettyi*) is now well established in New Zealand. *New Zealand Plant Protection*, 63, 282.
- Binkley, A.M. (1929) Transmission studies with the new psyllid-yellow disease of solanaceous plants. *Science*, 70, 615.
- Biondi, A., Guedes, R.N.C., Wan, F. & Desneux, N. (2018) Ecology, worldwide spread, and management of the invasive south american tomato pinworm, *Tuta absoluta*: Past, present, and future. *Annual Review of Entomology*, 63, 239–258.
- Birch, L.C. (1948) The intrinsic rate of natural increase of an insect population. *The Journal of Animal Ecology*, 17, 15–26.
- Blackman, R.L. & Eastop, V.F. (1994) *Aphids on the world's trees: An identification and information guide*. CAB International, Wallingford, Inglaterra.
- Blommers, L., Lobbes, P., Vink, P. & Wegdam, F. (1977) Studies on the response of *Amblyseius bibens* (Acarina: Phytoseiidae) to conditions of prey scarcity. *Entomophaga*, 22, 247–258.
- Bolckmans, K.J.F., Van Houten, Y.M., Van Baal, A.E., Timmer, R. & Morel, D.M. (2017) Mite composition comprising a predatory mite and immobilized prey contacted with a

- fungus reducing agent and methods and uses related to the use of said composition. Google Patents. Available from: <http://www.freepatentsonline.com/EP2612551.html>.
- Boller, E.F., Vogt, H., Ternes, P. & Malavolta, C. (2005) Working document on selectivity of pesticides. IOBC-WPRS, IOBC database on selectivity of pesticides. Available from https://www.iobc-wprs.org/ip_ipm/archive/03021_IOBC_WorkingDocumentPesticides_Explanations.pdf
- Bonizzoni, M., Gasperi, G., Chen, X. & James, A.A. (2013) The invasive mosquito species *Aedes albopictus*: Current knowledge and future perspectives. *Trends in Parasitology*, 29, 460–468.
- Bové J.M. (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology*, 88, 7–37.
- Bravo, M.E. & López, L.P. (2007) Principales plagas del chile de agua en los valles centrales de Oaxaca. *Agroproduce, Fundación Produce Oaxaca A.C.*, 7, 12–15.
- Broufas, G.D. & Koveos, D.S. (2000) Effect of different pollens on development, survivorship and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae). *Environmental Entomology*, 29, 743–749.
- Bucher, R., Binz, H., Menzel, F. & Entling, M.H. (2014) Spider cues stimulate feeding, weight gain and survival of crickets. *Ecological Entomology*, 39, 667–673.
- Bucher, R., Menzel, F. & Entling, M.H. (2015) Risk of spider predation alters food web structure and reduces local herbivory in the field. *Oecologia*, 178, 571–577.
- Buchman, J.L., Sengoda, V.G. & Munyaneza, J.E. (2011) Vector transmission efficiency of liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in Zebra Chip potato disease: effects of psyllid life stage and inoculation access period. *Journal of Economic Entomology*, 104, 1486–1495.
- Buitenhuis, R., Murphy, G., Shipp, L. & Scott-Dupree, C. (2015) *Amblyseius swirskii* in greenhouse production systems: a floricultural perspective. *Experimental and Applied Acarology*, 65, 451–464.
- Buitenhuis, R., Shipp, L., Scott-Dupree, C., Brommit, A. & Lee, W. (2014) Host plant effects on the behaviour and performance of *Amblyseius swirskii* (Acari: Phytoseiidae). *Experimental and Applied Acarology*, 62, 171–180.
- Burckhardt, D. (1986) Taxonomy and host plant relationships of the *Trioza apicalis* Förster complex (Hemiptera, Homoptera: Triozidae). *Insect Systematics & Evolution*, 16, 415–432.

- Burckhardt, D. (1994) Psyllid pests of temperate and subtropical crop and ornamental plants (Hemiptera, Psylloidea): a review. *Trends in Agricultural Sciences, Entomology*, 2, 173–186.
- Van Buskirk, J. (1989) Density-dependent cannibalism in larval dragonflies. *Ecology*, 70, 1442–1449.
- Butler, C.D. (2011) Management Strategies for the Potato Psyllid in California (Ph.D. thesis). University of California, Riverside, California, USA, 192 pp.
- Butler, C.D. & Trumble, J.T. (2012a) Identification and impact of natural enemies of *Bactericera cockerelli* (Hemiptera: Triozidae) in Southern California. *Journal of Economic Entomology*, 105, 1509–1519.
- Butler, C.D. & Trumble, J.T. (2012b) The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. *Terrestrial Arthropod Reviews*, 5, 87–111.
- Butt, T.M., Coates, C.J., Dubovskiy, I.M. & Ratcliffe, N.A. (2016) Entomopathogenic fungi: New insights into host-pathogen interactions. *Advances in Genetics*, 94, 307–364.
- Byrne, D.N. & Bellows, T.S. (1991) Whitefly biology. *Annual Review of Entomology*, 36, 431–457.
- Calabuig, A., Pekas, A. & Wäckers, F.L. (2018) The quality of nonprey food affects cannibalism, intraguild predation, and hyperpredation in two species of phytoseiid mites. *Journal of Economic Entomology*, 111, 72–77.
- Calvo, F.J., Torres-Ruiz, A., Velázquez-González, J., Rodríguez-Leyva, E. & Lomeli-Flores, J.R. (2018a) Improved sweetpotato whitefly and potato psyllid control in tomato by combining the mirid *Dicyphus hesperus* (Heteroptera: Miridae) with specialist parasitic wasps. *Journal of Economic Entomology*, 111, 549–555.
- Calvo, F.J., Torres-Ruiz, A., Velázquez-González, J.C., Rodríguez-Leyva, E. & Lomeli-Flores, J.R. (2016) Evaluation of *Dicyphus hesperus* for biological control of sweet potato whitefly and potato psyllid on greenhouse tomato. *BioControl*, 61, 415–424.
- Calvo, F.J., Velázquez-González, J.C., Velázquez-González, M.B. & Torres, A. (2018b) Supplemental releases of specialist parasitic wasps improve whitefly and psyllid control by *Dicyphus hesperus* in tomato. *BioControl*, 63, 629–639.
- Cañarte, E., Sarmiento, R.A., Venzon, M., Pedro-Neto, M., Ferreira Junior, D.F., Santos, F.A. & Pallini, A. (2017) Suitability and nutritional requirements of the predatory mite

- Typhlodromus transvaalensis*, a potential biological control agent of physic nut pest mites. *Biological Control*, 115, 165–172.
- Carvalho, F.P. (2017) Pesticides, environment, and food safety. *Food and Energy Security*, 6, 48–60.
- Casique-Valdez, R., Reyes-Martínez, A.Y., Sánchez-Peña, S.R., Bidochka, M.J., López-Arroyo, J.I., Casique-Valdes, R., Reyes-Martinez, A.Y., Sanchez-Peña, S.R., Bidochka, M.J. & Lopez-Arroyo, J.I. (2011) Pathogenicity of *Hirsutella citriformis* (Ascomycota: Cordycipitaceae) to *Diaphorina citri* (Hemiptera: Psyllidae) and *Bactericera cockerelli* (Hemiptera: Triozidae). *Florida Entomologist*, 94, 703–705.
- Castagnoli, M., Liguori, M., Simoni, S. & Duso, C. (2005) Toxicity of some insecticides to *Tetranychus urticae*, *Neoseiulus californicus* and *Tydeus californicus*. *BioControl*, 50, 611–622.
- Castilho, R.C., de Moraes, G.J., Silva, E.S. & Silva, L.O. (2009) Predation potential and biology of *Protogamasellopsis posnaniensis* Wisniewski & Hirschmann (Acari: Rhodacaridae). *Biological Control*, 48, 164–167.
- Castillo Carrillo, C.I., Fu, Z., Jensen, A.S. & Snyder, W.E. (2016) Arthropod pests and predators associated with bittersweet nightshade, a noncrop host of the potato psyllid (Hemiptera: Triozidae). *Environmental Entomology*, 45, 873–882.
- Cembrowski, A.R., Tan, M.G., Thomson, J.D. & Frederickson, M.E. (2014) Ants and ant scent reduce bumblebee pollination of artificial flowers. *The American Naturalist*, 183, 133–139.
- Chandler, D., Davidson, G. & Jacobson, R.J. (2005) Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Science and Technology*, 15, 37–54.
- Chauzat, M. & Faucon, J. (2007) Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France. *Pest Management Science*, 1106, 1100–1106.
- Chi, H. (1988) Life-table analysis incorporating both sexes and variable development rates among individuals. *Environmental Entomology*, 17, 26–34.
- Chi, H. (2017a) CONSUME-MSChart: computer program for consumption rate analysis based on the age stage, two-sex life table. National Chung Hsing University, Taichung Taiwan. Available from <http://140.120.197.173/Ecology/>.

- Chi, H. (2017b) TIMING-MSChart: Computer program for the population projection based on the age stage, two-sex life table analysis. National Chung Hsing University, Taichung Taiwan. Available from <http://140.120.197.173/Ecology/>.
- Chi, H. (2017c) TWOSEX-MSChart: A computer program for the age-stage, two-sex life table analysis. National Chung Hsing University, Taichung Taiwan. Available from <http://140.120.197.173/Ecology/>.
- Chi, H. & Liu, H. (1985) Two new methods for the study of insect population ecology. *Bulletin of the Institute of Zoology Academia Sinica*, 24, 225–240.
- Chi, H. & Su, H.-Y. (2006) Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead)(Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer)(Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. *Environmental Entomology*, 35, 10–21.
- Chi, H. & Yang, T.-C. (2003) Two-sex life table and predation rate of *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) fed on *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Environmental Entomology*, 32, 327–333.
- Chilima, C.Z. (1995) Cypress aphid control: first African release of *Pauesia juniperorum*. *FRIM Newsletter (Forestry Research Institute of Malawi)*, 74, 2.
- Choraży, A., Kropczyńska-Linkiewicz, D., Sas, D. & Escudero-Colomar, L.A. (2016) Distribution of *Amblydromalus limonicus* in northeastern Spain and diversity of phytoseiid mites (Acari: Phytoseiidae) in tomato and other vegetable crops after its introduction. *Experimental and Applied Acarology*, 69, 465–478.
- Chougule, N.P. & Bonning, B.C. (2012) Toxins for transgenic resistance to hemipteran pests. *Toxins*, 4, 405–429.
- Ciesla, W.M. (1991) Cypress aphid, *Cinara cupressi*, a new pest of conifers in eastern and southern Africa. *FAO Plant Protection Bulletin*, 39, 82–93.
- Cini, A., Ioriatti, C. & Anfora, G. (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology*, 65, 149–160.
- De Clercq, P. (2002) Dark clouds and their silver linings: exotic generalist predators in augmentative biological control. *Neotropical Entomology*, 31, 169–176.
- De Clercq, P., Arijs, Y., Van Meir, T., Van Stappen, G., Sorgeloos, P., Dewettinck, K., Rey, M., Grenier, S. & Febvay, G. (2005a) Nutritional value of brine shrimp cysts as a

- factitious food for *Orius laevigatus* (Heteroptera: Anthocoridae). *Biocontrol Science and Technology*, 15, 467–479.
- De Clercq, P., Bonte, M., Van Speybroeck, K., Bolckmans, K. & Deforce, K. (2005b) Development and reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. *Pest Management Science*, 61, 1129–1132.
- Collier, T. & Van Steenwyk, R. (2004) A critical evaluation of augmentative biological control. *Biological Control*, 31, 245–256.
- Collins, J.P. & Cheek, J.E. (1983) Effect of food and density on development of typical and Cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. *Integrative and Comparative Biology*, 23, 77–84.
- Compere, H. (1915) *Paratrioza cockerelli* (Sulc). *Monthly Bulletin of California State Commission of Horticulture*, 4, 574.
- Compere, H. (1916) Notes on the tomato psylla. *Monthly Bulletin of California State Commission of Horticulture*, 5, 189–191.
- Compere, H. (1943) A new species of *Metaphycus* parasite on psyllids. *Pan-Pacific Entomology*, 19, 71–73.
- Conceschi, M.R., D’Alessandro, C.P., Moral, R. de A., Demétrio, C.G.B. & Júnior, I.D. (2016) Transmission potential of the entomopathogenic fungi *Isaria fumosorosea* and *Beauveria bassiana* from sporulated cadavers of *Diaphorina citri* and *Toxoptera citricida* to uninfected *D. citri* adults. *BioControl*, 61, 567–577.
- Cranshaw, W. (2013) Entomophagus fungi (*Zoophthora radicans*). Available from: <http://www.forestryimages.org/browse/detail.cfm?imgnum=5488916>.
- Creighton, J.C. (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology*, 16, 1031–1036.
- Croft, B.A. & Croft, M.B. (1996) Intra- and interspecific predation among adult female phytoseiid mites (Acari : Phytoseiidae): Effects on survival and reproduction. *Biological Control*, 25, 853–858.
- Crowley, P.H. (1981) Dispersal and the stability of predator-prey interactions. *The American Naturalist*, 118, 673–701.
- Cui, L., Yuan, H., Wa, Q., Wang, Q. & Rui, C. (2018) Sublethal effects of the novel cis - nitromethylene neonicotinoid cycloxaprid on the cotton aphid *Aphis gossypii* Glover (Hemiptera : Aphididae). *Scientific Reports*, 8, 8915.

- Davidson, M.M., Nielsen, M.-C., Butler, R.C. & Silberbauer, R.B. (2016) Prey consumption and survival of the predatory mite, *Amblydromalus limonicus*, on different prey and host plants. *Biocontrol Science and Technology*, 26, 722–726.
- Day, R.K., Kairo, M.T.K., Abraham, Y.J., Kfir, R., Murphy, S.T., Mutitu, K.E. & Chilima, C.Z. (2003) Biological control of homopteran pests of conifers in Africa. In: Neuenschwander, P. & Borgemeister, C. (Eds). *Biological control in IPM systems in Africa*, CAB International, Wallingford, pp.101–112.
- Delisle, J.F., Brodeur, J. & Shipp, L. (2015a) Evaluation of various types of supplemental food for two species of predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae). *Experimental and Applied Acarology*, 65, 483–494.
- Delisle, J.F., Shipp, L. & Brodeur, J. (2015b) Apple pollen as a supplemental food source for the control of western flower thrips by two predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae), on potted chrysanthemum. *Experimental and Applied Acarology*, 65, 495–509.
- Delory, B.M., Weidlich, E.W.A., Meder, L., Lütje, A., van Duijnen, R., Weidlich, R. & Temperton, V.M. (2017) Accuracy and bias of methods used for root length measurements in functional root research. *Methods in Ecology and Evolution*, 8, 1594–1606.
- Detzel, A. & Wink, M. (1993) Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology*, 4, 8–18.
- Dias, C.R., Bernardo, A.M.G., Mencalha, J., Freitas, C.W.C., Sarmiento, R.A., Pallini, A. & Janssen, A. (2016) Antipredator behaviours of a spider mite in response to cues of dangerous and harmless predators. *Experimental and Applied Acarology*, 69, 263–276.
- Van Dinh, N., Sabelis, M.W. & Janssen, A. (1988) Influence of humidity and water availability on the survival of *Amblyseius idaeus* and *A. anonymus* (Acarina: Phytoseiidae). *Experimental & Applied Acarology*, 4, 27–40.
- Dogan, Y.O., Hazir, S., Yildiz, A., Butt, T.M. & Cakmak, I. (2017) Evaluation of entomopathogenic fungi for the control of *Tetranychus urticae* (Acari: Tetranychidae) and the effect of *Metarhizium brunneum* on the predatory mites (Acari: Phytoseiidae). *Biological Control*, 111, 6–12.
- Donka, A., Sermann, H. & Buttner, C. (2008) Effect of the entomopathogenic fungus *Lecanicillium muscarium* on the predatory mite *Phytoseiulus persimilis* as a non-target organism. *IOBC/wprs Bulletin*, 35, 109–112.

- Donka, A., Sermann, H. & Büttner, C. (2009) Side-effect of the entomopathogenic fungus *Lecanicillium muscarium* on the predatory mite *Phytoseiulus persimilis*. *IOBC/wprs Bulletin*, 45, 267–269.
- Duarte, M.V.A.A., Venzon, M., Bittencourt, M.C. d. de S. de S., Rodríguez-Cruz, F.A., Pallini, A. & Janssen, A. (2015) Alternative food promotes broad mite control on chilli pepper plants. *BioControl*, 60, 817–825.
- Duso, C., Malagnini, V., Pozzebon, A., Castagnoli, M., Liguori, M. & Simoni, S. (2008) Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari Tetranychidae, Phytoseiidae). *Biological Control*, 47, 16–21.
- Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D. & Blumenthal, D.M. (2016) Global threats from invasive alien species in the twenty-first century and national response capacities. *Nature Communications*, 7, 12485.
- Eckarsky, B.A.L., Ntosh, A.R., Taylor, B.W. & Dahl, J. (2002) Predator chemicals induce changes in mayfly life history traits: a whole-stream manipulation. *Ecology*, 83, 612–618.
- Edney-Browne, E., Brockerhoff, E.G. & Ward, D. (2018) Establishment patterns of non-native insects in New Zealand. *Biological Invasions*, 20, 1657–1669.
- Efron, B. & Tibshirani, R.J. (1993) *An introduction to the bootstrap*. CRC press, New York.
- English-Loeb, G., Norton, A.P. & Walker, M.A. (2002) Behavioral and population consequences of acarodomatia in grapes on phytoseiid mites (Mesostigmata) and implications for plant breeding. *Entomologia Experimentalis et Applicata*, 104, 307–319.
- Enkegaard, A., Sardar, M.A. & Brodsgaard, H.F. (1997) The predatory mite *Hypoaspis miles*: biological and demographic characteristics on two prey species. *Entomologia Experimentalis et Applicata*, 82, 135–146.
- Essig, E.O. (1917) The tomato and laurel psyllids. *Journal of Economic Entomology*, 10, 433–444.
- Étienne, J., Quilici, S., Marival, D. & Franck, A. (2001) Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera : Eulophidae). *Fruits*, 56, 307–315.
- Everett, K.R., Taylor, R.K., Romberg, M.K., Rees-George, J., Fullerton, R.A., Vanneste, J.L. & Manning, M.A. (2011) First report of *Pseudomonas syringae* pv. *actinidiae* causing kiwifruit bacterial canker in New Zealand. *Australasian Plant Disease Notes*, 6, 67–71.

- Faraji, F., Janssen, A., Sabelis, M.W., Dynamics, E. & Biology, S.P. (2002) Oviposition patterns in a predatory mite reduce the risk of egg predation caused by prey. *Ecological Entomology*, 27, 660–664.
- Farhadi, R., Allahyari, H. & Chi, H. (2011) Life table and predation capacity of *Hippodamia variegata* (Coleoptera: Coccinellidae) feeding on *Aphis fabae* (Hemiptera: Aphididae). *Biological Control*, 59, 83–89.
- Faria, M. & Wraight, S.P. (2001) Biological control of *Bemisia tabaci* with fungi. *Crop Protection*, 20, 767–778.
- Farooq, M. (2016) Lethal and sublethal effects of mixtures of entomopathogenic fungi and synthetic insecticides on biological aspects of *Musca domestica* L.. *Türkiye Entomoloji Dergisi*, 40, 211–225.
- Farooq, M. & Freed, S. (2016) Infectivity of housefly, *Musca domestica* (Diptera: Muscidae) to different entomopathogenic fungi. *Brazilian Journal of Microbiology*, 47, 807–816.
- Fernandez-Caldas, E., Iraola, V. & Carnes, J. (2007) Molecular and biochemical properties of storage mites (Except *Blomia* species). *Protein & Peptide Letters*, 14, 954–959.
- Ferrari, M.C.O., Wisenden, B.D. & Chivers, D.P. (2010) Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, 88, 698–724.
- Ferreira, J.A.M., Eshuis, B., Janssen, A. & Sabelis, M.W. (2008) Domatia reduce larval cannibalism in predatory mites. *Ecological Entomology*, 33, 374–379.
- Ferrero, M., Gigot, C., Tixier, M.S., van Houten, Y.M. & Kreiter, S. (2010) Egg hatching response to a range of air humidities for six species of predatory mites. *Entomologia Experimentalis et Applicata*, 135, 237–244.
- Ferro, D.N. & Southwick, E.E. (1984) Microclimates of small arthropods: estimating humidity within the leaf boundary layer. *Environmental Entomology*, 13, 926–929.
- Fidgett, M.J. & Stinson, C.S.A. (2008) Method for rearing predatory mites. WO Patent WO/2008/015393.
- Fischer, M.K. & Shingleton, A.W. (2001) Host plant and ants influence the honeydew sugar composition of aphids. *Functional Ecology*, 15, 544–550.
- Francisco, C., Barahona, S., Threlkeld, B.S., Avery, P.B., Francis, A.W. & Cave, R.D. (2018) Compatibility and efficacy of the lady beetle *Thalassa montezumae* and the entomopathogenic fungus *Isaria fumosorosea* for biological control of the green croton

- scale: laboratory and greenhouse investigations. *Arthropod-Plant Interactions*, 12, 715–723.
- Furlong, M.J. & Pell, J.K. (2005) Interactions between entomopathogenic fungi and arthropod natural enemies. In: Vega, F.E. & Blackwell, M. (Eds.) *Insect-fungal associations: ecology and evolution*. Oxford University Press, New York, pp. 51–73.
- Gaede, K. (1992) On the water balance of *Phytoseiulus persimilis* A.-H. and its ecological significance. *Experimental & Applied Acarology*, 15, 181–198.
- Ge, X., He, S., Zhu, C., Wang, T., Xu, Z. & Zong, S. (2018) Projecting the current and future potential global distribution of *Hyphantria cunea* (Lepidoptera: Arctiidae) using CLIMEX. *Pest Management Science*, 75, 160–169.
- Geary, I.J., Merfield, C.N., Hale, R.J., Shaw, M.D. & Hodge, S. (2016) Predation of nymphal tomato potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), by the predatory mite, *Anystis baccarum* L.(Trombidiformes: Anystidae). *New Zealand Entomologist*, 39, 110–116.
- Gerling, D., Alomar, Ò. & Arnó, J. (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Protection*, 20, 779–799.
- Gerson, U., Smiley, R.L. & Ochoa, R. (2003) *Mites (Acari) for pest control*. Blackwell Science Ltd., Malden, MA, USA, 539 pp.
- Gharalari, A.H., Nansen, C., Lawson, D.S., Gilley, J., Munyaneza, J.E. & Vaughn, K. (2009) Knockdown mortality, repellency, and residual effects of insecticides for control of adult *Bactericera cockerelli* (Hemiptera: Psyllidae). *Journal of Economic Entomology*, 102, 1032–1038.
- Ghasemzadeh, S., Leman, A. & Messelink, G.J. (2017) Biological control of *Echinothrips americanus* by phytoseiid predatory mites and the effect of pollen as supplemental food. *Experimental and Applied Acarology*, 73, 1–13.
- Ghazy, N.A., Osakabe, M., Waleed, M., Schausberger, P., Gotoh, T. & Amano, H. (2016) Phytoseiid mites under environmental stress. *Biological Control*, 96, 120–134.
- Gill, G. (2006) Tomato psyllid detected in New Zealand. *Biosecurity*, 69, 10–11.
- Gillespie, D.R. & McGregor, R.R. (2000) The functions of plant feeding in the omnivorous predator *Dicyphus hesperus*: water places limits on predation. *Ecological Entomology*, 25, 380–386.
- Gillespie, D.R., McGregor, R.R. & Sánchez, J.A. (2007) *Dicyphus hesperus* (Hemiptera: Miridae) as a success story in development of endemic natural enemies as biological

- control agents. In: Vincent, C.M., Goettel, M. & Lazarovits, G. (Eds). *Case studies in biological control: a global perspective*. CABI Publishing, Wallingford, UK, pp. 128–135.
- Gindin, G., Barash, I., Harari, N. & Raccach, B. (1994) Effect of endotoxic compounds isolated from *Verticillium lecanii* on the sweetpotato whitefly, *Bemisia tabaci*. *Phytoparasitica*, 22, 189–196.
- Glare, T.R. & O’Callaghan, M. (2017) Microbial biopesticides for control of invertebrates: Progress from New Zealand. *Journal of Invertebrate Pathology*, Doi:10.1016/j.jip.2017.11.014.
- Goleva, I., Gerken, S. & Zebitz, C.P.W. (2014) Influence of pollen feeding on body weight and body size of the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae). *Journal of Plant Diseases and Protection*, 121, 219–222.
- Goleva, I., Rubio Cadena, E.C., Ranabhat, N.B., Beckereit, C. & Zebitz, C.P.W. (2015) Dietary effects on body weight of predatory mites (Acari, Phytoseiidae). *Experimental and Applied Acarology*, 66, 541–553.
- Goleva, I. & Zebitz, C.P.W. (2013) Suitability of different pollen as alternative food for the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae). *Experimental and Applied Acarology*, 61, 259–283.
- Gonthier, D.J. (2012) Do herbivores eavesdrop on ant chemical communication to avoid predation? *PLoS ONE*, 7, e28703.
- Goodman, D. (1982) Optimal life histories, optimal notation, and the value of reproductive value. *The American Naturalist*, 119, 803–823.
- Grafton-Cardwell, E.E., Stelinski, L.L. & Stansly, P.A. (2013) Biology and management of asian citrus psyllid, vector of the huanglongbing pathogens. *Annual Review of Entomology*, 58, 413–432.
- Gratz, N.G. (2004) Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology*, 18, 215–227.
- Greer, G. & Saunders, C.M. (2012) *The costs of Psa-V to the New Zealand kiwifruit industry and the wider community*. Lincoln University. Agricultural Economics Research Unit.
- Grenier, S. & De Clercq, P. (2003) Comparison of artificially vs. naturally reared natural enemies and their potential for use in biological control. In: *Quality control and production of biological control agents: theory and testing procedures*. CABI Publishing, Wallingford, UK, pp 115–131.

- Grist, N.R. (1993) *Aedes albopictus*: the tyre-travelling tiger. *Journal of Infection*, 27, 1–4.
- Grostal, R. & O’Dowd, D.J. (1994) Plants, mites and mutualism: leaf domatia and the abundance and reproduction of mites on *Viburnum tinus* (Caprifoliaceae). *Oecologia*, 97, 308–315.
- Guenther, J., Goolsby, J. & Greenway, G. (2012) Use and cost of insecticides to control potato psyllids and Zebra Chip on potatoes. *Southwestern Entomologist*, 37, 263–270.
- Guenther, J. & Greenway, G. (2013) Zebra Chip economics. In: Workneh, F. & Rush, C.M. (Eds). *Proceedings of the 10th Annual Zebra Chip Reporting Session (November 7–10 2010)*. Dallas, TX, pp. 93–95.
- Gurulingappa, P., McGee, P.A. & Sword, G. (2011) Endophytic *Lecanicillium lecanii* and *Beauveria bassiana* reduce the survival and fecundity of *Aphis gossypii* following contact with conidia and secondary metabolites. *Crop Protection*, 30, 349–353.
- Halbert, S.E. & Manjunath, K.L. (2004) Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease in Citrus: A literature review and assessment of risk in Florida. *Florida Entomologist*, 87, 330–353.
- Halbert, S.E., Manjunath, K.L., Ramadugu, C., Brodie, M.W., Webb, S.E. & Lee, R.F. (2010) Trailers transporting oranges to processing plants move asian citrus psyllids. *Florida Entomologist*, 93, 33–38.
- Hale, O.M. (1973) Dried *Hermetia illucens* larvae (Diptera: Stratiomyidae) as a feed additive for poultry. *Journal of the Georgia Entomological Society*, 8, 16–20.
- Halliday, R.B. (2003) Health and safety issues related to mites in stored grain. In: *Proceedings of the Australian Postharvest Technical Conference*, pp. 116–118.
- Hänfling, B. & Kollmann, J. (2002) An evolutionary perspective of biological invasions. *Trends in Ecology & Evolution*, 17, 545–546.
- Van Haren, R.J.F., Steenhuis, M.M., Sabelis, M.W. & De Ponti, O.M.B. (1987) Tomato stem trichomes and dispersal success of *Phytoseiulus persimilis* relative to its prey *Tetranychus urticae*. *Experimental & Applied Acarology*, 3, 115–121.
- Hassan, M.F., Momen, F.M., Nasr, A.K., Mabrouk, A.H. & Ramadan, M.M. (2017) Development and reproduction of three predatory mites (Acari: Laelapidae and Rhodacaridae) on eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). *Acta Phytopathologica et Entomologica Hungarica*, 52, 97–106.

- Hawlena, D. & Schmitz, O.J. (2010) Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *Proceedings of the National Academy of Sciences*, 107, 15503–15507.
- Henry, T.J. & Wheeler Jr, A.G. (1988) Family Miridae Hahn, 1833 (= Capsidae Burmeister, 1835). The plant bugs. In: Henry, T.J. & Froeschner, R.C. (Eds), *Catalog of the Heteroptera, or true bugs of Canada and the continental United States*. EJ Brill, Leiden, pp. 251–507.
- Herbert, J.J. & Horn, D.J. (2008) Effect of ant attendance by *Monomorium minimum* (Buckley) (Hymenoptera: Formicidae) on predation and parasitism of the soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae). *Environmental Entomology*, 37, 1258–1263.
- Hermann, S.L. & Landis, D.A. (2017) Scaling up our understanding of non-consumptive effects in insect systems. *Current Opinion in Insect Science*, 20, 54–60.
- Hermann, S.L. & Thaler, J.S. (2014) Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. *Oecologia*, 176, 669–676.
- Hernández-moreno, A.S., Pérez-panduro, A., Lomeli-flores, J.R., Rodríguez-leyva, E., Juan, B. & Tovar, C. (2017) Aptitud Biológica de *Tamarixia triozae* Mediada por la Planta Hospedera. *Southwestern Entomologist*, 42, 225–236.
- Herre, E.A., Knowlton, N., Mueller, U.G. & Rehner, S.A. (1999) The evolution of mutualisms: Exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution*, 14, 49–53.
- Heslin, L.M., Kopittke, R.A. & Merritt, D.J. (2005) The role of insect cell lines in an artificial diet for the parasitoid wasp, *Trichogramma pretiosum*. *Biological Control*, 33, 186–193.
- Höcherl, N., Siede, R., Illies, I., Gäschenberger, H. & Tautz, J. (2012) Evaluation of the nutritive value of maize for honey bees. *Journal of Insect Physiology*, 58, 278–285.
- Hoda, F.M., Taha, H.A., Ibrahim, G.A. & El-Beheri, M.M. (1986) Biological observations on the predator mite, *Hypoaspis miles* Berlese (Acarina: Laelapidae). *Bulletin de la Societe Entomologique d' Egypte Soc Entomol Egypte*, 66, 103–106.
- Hoddle, M.S. (2004) Restoring balance: using exotic species to control invasive exotic species. *Conservation Biology*, 18, 38–49.

- Hodek, I. & Honěk, A. (2009) Scale insects, mealybugs, whiteflies and psyllids (Hemiptera, Sternorrhyncha) as prey of ladybirds. *Biological Control*, 51, 232–243.
- Hokkanen, H.M.T. & Hajek, A. (2003) *Environmental impacts of microbial insecticides: need and methods for risk assessment*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 269 pp.
- Hoogerbrugge, H., van Houten, Y.M., Knapp, M. & Bolckmans, K. (2011) Biological control of thrips and whitefly on strawberries with *Amblydromalus limonicus* and *Amblyseius swirskii*. *IOBC/wprs Bulletin*, 68, 65–69.
- Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, 50, 346–363.
- Hothorn, T., Hornik, K., Van De Wiel, M.A. & Zeileis, A. (2006) A lego system for conditional inference. *American Statistician*, 60, 257–263.
- Van Houten, Y.M. (1996) Biological control of western flower thrips on cucumber using the predatory mites *Amblyseius cucumeris* and *A. limonicus*. *IOBC/wprs Bulletin*, 19, 59–62.
- Van Houten, Y.M., van Rijn, P.C.J., Tanigoshi, L.K., van Stratum, P. & Bruin, J. (1995) Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. *Entomologia Experimentalis et Applicata*, 74, 225–234.
- Huang, H.-W., Chi, H. & Smith, C.L. (2017) Linking Demography and Consumption of *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) fed on *Solanum photeinocarpum* (Solanales: Solanaceae): With a new method to project the uncertainty of population growth and consumption. *Journal of Economic Entomology*, 111, 1–9.
- Huang, Y.-B. & Chi, H. (2011) The age-stage, two-sex life table with an offspring sex ratio dependent on female age. *Journal of Agriculture and Forestry*, 60, 337–345.
- Huang, Y.B. & Chi, H. (2012) Age-stage, two-sex life tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. *Insect Science*, 19, 263–273.
- Huffaker, C. (1958) Experimental studies on predation: dispersion factors and predator-prey oscillations. *Hilgardia*, 27, 343–383.
- Huffaker, C.B., Shea, K. & Herman, S. (1963) Experimental studies on predation: Complex dispersion and levels of food in an acarine predator-prey interaction. *Hilgardia*, 34, 305–330.

- Itagaki, N. & Koyama, K. (1986) Rearing of the predacious mite, *Amblyseius eharai* Amitai et Swirski (Acarina: Phytoseiidae) on a synthetic diet. *Japanese Journal of Applied Entomology and Zoology (Japan)*, 298–300.
- Jacobson, R.J., Chandler, D., Fenlon, J. & Russell, K.M. (2001a) Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants. *Biocontrol Science and Technology*, 11, 391–400.
- Jacobson, R.J., Croft, P. & Fenlon, J. (2001b) Suppressing establishment of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) in cucumber crops by prophylactic release of *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae). *Biocontrol Science and Technology*, 11, 27–34.
- James, D.G. (1993) Pollen, mould mites and fungi: improvements to mass rearing of *Typhlodromus doreenae* and *Amblyseius victoriensis*. *Experimental & Applied Acarology*, 17, 271–276.
- James, D.G. & Whitney, J. (1993) Cumbungi pollen as a laboratory diet for *Amblyseius victoriensis* (Womersley) and *Typhlodromus Doreenae* Schicha (Acari: Phytoseiidae). *Australian Journal of Entomology*, 32, 5–6.
- Janssen, A. & Sabelis, M.W. (2015) Alternative food and biological control by generalist predatory mites: The case of *Amblyseius swirskii*. *Experimental and Applied Acarology*, 65, 413–418.
- Janssen, A., Sabelis, M.W., Magalhães, S., Montserrat, M. & van der Hammen, T. (2007) Habitat structure affects intraguild predation. *Ecology*, 88, 2713–2719.
- Janssens, L. & Stoks, R. (2013a) Predation risk causes oxidative damage in prey. *Biology Letters*, 9, 20130350.
- Janssens, L. & Stoks, R. (2013b) Synergistic effects between pesticide stress and predator cues: Conflicting results from life history and physiology in the damselfly *Enallagma cyathigerum*. *Aquatic Toxicology*, 132–133, 92–99.
- Jarrahi, A. & Safavi, S.A. (2016a) Fitness costs to *Helicoverpa armigera* after exposure to sub-lethal concentrations of *Metarhizium anisopliae* sensu lato: Study on F1 generation. *Journal of Invertebrate Pathology*, 138, 50–56.
- Jarrahi, A. & Safavi, S.A. (2016b) Sublethal effects of *Metarhizium anisopliae* on life table parameters of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* larvae at different time intervals. *BioControl*, 61, 167–175.

- Jensen, D. (1957) Parasites of the Psyllidae. *Hilgardia*, 27, 71–99.
- Ji, J., Zhang, Y.X., Lin, J.Z., Chen, X., Sun, L. & Saito, Y. (2015) Life histories of three predatory mites feeding upon *Carpoglyphus lactis* (Acari, Phytoseiidae; Carpglyphidae). *Systematic and Applied Acarology*, 20, 491–496.
- Ji, J., Zhang, Y.X., Saito, Y., Takada, T. & Tsuji, N. (2016) Competitive and predacious interactions among three phytoseiid species under experimental conditions (Acari: Phytoseiidae). *Environmental Entomology*, 45, 46–52.
- Jiménez, M.M., Fresno, M.J. & Selles, E. (1994) The galenic behaviour of a dermatopharmaceutical excipient containing honey. *International Journal of Cosmetic Science*, 16, 211–226.
- Jiu, M., Zhou, X.P., Tong, L., Xu, J., Yang, X., Wan, F.H. & Liu, S.S. (2007) Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE*, 2, e182.
- Johnson, T.E. (1971) The effectiveness of *Tetrastichus triozae* Burks (Hymenoptera: Eulophidae) as a biological control agent of *Paratrioza cockerelli* (Sulc.) (Homoptera: Psyllidae) in north central Colorado (M.S. thesis). Fort Collins, Colorado, USA, 45 pp.
- Jones, D.R. (2005) Plant viruses transmitted by Thrips. *European Journal of Plant Pathology*, 113, 119–157.
- Kairo, M.T.K. & Murphy, S.T. (2005) Comparative studies on populations of *Pauesia juniperorum* (Hymenoptera: Braconidae), a biological control agent for *Cinara cupressivora* (Hemiptera: Aphididae). *Bulletin of Entomological Research*, 95, 597–603.
- Kale, A. (2011) Report on the economic and business impacts of potato psyllid on the potato industry. A report prepared for Potatoes NZ. ELAK consultants Ltd., 10 pp.
- Kar, F., Lin, S. & Zhang, Z.-Q. (2015) *Neocypholaelaps novaehollandiae* Evans (Acari: Ameroseiidae) rediscovered: Experiments on its life history and behaviour. *New Zealand Entomologist*, 38, 126–133.
- Kean, A.M., Nielsen, M.C., Davidson, M.M., Butler, R.C. & Vereijssen, J. (2019) Host plant influences establishment and performance of *Amblydromalus limonicus*, a predator for *Bactericera cockerelli*. *Pest Management Science*, 75, 787–792.
- Kenis, M., Auger-Rozenberg, M., Roques, A., Timms, L., Perec, M., Cock, M.J.W., Settele, J., Augustin, S. & Lopez-Caamonde, C. (2009) Ecological effects of invasive alien insects. *Biological Invasions*, 11, 21–45.

- Kennett, C.E. & Hamai, J. (1980) Oviposition and development in predaceous mites fed with artificial and natural diets (Acari: Phytoseiidae). *Entomologia Experimentalis et Applicata*, 28, 116–122.
- Khanamani, M., Fathipour, Y., Asghar Talebi, A. & Mehrabadi, M. (2017a) How pollen supplementary diet affect life table and predation capacity of *Neoseiulus californicus* on two-spotted spider mite. *Systematic and Applied Acarology*, 22, 135–147.
- Khanamani, M., Fathipour, Y., Talebi, A.A. & Mehrabadi, M. (2017b) Evaluation of different artificial diets for rearing the predatory mite *Neoseiulus californicus* (Acari: Phytoseiidae): Diet-dependent life table studies. *Acarologia*, 57, 407–419.
- Khanamani, M., Fathipour, Y., Talebi, A.A. & Mehrabadi, M. (2017c) Linking pollen quality and performance of *Neoseiulus californicus* (Acari: Phytoseiidae) in two-spotted spider mite management programmes. *Pest Management Science*, 73, 452–461.
- Khanamani, M., Fathipour, Y., Talebi, A.A. & Mehrabadi, M. (2017d) Quantitative analysis of long-term mass rearing of *Neoseiulus californicus* (Acari: Phytoseiidae) on almond pollen. *Journal of Economic Entomology*, 110, 1442–1450.
- Kirk, W.D.J. & Terry, L.I. (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology*, 5, 301–310.
- Kishimoto, H. (2015) Development and oviposition of eight native phytoseiid species (Acari: Phytoseiidae) reared on eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Journal of the Acarological Society of Japan*, 24, 71–76.
- Knapp, M., van Houten, Y., Hoogerbrugge, H., Bolckmans, K., Houten, Y. Van, Hoogerbrugge, H. & Bolckmans, K. (2013) *Amblydromalus limonicus* (Acari: Phytoseiidae) as a biocontrol agent: Literature review and new Findings. *Acarologia*, 53, 191–202.
- Knowlton, G.E. (1933) Ladybird beetles as predators of the potato psyllid. *The Canadian Entomologist*, 65, 241–243.
- Koch, R.L. (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, 3, 32.
- Kolokytha, P.D., Fantinou, A.A. & Papadoulis, G.T. (2011a) Effect of several different pollens on the bio-ecological parameters of the predatory mite *Typhlodromus athenas* Swirski and Ragusa (Acari: Phytoseiidae). *Environmental Entomology*, 40, 597–604.

- Kolokytha, P.D., Fantinou, A.A. & Papadoulis, G.T. (2011b) Temperature and diet effects on immature development of predatory mite *Typhlodromus athenas* Swirski and Ragusa (Acari: Phytoseiidae). *Environmental Entomology*, 40, 1577–1584.
- Kumar, V., Wekesa, V.W., Avery, P.B., Powell, C.A., McKenzie, C.L. & Osborne, L.S. (2014) Effect of pollens of various ornamental pepper cultivars on the development and reproduction of *Amblyseius Swirskii* (Acari: Phytoseiidae). *Florida Entomologist*, 97, 367–373.
- Kumar, V., Xiao, Y., McKenzie, C.L. & Osborne, L.S. (2015) Early establishment of the phytoseiid mite *Amblyseius swirskii* (Acari: Phytoseiidae) on pepper seedlings in a Predator-in-First approach. *Experimental and Applied Acarology*, 65, 465–481.
- Kutuk, H. (2017) Performance of the predator *Amblyseius swirskii* (Acari: Phytoseiidae) on greenhouse eggplants in the absence and presence of pine *Pinus brutia* (Pinales:Pinaceae) pollen. *Entomological Research*, 47, 263–269.
- Kütük, H., Karacaoğlu, M., Tüfekli, M. & Villanueva, R. (2016) Failure of biological control of *Frankliniella occidentalis* on protected eggplants using *Amblyseius swirskii* in the Mediterranean region of Turkey. *Turkish Journal of Agriculture and Forestry*, 40, 13–17.
- Lacey, L.A., Frutos, R., Kaya, H.K. & Vail, P. (2001) Insect pathogens as biological control agents: Do they have a future? *Biological Control*, 248, 230–248.
- Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M. & Goettel, M.S. (2015) Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology*, 132, 1–41.
- Lacey, L.A., de la Rosa, F. & Horton, D.R. (2009) Insecticidal activity of entomopathogenic fungi (Hypocreales) for potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae): Development of bioassay techniques, effect of fungal species and stage of the psyllid. *Biocontrol Science and Technology*, 19, 957–970.
- Lacey, L.A., Liu, T.-X., Buchman, J.L., Munyaneza, J.E., Goolsby, J.A. & Horton, D.R. (2011) Entomopathogenic fungi (Hypocreales) for control of potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) in an area endemic for Zebra Chip disease of potato. *Biological Control*, 56, 271–278.
- Lacey, L.A., Liu, T.X., Buchman, J.L., Munyaneza, J.E. & Goolsby, J.A. (2010) Entomopathogenic fungi (Hypocreales) for control of potato psyllid. In: Workneh, F. & Rush, C.M. (Eds). *Proceedings of the 10th Annual Zebra Chip Reporting Session (November 7–10 2010)*. Dallas, TX, pp. 74–77.

- Lee, M.H. & Zhang, Z.-Q. (2018) Assessing the augmentation of *Amblydromalus limonicus* with the supplementation of pollen, thread, and substrates to combat greenhouse whitefly populations. *Scientific Reports*, 8, 12189.
- Lee, M.H. & Zhang, Z.Q. (2016) Habitat structure and its influence on populations of *Amblydromalus limonicus* (Acari: Phytoseiidae). *Systematic and Applied Acarology*, 21, 1361–1378.
- Leman, A. & Messelink, G.J. (2015) Supplemental food that supports both predator and pest: A risk for biological control? *Experimental and Applied Acarology*, 65, 511–524.
- van Lenteren, J.C. (2003) Commercial availability of biological control agents. In: Van Lenteren, J.C. (Ed), *Quality control and production of biological control agents: theory and testing procedures*. CABI Publication, Wallingford, UK, pp. 167–178.
- Van Lenteren, J.C. (2000) A greenhouse without pesticides: Fact or fantasy? *Crop Protection*, 19, 375–384.
- Van Lenteren, J.C. (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, 57, 1–20.
- Leslie, P.H. (1945) On the use of matrices in certain population mathematics. *Biometrika*, 33, 183–212.
- Lewis, E.G. (1942) On the generation and growth of a population. *Sankhyā: The Indian Journal of Statistics*, 6, 93–96.
- Li, G.Y. & Zhang, Z.Q. (2016) Some factors affecting the development, survival and prey consumption of *Neoseiulus cucumeris* (Acari: Phytoseiidae) feeding on *Tetranychus urticae* eggs (Acari: Tetranychidae). *Systematic and Applied Acarology*, 21, 555–566.
- Liefting, L.W., Perez-Egusquiza, Z.C. & Clover, G.R.G. (2008) A new ‘Candidatus Liberibacter’ species in *Solanum tuberosum* in New Zealand. *Plant Disease*, 92, 1474–1474.
- Liefting, L.W., Sutherland, P.W., Ward, L.I., Paice, K.L., Weir, B.S. & Clover, G.R.G. (2009a) A new ‘Candidatus Liberibacter’ species associated with diseases of solanaceous crops. *Plant Disease*, 93, 208–214.
- Liefting, L.W., Weir, B.S., Pennycook, S.R. & Clover, G.R.G. (2009b) ‘Candidatus Liberibacter solanacearum’, associated with plants in the family Solanaceae. *International Journal of Systematic and Evolutionary Microbiology*, 59, 2274–2276.

- Lima, S.L., Bednekoff, P.A., The, S., Naturalist, A., June, N., Lima, S.L. & Bednekoff, P.A. (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *The American Naturalist*, 153, 649–659.
- Lin, G., Tanguay, A., Guertin, C., Todorova, S. & Brodeur, J. (2017) A new method for loading predatory mites with entomopathogenic fungi for biological control of their prey. *Biological Control*, 115, 105–111.
- Lin, K.-H. (1956) Observations on yellow shoot of Citrus. Etiological studies of yellow shoot of Citrus. *Acta Phytopathologica Sinica*, 2, 1–42.
- List, G.M. & Daniels, L.B. (1934) A promising control for psyllid yellows of potatoes. *Science (Washington)*, 79, 79.
- Liu, D. & Trumble, J.T. (2007) Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerelli*). *Entomologia Experimentalis et Applicata*, 123, 35–42.
- Liu, J., Li, M., Li, J., Huang, C., Zhou, X., Xu, F. & Liu, S. (2010) Viral infection of tobacco plants improves performance of *Bemisia tabaci* but more so for an invasive than for an indigenous biotype of the whitefly. *Journal of Zhejiang University Science B*, 11, 30–40.
- Liu, S., Barro, P.J. De, Xu, J., Luan, J. & Zang, L. (2007) Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science*, 318, 1769–1772.
- Liu, T.-X., Zhang, Y.-M., Peng, L.-N., Rojas, P. & Trumble, J.T. (2012) Risk assessment of selected insecticides on *Tamarixia triozae* (Hymenoptera: Eulophidae), a parasitoid of *Bactericera cockerelli* (Hemiptera: Triozidae). *Journal of Economic Entomology*, 105, 490–496.
- Lomeli-Flores, J.R. & Bueno, R. (2002) Nuevo registro de *Tamarixia triozae* (Burks) parasitoide del psílido del tomate *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae) en México. *Folia Entomologica Mexicana*, 3, 375–376.
- Lopes, P.C., Kanno, R.H., Sourassou, N.F. & De Moraes, G.J. (2018) Effect of temperature and diet on the morphology of *Euseius concordis* (Acari: Phytoseiidae). *Systematic and Applied Acarology*, 23, 1322–1332.
- Lorenzon, M., Pozzebon, A. & Duso, C. (2012) Effects of potential food sources on biological and demographic parameters of the predatory mites *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni*. *Experimental and Applied Acarology*, 58, 259–278.

- Loughner, R., Nyrop, J., Wentworth, K. & Sanderson, J. (2011) Effects of supplemental pollen and fibers on canopy abundance of *Amblyseius swirskii*. *IOBC/WPRS Bulletin*, 68, 105–109.
- Loughner, R., Wentworth, K., Loeb, G. & Nyrop, J. (2010) Leaf trichomes influence predatory mite densities through dispersal behavior. *Entomologia Experimentalis et Applicata*, 134, 78–88.
- Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. (2000) *100 of the world's worst invasive alien species: a selection from the global invasive species database*. The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), Auckland.
- Ludwig, S.W. & Oetting, R.D. (2001) Susceptibility of natural enemies to infection by *Beauveria bassiana* and impact of insecticides on *Ipheseius degenerans* (Acari: Phytoseiidae). *Journal of Agricultural and Urban Entomology*, 18, 169–178.
- Luna-Cruz, A., Rodríguez-Leyva, E., Lomeli-Flores, J.R., Ortega-Arenas, L.D., Bautista-Martínez, N. & Pineda, S. (2015) Toxicity and residual activity of insecticides against *Tamarixia triozae* (Hymenoptera: Eulophidae), a parasitoid of *Bactericera cockerelli* (Hemiptera: Triozidae). *Journal of Economic Entomology*, 108, 2289–2295.
- Luna-Cruz, A., Lomeli-Flores, J.R., Rodríguez-Leyva, E., Ortega-Arenas, L.D. & Huerta-de la Peña, A. (2011) Toxicidad de cuatro insecticidas sobre *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) y su hospedero *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae). *Acta zoológica Mexicana*, 27, 509–526.
- Ma, D., Gorman, K., Devine, G., Luo, W. & Denholm, I. (2007) The biotype and insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading cropping systems in Xinjiang Uygur Autonomous Region, northwestern China. *Crop Protection*, 26, 612–617.
- Ma, M., Fan, Q.-H. & Zhang, Z.-Q. (2018) Morphological ontogeny of *Amblydromalus limonicus* (Acari: Phytoseiidae). *Systematic & Applied Acarology*, 23, 1741–1765.
- MacDonald, F.H., Connolly, P.G., Larsen, N.J. & Walker, G.P. (2016) The voracity of five insect predators on *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) (tomato potato psyllid; TPP). *New Zealand Entomologist*, 39, 15–22.
- MacDonald, F.H. & Walker, G.P. (2012) The interactions between natural enemies and their role in controlling *Bactericera cockerelli* in potatoes. *New Zealand Plant Protection*, 65, 293.

- Maniania, N.K., Ekesi, S., Kungu, M.M., Salifu, D. & Srinivasan, R. (2016) The effect of combined application of the entomopathogenic fungus *Metarhizium anisopliae* and the release of predatory mite *Phytoseiulus longipes* for the control of the spider mite *Tetranychus evansi* on tomato. *Crop Protection*, 90, 49–53.
- Marafeli, P.P., Reis, P.R., Silveira, E.C. da, Souza-Pimentel, G.C. & de Toledo, M.A. (2014) Life history of *Neoseiulus californicus* (McGregor, 1954) (Acari : Phytoseiidae) fed with castor bean (*Ricinus communis* L.) pollen in laboratory conditions. *Journal Biology Brazilian*, 74, 691–697.
- Martínez, A.M., Baena, M., Figueroa, J.I., Del Estal, P., Medina, M., Guzmán-Lara, E. & Pineda, S. (2014) First record of *Engytatus varians* (Distant) (Hemiptera: Heteroptera: Miridae) in México and its predation on *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae): a survey of its distribution and habits. *Acta Zoológica Mexicana*, 30, 617–624.
- Martínez, A., Chavarrieta, J., Morales, S., Díaz, O., Bujanos, R., Caudillo, K., Figueroa, J., Díaz, O., Bujanos, R., Gomez, B.K., Uela, E., Pineda, S. & Vin, E. (2015) Behavior of *Tamarixia triozae* females (Hymenoptera: Eulophidae) attacking *Bactericera cockerelli* (Hemiptera: Triozidae) and effects of three pesticides on this parasitoid. *Environmental Entomology*, 44, 3–11.
- Mauchline, N.A. & Stannard, K.A. (2013) Evaluation of selected entomopathogenic fungi and bio-insecticides against *Bactericera cockerelli* (Hemiptera). *New Zealand Plant Protection*, 66, 324–332.
- McFarland, C.D. & Hoy, M.A. (2001) Survival of *Diaphorina citri* (Homoptera: Psyllidae), and its two parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), under different relative humidities and temperature regimes. *Florida Entomologist*, 84, 227–233.
- McMurtry, J.A. & Croft, B.A. (1997) Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology*, 42, 291–321.
- McMurtry, J.A., Moraes, G.J. & Sourassou, N.F. (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. *Systematic and Applied Acarology*, 18, 297–320.
- McMurtry, J.A. & Scriven, G.T. (1965) Life-history studies of *Amblyseius limonicus*, with comparative observations on *Amblyseius hibisci* (Acarina: Phytoseiidae). *Annals of the Entomological Society of America*, 58, 106–111.

- McMurtry, J.A. & Scriven, G.T. (1966) Effects of artificial foods on reproduction and development of four species of phytoseiid mites. *Annals of the Entomological Society of America*, 59, 267–269.
- Medd, N.C. & Greatrex, R.M. (2014) An evaluation of three predatory mite species for the control of greenhouse whitefly (*Trialeurodes vaporariorum*). *Pest Management Science*, 70, 1492–1496.
- Medeiros, M.N. de, Belmonte, R., Soares, B.C.C., Medeiros, L.N. de, Canetti, C., Freire-de-Lima, C.G., Maya-Monteiro, C.M., Bozza, P.T., Almeida, I.C., Masuda, H., Kurtenbach, E. & Machado, E.A. (2009) Arrest of oogenesis in the bug *Rhodnius prolixus* challenged with the fungus *Aspergillus niger* is mediated by immune response-derived PGE2. *Journal of Insect Physiology*, 55, 150–157.
- Messelink, G.J., Van Steenpaal, S.E.F. & Ramakers, P.M.J. (2006) Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. *BioControl*, 51, 753–768.
- Meyerson, L.A. & Mooney, H.A. (2007) Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment*, 5, 199–208.
- Michaud, J.P. (2004) Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. *Biological Control*, 29, 260–269.
- Midthassel, A., Leather, S.R. & Baxter, I.H. (2013) Life table parameters and capture success ratio studies of *Typhlodromips swirskii* (Acari: Phytoseiidae) to the factitious prey *Suidasia medanensis* (Acari: Suidasidae). *Experimental and Applied Acarology*, 61, 69–78.
- Midthassel, A., Leather, S.R., Wright, D.J. & Baxter, I.H. (2016) Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: Two potentially complimentary biocontrol agents. *BioControl*, 61, 437–447.
- Moghadas, M., Saboori, A., Allahyari, H. & Zahedi Golpayegani, A. (2014) Life table and predation capacity of *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) feeding on *Tetranychus urticae* (Acari: Tetranychidae) on rose. *International Journal of Acarology*, 40, 501–508.
- Mohammed, A.A. (2018) *Lecanicillium muscarium* and *Adalia bipunctata* combination for the control of black bean aphid, *Aphis fabae*. *BioControl*, 63, 1–11.

- Momen, F.M. (2004) Suitability of the pollen grains, *Ricinus communis* and *Helianthus annuus* as food for six Species of phytoseiid mites (Acari: Phytoseiidae). *Acta Phytopathologica et Entomologica Hungarica*, 39, 415–422.
- Momen, F.M. & El-Laithy, A.Y. (2007) Suitability of the flour moth *Ephesia kuehniella* (Lepidoptera: Pyralidae) for three predatory phytoseiid mites (Acari: Phytoseiidae) in Egypt. *International Journal of Tropical Insect Science*, 27, 102–107.
- Montalva, C., Rojas, E., Ruiz, C. & Lanfranco, D. (2010) The cypress aphid in Chile: A review of the current situation and preliminary data of the biological control. *Bosque*, 31, 81–88.
- Montalva, C., Valenzuela, E., Barta, M., Rojas, E., Arismendi, N., Rodrigues, J. & Humber, R.A. (2017) *Lecanicillium attenuatum* isolates affecting the invasive cypress aphid (*Cinara cupressi*) in Chile. *BioControl*, 62, 625–637.
- Morales, S.I., Martínez, A.M., Viñuela, E., Chavarrieta, J.M., Figueroa, J.I., Schneider, M.I., Tamayo, F. & Pineda, S. (2018) Lethal and sublethal effects on *Tamarixia triozae* (Hymenoptera: Eulophidae), an ectoparasitoid of *Bactericera cockerelli* (Hemiptera: Triozidae), of three insecticides used on solanaceous crops. *Journal of Economic Entomology*, 111, 1048–1055.
- Mori, H. & Chant, D.A. (1966) The influence of prey density, relative humidity, and starvation on the predacious behavior of *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). *Canadian Journal of Zoology*, 44, 483–491.
- Munyaneza, J.E. (2010) Psyllids as vectors of emerging bacterial diseases of annual crops. *Southwestern Entomologist*, 35, 471–477.
- Munyaneza, J.E. (2012) Zebra Chip disease of potato: biology, epidemiology, and management. *American Journal of Potato Research*, 89, 329–350.
- Munyaneza, J.E. (2015) Zebra Chip disease, *Candidatus Liberibacter*, and potato psyllid: A global threat to the potato industry. *American Journal of Potato Research*, 92, 230–235.
- Munyaneza, J.E., Buchman, J.L., Upton, J.E., Goolsby, J., Crosslin, J.M., Bester, G., Miles, G.P. & Sengoda, V.G. (2008) Impact of different potato psyllid populations on Zebra Chip disease incidence, severity, and potato yield. *Subtropical Plant Science*, 60, 27–37.
- Munyaneza, J.E., Crosslin, J.M. & Buchman, J.L. (2009) Seasonal occurrence and abundance of the potato psyllid, *Bactericera cockerelli*, in South Central Washington. *American Journal of Potato Research*, 86, 513–518.

- Munyanzeza, J.E., Crosslin, J.M. & Upton, J.E. (2007a) Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with “Zebra Chip” a new potato disease in Southwestern United States and Mexico. *Journal of Economic Entomology*, 100, 656–663.
- Munyanzeza, J.E., Fisher, T.W., Sengoda, V.G., Garczynski, S.F., Nissinen, A. & Lemmetty, A. (2010a) Association of “*Candidatus Liberibacter solanacearum*” with the psyllid, *Trioza apicalis* (Hemiptera: Triozidae) in Europe. *Journal of Economic Entomology*, 103, 1060–1070.
- Munyanzeza, J.E., Fisher, T.W., Sengoda, V.G., Garczynski, S.F., Nissinen, A. & Lemmetty, A. (2010b) First report of “*Candidatus Liberibacter solanacearum*” associated with psyllid-affected carrots in Europe. *Plant Disease*, 94, 639.
- Munyanzeza, J.E., Goolsby, J.M., Crosslin, J.M. & Upton, J.E. (2007b) Further evidence that Zebra Chip potato disease in the lower rio grande valley of Texas is associated with *Bactericera cockerelli*. *Subtropical Plant Science*, 59, 30–37.
- Murphy, S.T., Chilima, C.Z., Cross, A.E., Abraham, Y.J., Kairo, M.T.K., Allard, G.B. & Day, R.K. (1994) Exotic conifer aphids in Africa: Ecology and biological control. In: Leather, S.R., Watt, A.D., Mills, N.J. & Walters, K.F.A. (Eds) *Individuals populations and patterns in ecology*. Intercept, Andover, UK, pp. 63–76.
- Mustafa, T., Alvarez, J.M. & Munyanzeza, J.E. (2015) Effect of Cyantraniliprole on probing behavior of the potato psyllid (Hemiptera: Triozidae) as measured by the electrical penetration graph technique. *Journal of Economic Entomology*, 108, 2529–2535.
- Nasr, A.-E.K., Momen, F.M., Metwally, A.-E.M., Gesraha, M., Abdallah, A.A. & Saleh, K.M. (2015) Suitability of *Corcyra cephalonica* eggs (Lepidoptera: Pyralidae) for the development, reproduction and survival of four predatory mites of the family Phytoseiidae (Acari: Phytoseiidae). *Gesunde Pflanzen*, 67, 175–181.
- Navarrete, B., McAuslane, H., Deyrup, M. & Peña, J.E. (2013) Ants (Hymenoptera: Formicidae) associated with *Diaphorina citri* (Hemiptera: Liviidae) and their role in its biological control. *Florida Entomologist*, 96, 590–597.
- Navarro-Campos, C., Wäckers, F.L. & Pekas, A. (2016) Impact of factitious foods and prey on the oviposition of the predatory mites *Gaeolaelaps aculeifer* and *Stratiolaelaps scimitus* (Acari: Laelapidae). *Experimental and Applied Acarology*, 70, 69–78.
- Nelson, W.R., Sengoda, V.G., Alfaro-Fernandez, A.O., Font, M.I., Crosslin, J.M. & Munyanzeza, J.E. (2013) A new haplotype of “*Candidatus Liberibacter solanacearum*”

- identified in the Mediterranean region. *European Journal of Plant Pathology*, 135, 633–639.
- New, T.R. (2017) *Alien species and insect conservation*. Springer, Switzerland, 230 pp.
- Nguyen, D.T., Bouguet, V., Spranghers, T., Vangansbeke, D. & De Clercq, P. (2015a) Beneficial effect of supplementing an artificial diet for *Amblyseius swirskii* with *Hermetia illucens* haemolymph. *Journal of Applied Entomology*, 139, 342–351.
- Nguyen, D.T., Vangansbeke, D. & De Clercq, P. (2014) Artificial and factitious foods support the development and reproduction of the predatory mite *Amblyseius swirskii*. *Experimental and Applied Acarology*, 62, 181–194.
- Nguyen, D.T., Vangansbeke, D. & De Clercq, P. (2015b) Performance of four species of phytoseiid mites on artificial and natural diets. *Biological Control*, 80, 56–62.
- Nguyen, D.T., Vangansbeke, D., Lü, X. & De Clercq, P. (2013) Development and reproduction of the predatory mite *Amblyseius swirskii* on artificial diets. *BioControl*, 58, 369–377.
- Nguyen, T.V. & Shih, C.I.T. (2010) Development of *Neoseiulus womersleyi* (Schicha) and *Euseius ovalis* (Evans) feeding on four tetranychid mites (Acari: Phytoseiidae, Tetranychidae) and pollen. *Journal of Asia-Pacific Entomology*, 13, 289–296.
- Nguyen, T.V. & Shih, C.I.T. (2012) Life-table parameters of *Neoseiulus womersleyi* (Schicha) and *Euseius ovalis* (Evans) (Acari: Phytoseiidae) feeding on six food sources. *International Journal of Acarology*, 38, 197–205.
- Ninkovic, V., Feng, Y., Olsson, U. & Pettersson, J. (2013) Ladybird footprints induce aphid avoidance behavior. *Biological Control*, 65, 63–71.
- Nomikou, M., Janssen, A., Schraag, R. & Sabelis, M.W. (2002) Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. *Experimental and Applied Acarology*, 27, 57–68.
- Nomikou, M., Janssen, A., Schraag, R. & Sabelis, M.W. (2004) Vulnerability of *Bemisia tabaci* immatures to phytoseiid predators: Consequences for oviposition and influence of alternative food. *Entomologia Experimentalis et Applicata*, 110, 95–102.
- Nomikou, M., Sabelis, M.W. & Janssen, A. (2010) Pollen subsidies promote whitefly control through the numerical response of predatory mites. *BioControl*, 55, 253–260.
- Novak, H. (1994) The influence of ant attendance on larval parasitism in hawthorn psyllids (Homoptera: Psyllidae). *Oecologia*, 99, 72–78.

- Numa Vergel, S.J., Bustos, R.A., Rodríguez, C.D. & Cantor, R.F. (2011) Laboratory and greenhouse evaluation of the entomopathogenic fungi and garlic-pepper extract on the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* and their effect on the spider mite *Tetranychus urticae*. *Biological Control*, 57, 143–149.
- O’Connell, D.M., Wratten, S.D., Pugh, A.R. & Barnes, A.M. (2012) ‘New species association’ biological control? Two coccinellid species and an invasive psyllid pest in New Zealand. *Biological Control*, 62, 86–92.
- Ochieng’, R.S., Oloo, G.W. & Amboga, E.O. (1987) An artificial diet for rearing the phytoseiid mite, *Amblyseius teke* Pritchard and Baker. *Experimental & Applied Acarology*, 3, 169–173.
- Ogawa, Y. & Osakabe, M. (2008) Development, long-term survival, and the maintenance of fertility in *Neoseiulus californicus* (Acari: Phytoseiidae) reared on an artificial diet. *Experimental and Applied Acarology*, 45, 123–136.
- Ogden, S.C. (2011) Tomato potato psyllid and liberibacter in New Zealand—impact and research programme overview. In: Workneh, F., Rashed, A. & Rush, C.M. (Eds) *Proceedings of the 11th annual Zebra Chip reporting session (November 6–9, 2011)*. San Antonio, TX, pp. 6–9.
- Oliver, T.H., Jones, I., Cook, J.M. & Leather, S.R. (2008) Avoidance responses of an aphidophagous ladybird, *Adalia bipunctata*, to aphid-tending ants. *Ecological Entomology*, 33, 523–528.
- Onzo, A., Bello, I.A. & Hanna, R. (2013) Effects of the entomopathogenic fungus *Neozygites tanajoae* and the predatory mite *Typhlodromalus aripo* on cassava green mite densities: Screenhouse experiments. *BioControl*, 58, 397–405.
- Onzo, A., Hanna, R., Negloh, K., Toko, M. & Sabelis, M.W. (2005) Biological control of cassava green mite with exotic and indigenous phytoseiid predators—Effects of intraguild predation and supplementary food. *Biological Control*, 33, 143–152.
- Orales, M.A. (2000) Mechanisms and density dependence of benefit in an ant—membracid mutualism. *Ecology*, 81, 482–489.
- Orondo, S.B.O. & Day, R.K. (1994) Cypress aphid (*Cinara cupressi*) damage to a cypress (*Cupressus lusitanica*) stand in Kenya. *International Journal of Pest Management*, 40, 141–144.
- Oveja, M.F., Riudavets, J., Arnó, J. & Gabarra, R. (2016) Does a supplemental food improve the effectiveness of predatory bugs on cucumber? *BioControl*, 61, 47–56.

- Overmeer, W.P.J. (1985) Rearing and handling. *In*: Helle, W. & Sabelis, M.W. (Eds). *Spider mites: their biology, natural enemies and control*, Elsevier, Amsterdam, 1, 161–170.
- Özgökçe, M.S., Chi, H., Atlıhan, R. & Kara, H. (2018) Demography and population projection of *Myzus persicae* (Sulz.) (Hemiptera: Aphididae) on five pepper (*Capsicum annuum* L.) cultivars. *Phytoparasitica*, 46, 153–167.
- Palumbo, J.C., Toscano, N.C., Blua, M.J. & Yoshida, H.A. (2000) Impact of *Bemisia* whiteflies (Homoptera: Aleyrodidae) on alfalfa growth, forage yield, and quality. *Journal of Economic Entomology*, 93, 1688–1694.
- Papadopoulos, G.D. & Papadoulis, G.T. (2008) Effect of seven different pollens on bio-ecological parameters of the predatory mite *Typhlodromus foenilis* (Acari: Phytoseiidae). *Environmental Entomology*, 37, 340–7.
- Parker, I.M., Simberloff, D., Lonsdale, W.M., Goodell, K., Wonham, M., Kareiva, P.M., Williamson, M.H., Von Holle, B., Moyle, P.B. & Byers, J.E. (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, 1, 3–19.
- Patel KS, Effectiveness of two predatory mites *Amblydromalus limonicus* and *Neoseiulus cucumeris* in controlling tomato/potato psyllids (*Bactericera cockerelli*). University of Auckland, Auckland, pp.113 (2017).
- Patel, K. & Zhang, Z.-Q. (2017a) Functional and numerical responses of *Amblydromalus limonicus* and *Neoseiulus cucumeris* to eggs and first instar nymph of tomato/potato psyllid (*Bactericera cockerelli*). *Systematic & Applied Acarology*, 22, 1476–1488.
- Patel, K. & Zhang, Z.-Q. (2017b) Prey preference and reproduction of predatory mites, *Amblydromalus limonicus* and *Neoseiulus cucumeris*, on eggs and 1st instar nymphs of the tomato/potato psyllid. *International Journal of Acarology*, 43, 468–474.
- Pelizza, S.A., Scorsetti, A.C. & Tranchida, M.C. (2013) The sublethal effects of the entomopathogenic fungus *Leptolegnia chapmanii* on some biological parameters of the dengue vector *Aedes aegypti*. *Journal of Insect Science*, 13, 22.
- Pell, J.K., Baverstock, J., Roy, H.E., Ware, R.L. & Majerus, M.E.N. (2008) Intraguild predation involving *Harmonia axyridis*: A review of current knowledge and future perspectives. *BioControl*, 53, 147–168.
- Peña, M.A. & Altmann, S.H. (2009) Use of satellite-derived hyperspectral indices to identify stress symptoms in an *Austrocedrus chilensis* forest infested by the aphid *Cinara cupressi*. *International Journal of Pest Management*, 55, 197–206.

- Pérez-González, O. & Sánchez-Peña, S.R. (2017) Compatibility *in Vitro* and *in Vivo* of the entomopathogenic fungi *Beauveria bassiana* and *Hirsutella citriformis* with selected insecticides. *Southwestern Entomologist*, 42, 707–718.
- Perring, T.M. (2001) The *Bemisia tabaci* species complex. *Crop Protection*, 20, 725–737.
- Persons, M.H., Walker, S.E. & Rypstra, A.L. (2001) Fitness costs and benefits of antipredator behavior mediated by chemotactile cues in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). *Behavioral Ecology*, 13, 386–392.
- Pina, T., Argolo, P.S., Urbaneja, A. & Jacas, J.A. (2012) Effect of pollen quality on the efficacy of two different life-style predatory mites against *Tetranychus urticae* in citrus. *Biological Control*, 61, 176–183.
- Pletsch, D.J. (1947) The potato psyllid, *Paratrioza cockerelli* (Sulc), its biology and control. *Montana Agricultural Experiment Station Bulletin*, 446, 1–95.
- Polis, G.A. (1981) The evolution and dynamics of intraspecific predation. *Annual Review of Ecology and Systematics*, 12, 225–251.
- Pozzebon, A. & Duso, C. (2010) Pesticide side-effects on predatory mites: the role of trophic interactions. In: Sabelis, M.W. & Bruin, J. (Eds). *Trends in acarology: Proceedings of the 12th international congress*. Springer, Netherlands, pp. 465–469.
- Pozzebon, A., Loeb, G.M. & Duso, C. (2015) Role of supplemental foods and habitat structural complexity in persistence and coexistence of generalist predatory mites. *Scientific Reports*, 5, 14997.
- Prager, S.M., Kund, G. & Trumble, J. (2016) Low-input, low-cost IPM program helps manage potato psyllid. *California Agriculture*, 70, 89–95.
- Prager, S.M. & Trumble, J.T. (2017) Psyllids: biology, ecology, and management. In: Wakil, W., Brust, G. & Perring, T. (Eds). *Sustainable management of arthropod pests of tomato*. Academic Press, New York, pp.161–181.
- Preisser, E.L., Bolnick, D.I. & Benard, M.F. (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86, 501–509.
- Pugh, A.R., O’Connell, D.M. & Wratten, S.D. (2015) Further evaluation of the southern ladybird (*Cleobora mellyi*) as a biological control agent of the invasive tomato-potato psyllid (*Bactericera cockerelli*). *Biological Control*, 90, 157–163.
- Pysek, P. & Richardson, D.M. (2010) Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources*, 35, 25–55.

- Pytlak, T., Fiedler, Ž. & Sosnowska, D. (2014) The influence of different spore concentrations of entomopathogenic and nematopathogenic fungi on survival of the predatory mite *Amblydromalus limonicus* (Garman & McGregor). *Progress in Plant Protection*, 54, 467–470.
- Qu, S. & Wang, S. (2018) Interaction of entomopathogenic fungi with the host immune system. *Developmental and Comparative Immunology*, 83, 96–103.
- Ramakers, P.M.J. (1983) Mass production and introduction of *Amblyseius mackenziei* and *Amblyseius cucumeris*. *IOBC/wprs Bulletin*, 6, 203–206.
- Ramakers, P.M.J. & Van-Lieburg, M.J. (1982) Start of commercial production and introduction of *Amblyseius mckenziei* Sch. and Pr. (Acarina: Phytoseiidae) for the control of *Thrips tabaci* Lind. (Thysanoptera: Thripidae) in glasshouses. *Rijksuniversiteit Faculteit Landbouwwetenschappen, Gent*, 47, 541–545.
- Ramírez-Ahuja, M. de L., Rodríguez-Leyva, E., Lomeli-Flores, J.R., Torres-Ruiz, A. & Guzmán-Franco, A.W. (2017) Evaluating combined use of a parasitoid and a zoophytophagous bug for biological control of the potato psyllid, *Bactericera cockerelli*. *Biological Control*, 106, 9–15.
- Ranabhat, N.B., Goleva, I. & Zebitz, C.P.W. (2014) Life tables of *Neoseiulus cucumeris* exclusively fed with seven different pollens. *BioControl*, 59, 195–203.
- Rao, K.S., Padmanabhan, A. & Vishnupriya, R. (2018) Recent update on the role of predatory mites in biological control programmes. *Research Journal of Agricultural Sciences*, 9, 473–479.
- Rasmy, A.H. & El-Banhawy, E.M. (1974) Behaviour and bionomics of the predatory mite, *Phytoseius plumifer* [Acarina: Phytoseiidae] as affected by physical surface features of host plants. *Entomophaga*, 19, 255–257.
- Reiter, P. (2010) Yellow fever and dengue: A threat to Europe? *Eurosurveillance*, 15, 11–17.
- Remaudiere, G. & Remaudiere, M. (1997) *Catalogue des Aphididae du Monde (Catalogue of the world's Aphididae)*. Homoptera. Aphidoidea INRA editions, Paris, 473 pp.
- Riahi, E., Fathipour, Y., Talebi, A.A. & Mehrabadi, M. (2016) Pollen quality and predator viability: Life table of *Typhlodromus bagdasarjani* on seven different plant pollens and two-spotted spider mite. *Systematic and Applied Acarology*, 21, 1399–1412.
- Riahi, E., Fathipour, Y., Talebi, A.A. & Mehrabadi, M. (2017a) Linking life table and consumption rate of *Amblyseius swirskii* (Acari: Phytoseiidae) in presence and absence of different pollens. *Annals of the Entomological Society of America*, 110, 244–253.

- Riahi, E., Fathipour, Y., Talebi, A.A. & Mehrabadi, M. (2017b) Natural diets versus factitious prey: Comparative effects on development, fecundity and life table of *Amblyseius swirskii* (Acari: Phytoseiidae). *Systematic and Applied Acarology*, 22, 711–723.
- Ricciardi, A., Blackburn, T.M., Carlton, J.T., Dick, J.T.A., Hulme, P.E., Iacarella, J.C., Jeschke, J.M., Liebhold, A.M., Lockwood, J.L., MacIsaac, H.J., Pyšek, P., Richardson, D.M., Ruiz, G.M., Simberloff, D., Sutherland, W.J., Wardle, D.A. & Aldridge, D.C. (2017) Invasion science: A horizon scan of emerging challenges and opportunities. *Trends in Ecology and Evolution*, 32, 464–474.
- Richards, B.L. (1928) A new and destructive disease of the potato in Utah and its relation to the potato psylla. *Phytopathology*, 18, 140–141.
- Richards, B.L. (1931) Further studies with psyllid yellows of the potato. *Phytopathology*, 21, 103.
- Richardson, M.L., Mitchell, R.F., Reagel, P.F. & Hanks, L.M. (2010) Causes and consequences of cannibalism in noncarnivorous insects. *Annual Review of Entomology*, 55, 39–53.
- Riddick, E.W. (2009) Benefits and limitations of factitious prey and artificial diets on life parameters of predatory beetles, bugs, and lacewings: A mini-review. *BioControl*, 54, 325–339.
- Van Rijn, P.C.J., Van Houten, Y.M. & Sabelis, M.W. (1999) Pollen improves thrips control with predatory mites. *IOBC/wprs Bulletin*, 22, 209–212.
- Van Rijn, P.C.J. & Sabelis, M.W. (1990) Pollen availability and its effect on the maintenance of populations of *Amblyseius cucumeris*, a predator of thrips. *Medical Faculty Landbouwkundige Rijksuniversiteit Gent*, 55, 335–341.
- Van Rijn, P.C.J. & Tanigoshi, L.K. (1999) Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): Dietary range and life history. *Experimental and Applied Acarology*, 23, 785–802.
- Van Rijn, P.C.J., Houten, Y.M. & Sabelis, M.W. (2002) Plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology*, 83, 2664–2679.
- Rios-velasco, C., Pérez-corrall, D.A., Salas-marina, M.Á., Berlanga-reyes, D.I., Ornelas-paz, J.J., Muñiz, C.H.A., Cambero-campos, J. & Jacobo-cuellar, J.L. (2014) Pathogenicity of the Hypocreales fungi *Beauveria bassiana* and *Metarhizium anisopliae* against insect pests of tomato. *Southwestern Entomologist*, 39, 739–750.

- Roberts, D.W. & Hajek, A.E. (1992) Entomopathogenic fungi as bioinsecticides. *In: Leatham, G.F. (Ed.). Frontiers in industrial mycology*. Chapman & Hall, Inc., Routledge, pp. 144–159.
- Roda, A., Nyrop, J. & English-Loeb, G. (2003) Leaf pubescence mediates the abundance of non-prey food and the density of the predatory mite *Typhlodromus pyri*. *Experimental and Applied Acarology*, 29, 193–211.
- Roda, A., Nyrop, J., English-Loeb, G. & Dicke, M. (2001) Leaf pubescence and two-spotted spider mite webbing influence phytoseiid behavior and population density. *Oecologia*, 129, 551–560.
- Roditakis, E., Roditakis, N.E. & Tsagkarakou, A. (2005) Insecticide resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) populations from Crete. *Pest Management Science*, 61, 577–582.
- Rodríguez-Cruz, F.A., Venzon, M. & Pinto, C.M.F. (2013) Performance of *Amblyseius herbicolus* on broad mites and on castor bean and sunnhemp pollen. *Experimental and Applied Acarology*, 60, 497–507.
- Rojas, P., Rodríguez-Leyva, E., Refugio Lomeli-Flores, J. & Liu, T.X. (2015) Biology and life history of *Tamarixia triozae*, a parasitoid of the potato psyllid *Bactericera cockerelli*. *BioControl*, 60, 27–35.
- Roques, A., Rabitsch, W., Rasplus, J.Y., Lopez-Vaamonde, C., Nentwig, W. & Kenis, M. (2009) Alien terrestrial invertebrates of Europe. *In: Drake, J.A. (Ed) Handbook of alien species in Europe*. Amsterdam, Springer, pp 63–79.
- Rosson, P. (2009) Economic impacts of Zebra Chip on Texas. CNAS Issue Brief 2009-01. January 27, 2009.
- Rotem, K.A. & Agrawal, A.A. (2003) Density dependent population growth of the two-spotted spider mite, *Tetranychus urticae*, on the host plant *Leonurus cardiaca*. *Oikos*, 103, 559–565.
- Roush, R.T. & Daly, J.C. (1990) The role of population genetics in resistance research and management. *In: Roush, R.T. & Tabashnik, B.E. (Eds). Pesticide resistance in arthropods*. Springer, Boston, MA, pp. 97–152.
- Roux, O., Vantaux, A., Roche, B., Yameogo, K.B., Diabate, A., Simard, F., Lefevre, T., Dabire, K.R., Diabate, A., Simard, F. & Lefevre, T. (2015) Evidence for carry-over effects of predator exposure on pathogen transmission potential. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20152430.

- Roy, H.E. & Wajnberg, E. (2008) From biological control to invasion: The ladybird *Harmonia axyridis* as a model species. *Biocontrol*, 53, 1–4.
- Sabelis, M.W. (1981) Biological control of two-spotted spider mites using phytoseiid predators. Part I: Modelling the predator-prey interaction at the individual level (Ph.D. thesis). Wageningen Agricultural University, Wageningen, the Netherlands, 480 pp.
- Saito, T. & Brownbridge, M. (2016) Compatibility of soil-dwelling predators and microbial agents and their efficacy in controlling soil-dwelling stages of western flower thrips *Frankliniella occidentalis*. *Biological Control*, 92, 92–100.
- Saito, T. & Brownbridge, M. (2018) Compatibility of foliage-dwelling predatory mites and mycoinsecticides, and their combined efficacy against western flower thrips *Frankliniella occidentalis*. *Journal of Pest Science*, 91, 1291–1300.
- Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Norman, C., Mccauley, D.E., Neil, P.O., Parker, I.M., Thompson, J.N., Weller, S.G., Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, M., Molofsky, J., With, K.A., Cabin, R.J., Cohen, J.E., Norman, C., Mccauley, D.E., Neil, P.O., Parker, M., Thompson, J.N. & Weller, S.G. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, 32, 305–332.
- Samaras, K., Pappas, M.L., Fytas, E. & Broufas, G.D. (2015) Pollen suitability for the development and reproduction of *Amblydromalus limonicus* (Acari: Phytoseiidae). *BioControl*, 60, 773–782.
- Sánchez-Peña, S.R., Casas-De-Hoyo, E., Hernandez-Zul, R. & Wall, K.M. (2007) A comparison of the activity of soil fungal isolates against three insect pests. *Journal of Agricultural and Urban Entomology*, 24, 43–48.
- Schausberger, P. (2003) Cannibalism among phytoseiid mites: A review. *Experimental and Applied Acarology*, 29, 173–191.
- Schausberger, P. & Croft, B.A. (2000a) Cannibalism and intraguild predation among phytoseiid mites: Are aggressiveness and prey preference related to diet specialization? *Experimental and Applied Acarology*, 24, 709–725.
- Schausberger, P. & Croft, B.A. (2000b) Nutritional benefits of intraguild predation and cannibalism among generalist and specialist phytoseiid mites. *Ecological Entomology*, 25, 473–480.
- Schmidt, R.A. (2014) Leaf structures affect predatory mites (Acari: Phytoseiidae) and biological control: A review. *Experimental and Applied Acarology*, 62, 1–17.

- Schowalter, T.D. & Ring, D.R. (2017) Biology and management of the fall webworm, *Hyphantria cunea* (Lepidoptera: Erebidae). *Journal of Integrated Pest Management*, 8, 1–6.
- Seiedy, M. (2014) Feeding preference of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) towards untreated and *Beauveria bassiana*-treated *Tetranychus urticae* (Acari: Tetranychidae) on cucumber leaves. *Persian Journal of Acarology*, 3, 91–97.
- Seiedy, M. (2015) Compatibility of *Amblyseius swirskii* (Acari: Phytoseiidae) and *Beauveria bassiana* for biological control of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Systematic and Applied Acarology*, 20, 731–738.
- Seiedy, M. & Moezipour, M. (2017) The entomopathogenic fungus *Beauveria bassiana* and its compatibility with *Phytoseiulus persimilis* (Acari: Phytoseiidae): Effects on *Tetranychus urticae* (Acari: Tetranychidae) Marjan. *Persian Journal of Acarology*, 6, 329–338.
- Seiedy, M., Saboori, A. & Allahyari, H. (2012a) Interactions of two natural enemies of *Tetranychus urticae*, the fungal entomopathogen *Beauveria bassiana* and the predatory mite, *Phytoseiulus persimilis*. *Biocontrol Science and Technology*, 22, 873–882.
- Seiedy, M., Saboori, A., Allahyari, H., Talaei-Hassanloui, R. & Tork, M. (2012b) Functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae) on untreated and *Beauveria bassiana*-treated adults of *Tetranychus urticae* (Acari: Tetranychidae). *Journal of Insect Behavior*, 25, 543–553.
- Seiedy, M., Saboori, A. & Zahedi-Golpayegani, A. (2013) Olfactory response of *Phytoseiulus persimilis* (Acari: Phytoseiidae) to untreated and *Beauveria bassiana*-treated *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology*, 60, 219–227.
- Seiedy, M., Tork, M. & Deyhim, F. (2015) Effect of the entomopathogenic fungus *Beauveria bassiana* on the predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae) as a nontarget organism. *Systematic and Applied Acarology*, 20, 241–250.
- Sengoda, V.G., Cooper, W.R., Swisher, K.D., Henne, D.C. & Munyaneza, J.E. (2014) Latent period and transmission of “*Candidatus liberibacter solanacearum*” by the potato psyllid *Bactericera cockerelli* (Hemiptera: Triozidae). *PLoS ONE*, 9, 1–10.
- Seyed-Talebi, F.-S., Kheradmand, K., Talaei-Hassanloui, R. & Talebi-Jahromi, K. (2012) Sublethal effects of *Beauveria bassiana* on life table parameters of two-spotted spider

- mite, *Tetranychus urticae* (Acari: Tetranychidae). *Biocontrol Science and Technology*, 22, 293–303.
- Shaef, M. & Lim, U.T. (2017) Synergism of *Beauveria bassiana* and *Phytoseiulus persimilis* in control of *Tetranychus urticae* on bean plants. *Systematic & Applied Acarology*, 22, 1924–1935.
- Shakya, S., Weintraub, P.G. & Coll, M. (2009) Effect of pollen supplement on intraguild predatory interactions between two omnivores: The importance of spatial dynamics. *Biological Control*, 50, 281–287.
- Shang, S., Chen, Y. & Bai, Y. (2018) The pathogenicity of entomopathogenic fungus *Acremonium hansfordii* to two-spotted spider mite, *Tetranychus urticae* and predatory mite *Neoseiulus barkeri*. *Systematic & Applied Acarology*, 23, 2173–2183.
- Sharma, S., Agarwal, G.P. & Rajak, R.C. (1994) Pathophysiological alterations caused in *Heliothis armigera* by toxic metabolites of *Beauveria bassiana* (Bals) Vuill. *Indian Journal of Experimental Biology*, 32, 168–171.
- Shehata, K.K. & Weismann, L. (1972) Rearing the predacious mite *Phytoseiulus persimilis* Athias-Henriot on artificial diet (Acarina: Phytoseiidae). *Biologia Bratislava*, 27, 609–615.
- Shereef, G.M., Soliman, Z.R. & Afifi, A.M. (1980) Economic importance of the mite, *Hypoaspis miles* (Berlese) (Mesostigmata: Laelapidae) and its life history. *Bulletin de la Societe Entomologique d' Egypt*, 30, 130–138.
- Shih, C.I.T., Chang, H.Y., Hsu, P.H. & Hwang, Y.F. (1993) Responses of *Amblyseius ovalis* (Evans) (Acarina: Phytoseiidae) to natural food resources and two artificial diets. *Experimental and Applied Acarology*, 17, 503–519.
- Shipp, J.L., Wang, K. & Binns, M. (2000) Economic injury level western flower thrips (Thysanoptera: Thripidae) on greenhouse cucumber. *Journal of Economic Entomology*, 93, 1732–1740.
- Sidhu, C.S. & Wilson Rankin, E.E. (2016) Honey bees avoiding ant harassment at flowers using scent cues. *Environmental Entomology*, 45, 420–426.
- Simoni, S., Nannelli, R., Goggioli, D., Guidi, S. & Castagnoli, M. (2006) Biological and demographic parameters of *Neoseiulus californicus* (McGregors) (Acari Phytoseiidae) reared on two astigmatid mites. *Redia*, 89, 59–63.
- Sitvarin, M.I., Breen, K. & Rypstra, A.L. (2015) Predator cues have contrasting effects on lifespan of *Pardosa milvina* (Araneae: Lycosidae). *Journal of Arachnology*, 43, 107–110.

- Škaloudová, B., Zemek, R. & Křivan, V. (2007) The effect of predation risk on an acarine system. *Animal Behaviour*, 74, 813–821.
- Sloggett, J.J. & Majerus, M.E.N. (2003) Adaptations of *Coccinella magnifica*, a myrmecophilous coccinellid to aggression by wood ants (*Formica rufa* group). II. Larval behaviour, and ladybird oviposition location. *European Journal of Entomology*, 100, 337–344.
- Slos, S. & Stoks, R. (2008) Predation risk induces stress proteins and reduces antioxidant defense. *Functional Ecology*, 22, 637–642.
- Stadler, B. & Dixon, A.F.G. (2005) Ecology and evolution of aphid-ant interactions. *Annual Review of Ecology, Evolution, and Systematics*, 36, 345–372.
- Van Stappen, G. (1996) Use of cysts. In: Lavens, P. & Sorgeloos, P. (Eds), *Manual on the production and use of live food for aquaculture*. FAO Fisheries Technical Paper No. 361, Rome, pp. 107–136.
- Steel, R.G.D., Torrie, J.H. & Dickey, D.A. (1980) *Principles and procedures of statistics: A biometrical approach*. McGraw-Hill, New York.
- Stewart-Jones, A., Pope, T.W., Fitzgerald, J.D. & Poppy, G.M. (2008) The effect of ant attendance on the success of rosy apple aphid populations, natural enemy abundance and apple damage in orchards. *Agricultural and Forest Entomology*, 10, 37–43.
- Styrsky, J.D. & Eubanks, M.D. (2007) Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B: Biological Sciences*, 274, 151–164.
- Su, K.F.Y.Y. & Li, D. (2006) Female-biased predation risk and its differential effect on the male and female courtship behaviour of jumping spiders. *Animal Behaviour*, 71, 531–537.
- Sudo, M. & Osakabe, M. (2013) Stellate hairs on leaves of a deciduous shrub *Viburnum erosum* var. *punctatum* (Adoxaceae) effectively protect *Brevipalpus obovatus* (Acari: Tenuipalpidae) eggs from the predator *Phytoseius nipponicus* (Acari: Phytoseiidae). *Experimental and Applied Acarology*, 60, 299–311.
- Sulc, K. (1909) *Trioza cockerelli* n. sp., a novelty from North America, being also of economic importance. *Acta Societatis Entomologicae Bohemiae*, 6, 102–108.
- Sun, W., Sarkar, S.C., Xu, X., Lei, Z., Wu, S. & Meng, R. (2018) The entomopathogenic fungus *Beauveria bassiana* used as granules has no impact on the soil-dwelling predatory mite *Stratiolaelaps scimitus*. *Systematic & Applied Acarology*, 23, 2165–2172.

- Tamayo-Mejía, F., Tamez-Guerra, P., Guzmán-Franco, A.W. & Gomez-Flores, R. (2015) Can *Beauveria bassiana* Bals. (Vuill) (Ascomycetes: Hypocreales) and *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) be used together for improved biological control of *Bactericera cockerelli* (Hemiptera: Triozidae)? *Biological Control*, 90, 42–48.
- Tamayo-Mejía, F., Tamez-Guerra, P., Guzmán-Franco, A.W., Gomez-Flores, R. & Cruz-Cota, L.R. (2014) Efficacy of entomopathogenic fungi (Hypocreales) for *Bactericera cockerelli* (Sulc.) (Hemiptera: Triozidae) control in the laboratory and field. *Southwestern Entomologist*, 39, 271–284.
- Teulon, D.A.J., Workman, P.J., Thomas, K.L. & Nielsen, M.C. (2009) *Bactericera cockerelli*: Incursion, dispersal and current distribution on vegetable crops in New Zealand. *New Zealand Plant Protection*, 62, 136–144.
- Thaler, J.S., McArt, S.H. & Kaplan, I. (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proceedings of the National Academy of Sciences*, 109, 12075–12080.
- Thinakaran, J., Yang, X.B., Munyaneza, J.E., Rush, C.M. & Henne, D.C. (2015) Comparative biology and life tables of “*Candidatus Liberibacter Solanacearum*”-infected and -free *Bactericera cockerelli* (Hemiptera: Triozidae) on potato and silverleaf nightshade. *Annals of the Entomological Society of America*, 108, 459–467.
- Thomas, K.L., Jones, D.C., Kumarasinghe, L.B., Richmond, J.E., Gill, G.S.C. & Bullians, M.S. (2011) Investigation into the entry pathway for tomato potato psyllid *Bactericera cockerelli*. *New Zealand Plant Protection*, 64, 259–268.
- Throne, J.E., Weaver, D.K., Chew, V. & Baker, J.E. (1995) Probit analysis of correlated data: multiple observations over time at one concentration. *Journal of Economical Entomology*, 88, 1510–1512.
- Tigeros, N., Norris, R.H., Wang, E.H. & Thaler, J.S. (2017) Maternally induced intraclutch cannibalism: an adaptive response to predation risk? *Ecology Letters*, 20, 487–494.
- Tilney, P.M., Van Wyk, A.E. & Van Der Merwe, C.F. (2012) Structural evidence in *Plectroniella armata* (Rubiaceae) for possible material exchange between domatia and mites. *PLoS ONE*, 7, 1–6.
- Tolley, I.S. (1990) The relation of nursery production with orchard planning and management. *In: Rehabilitation of Citrus Industry in the Asia Pacific Region. Proceeding of the 4th International Asia Pacific Conference on Citriculture Rehabilitation (4–10th Feb. 1990)*. Chiang Mai, Thailand, pp. 77–82.

- Torrado-León, E., Montoya-Lerma, J. & Valencia-Pizo, E. (2006) Sublethal effects of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) on the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) under laboratory conditions. *Mycopathologia*, 162, 411–419.
- Torres Acosta, R.I., Humber, R.A. & Sánchez-Peña, S.R. (2016) *Zoophthora radicans* (Entomophthorales), a fungal pathogen of *Bagrada hilaris* and *Bactericera cockerelli* (Hemiptera: Pentatomidae and Triozidae): Prevalence, pathogenicity, and interplay of environmental influence, morphology, and sequence data on. *Journal of Invertebrate Pathology*, 139, 82–91.
- Tran, L. (2012) Population phenology, life table and forecasting models of tomato-potato psyllid (*Bactericera cockerelli*) and the efficiency of a selected natural enemy for its control (Ph.D. thesis). Lincoln University, New Zealand.
- Trandem, N., Berdinesen, R., Pell, J.K. & Klingens, I. (2016) Interactions between natural enemies: Effect of a predatory mite on transmission of the fungus *Neozygites floridana* in two-spotted spider mite populations. *Journal of Invertebrate Pathology*, 134, 35–37.
- Tsolakis, H., Principato, D., Jordà Palomero, R. & Lombardo, A. (2016) Biological and life table parameters of *Typhlodromus laurentii* and *Iphiseius degenerans* (Acari, Phytoseiidae) fed on *Panonychus citri* and pollen of *Oxalis pes-caprae* under laboratory conditions. *Experimental and Applied Acarology*, 70, 205–218.
- Ullah, M.S. & Lim, U.T. (2017) Laboratory evaluation of the effect of *Beauveria bassiana* on the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Journal of Invertebrate Pathology*, 148, 102–109.
- Umeya, K. (1977) Invasion and establishment of a new insect pest in Japan. *Adaptation and Speciation in the Fall Webworm*, 1–12.
- Vangansbeke, D., Nguyen, D.T., Audenaert, J., Gobin, B., Tirry, L. & De Clercq, P. (2016a) Establishment of *Amblyseius swirskii* in greenhouse crops using food supplements. *Systematic and Applied Acarology*, 21, 1174–1184.
- Vangansbeke, D., Nguyen, D.T., Audenaert, J., Verhoeven, R., Deforce, K., Gobin, B., Tirry, L. & De Clercq, P. (2014a) Diet-dependent cannibalism in the omnivorous phytoseiid mite *Amblydromalus limonicus*. *Biological Control*, 74, 30–35.
- Vangansbeke, D., Nguyen, D.T., Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2014b) Performance of the predatory mite *Amblydromalus limonicus* on factitious foods. *BioControl*, 59, 67–77.

- Vangansbeke, D., Nguyen, D.T., Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2014c) Food supplementation affects interactions between a phytoseiid predator and its omnivorous prey. *Biological Control*, 76, 95–100.
- Vangansbeke, D., Nguyen, D.T., Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2016b) Supplemental food for *Amblyseius swirskii* in the control of thrips: Feeding friend or foe? *Pest Management Science*, 72, 466–473.
- Vantornhout, I., Minnaert, H., Tirry, L. & De Clercq, P. (2004) Effect of pollen, natural prey and factitious prey on the development of *Iphiseius degenerans*. *BioControl*, 49, 627–644.
- Vereijssen, J., Smith, G.R. & Weintraub, P.G. (2018) *Bactericera cockerelli* (Hemiptera: Triozidae) and *Candidatus Liberibacter solanacearum* in potatoes in New Zealand: Biology, transmission, and implications for management. *Journal of Integrated Pest Management*, 9, 1–21.
- Vidal, C., Fargues, J., Ekesi, S. & Maniania, N.K. (2007) Climatic constraints for fungal bioinsecticides. In: Ekesi, S., Maniania, N.K. (Eds.). *Use of entomopathogenic fungi in biological pest management*. Research Signpost, Kerala, India, pp. 39–55.
- Villegas-Rodriguez, F., Diaz-Gomez, O., Sergio Casas-Flores, J., Monreal-Vargas, T.C., Tamayo-Mejia, F. & Aguilar-Medel, S. (2017) Activity of two entomopathogenic fungi, molecularly identified, on *Bactericera cockerelli*. *Revista Colombiana de Entomología*, 43, 27–33.
- Villegas-Rodriguez, F., Marin-Sanchez, J., Delgado-Sanchez, P., Torres-Castillo, J.A. & Alvarado-Gomez, O.G. (2014) Management of *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) in greenhouses with entomopathogenic fungi (Hypocreales). *Southwestern Entomologist*, 39, 613–624.
- Wäckers, F.L. (2005) Suitability of (extra-) floral nectar, pollen and honeydew as insect food sources. In: Wäckers, F.L., Van Rijn, P.C.J. & Bruin, J. (Eds). *Plant-provided food for carnivorous insects: A protective mutualism and its applications*. Cambridge university press, Cambridge, UK, pp. 17–74.
- Wagner, D.L. & Van Driesche, R.G. (2010) Threats posed to rare or endangered insects by invasions of nonnative species. *Annual Review of Entomology*, 55, 547–568.
- Walker, G.P., MacDonald, F.H., Larsen, N.J. & Wallace, A.R. (2011) Monitoring *Bactericera cockerelli* and associated insect populations in potatoes in South Auckland. *New Zealand Plant Protection*, 64, 269–275.

- Walker, G.P., MacDonald, F.H., Wright, P.J., Puketapu, A.J., Gardner-Gee, R., Connolly, P.G. & Anderson, J.A.D. (2015) Development of action thresholds for management of *Bactericera cockerelli* and Zebra Chip disease in potatoes at Pukekohe, New Zealand. *American Journal of Potato Research*, 92, 266–275.
- Walls, S.C. (1998) Density dependence in a larval salamander: The effects of interference and food limitation. *Copeia*, 4, 926–935.
- Walter, D.E. & O’Dowd, D.J. (1992) Leaf morphology and predators: Effect of leaf domatia on the abundance of predatory mites (Acari: Phytoseiidae). *Environmental Entomology*, 21, 478–484.
- Wang, D., Zang, L.-S., Zhang, Y., Wang, H. & Lei, Z.R. (2014) Sublethal effects of *Beauveria bassiana* Balsamo on life table parameters of subsequent generations of *Bemisia tabaci* Gennadius. *Scientia Agricultura Sinica*, 47, 3588–3595.
- Wang, S.Y., Chi, H. & Liu, T.X. (2016) Demography and parasitic effectiveness of *Aphelinus asychis* reared from *Sitobion avenae* as a biological control agent of *Myzus persicae* reared on chili pepper and cabbage. *Biological Control*, 92, 111–119.
- Wang, Y., Yu, N., Chu, F., Korai, S.K. & Liu, Z. (2018) Sublethal effects of *Beauveria bassiana* sensu lato isolate NJBb2101 on biological fitness and insecticide sensitivity of parental and offspring generations of brown planthopper, *Nilaparvata lugens*. *Biological Control*, 121, 44–49.
- Weintraub, P., Kleitman, S., Mori, R., Gan-Mor, S., Ganot, L. & Palevsky, E. (2009) Novel application of pollen to augment the predator *Amblyseius swirskii* on greenhouse sweet pepper. *IOBC/wprs Bulletin*, 50, 119–124.
- Wekesa, V.W., Moraes, G.J., Knapp, M. & Delalibera, I. (2007) Interactions of two natural enemies of *Tetranychus evansi*, the fungal pathogen *Neozygites floridana* (Zygomycetes: Entomophthorales) and the predatory mite, *Phytoseiulus longipes* (Acari: Phytoseiidae). *Biological Control*, 41, 408–414.
- Wen, M.-F., Chi, H., Lian, Y.-X., Zheng, Y.-H., Fan, Q.-H. & You, M.-S. (2017) Population characteristics of *Macrocheles glaber* (Acari: Macrochelidae) and *Stratiolaelaps scimitus* (Acari: Laelapidae) reared on a mushroom fly *Coboldia fuscipes* (Diptera: Scatopsidae). *Insect Science*, DOI 10.1111/1744-7917.12511.
- Werner, E.E. & Peacor, S.D. (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology*, 84, 1083–1100.

- Wildy, E.L., Chivers, D.P., Kiesecker, J.M. & Blaustein, A.R. (2001) The effects of food level and conspecific density on biting and cannibalism in larval long-toed salamanders, *Ambystoma macrodactylum*. *Oecologia*, 128, 202–209.
- Wong, S.K. & Frank, S.D. (2013) Pollen increases fitness and abundance of *Orius insidiosus* Say (Heteroptera: Anthocoridae) on banker plants. *Biological Control*, 64, 45–50.
- Workman, P.J., Dymock, J.J., Martin, N.A., Ennis, I.L. & Popay, A.J. (1994) The potential for biological control of thrips in greenhouse capsicums using the predatory mite *Amblyseius cucumeris*. In: *Proceedings of the New Zealand Plant Protection Conference*, pp. 139–143.
- Wright, P.J., Walker, G.P., MacDonald, F.H., Gardner-Gee, R. & Hedderley, D.I. (2017) Mineral oil foliar applications in combination with insecticides affect tomato potato psyllid (*Bactericera cockerelli*) and beneficial insects in potato crops. *New Zealand Journal of Crop and Horticultural Science*, 45, 263–276.
- Wu, H. (1994) Preliminary studies on *Anystis baccarun* (Linnaeus) (Acari: Anystidae). *Natural Enemies of Insects*, 16, 101–106.
- Wu, S., Gao, Y., Smaghe, G., Xu, X. & Lei, Z. (2016a) Interactions between the entomopathogenic fungus *Beauveria bassiana* and the predatory mite *Neoseiulus barkeri* and biological control of their shared prey/host *Frankliniella occidentalis*. *Biological Control*, 98, 43–51.
- Wu, S., Gao, Y., Xu, X., Wang, D., Li, J., Wang, H., Wang, E. & Lei, Z. (2015) Feeding on *Beauveria bassiana*-treated *Frankliniella occidentalis* causes negative effects on the predatory mite *Neoseiulus barkeri*. *Scientific Reports*, 5, 12033.
- Wu, S., Gao, Y., Zhang, Y., Wang, E., Xu, X. & Lei, Z. (2014) An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus barkeri*: Evidence from laboratory bioassay and scanning electron microscopic observation. *PLoS ONE*, 9, e84732.
- Wu, S., Guo, J., Xing, Z., Gao, Y., Xu, X. & Lei, Z. (2018a) Comparison of mechanical properties for mite cuticles in understanding passive defense of phytoseiid mite against fungal infection. *Materials & Design*, 140, 241–248.
- Wu, S., He, Z., Wang, E., Xu, X. & Lei, Z. (2017) Application of *Beauveria bassiana* and *Neoseiulus barkeri* for improved control of *Frankliniella occidentalis* in greenhouse cucumber. *Crop Protection*, 96, 83–87.

- Wu, S., Xie, H., Li, M., Xu, X. & Lei, Z. (2016b) Highly virulent *Beauveria bassiana* strains against the two-spotted spider mite, *Tetranychus urticae*, show no pathogenicity against five phytoseiid mite species. *Experimental and Applied Acarology*, 70, 421–435.
- Wu, S., Xing, Z., Sun, W., Xu, X., Meng, R. & Lei, Z. (2018b) Effects of *Beauveria bassiana* on predation and behavior of the predatory mite *Phytoseiulus persimilis*. *Journal of Invertebrate Pathology*, 153, 51–56.
- Wu, S., Youngman, R.R., Kok, L.T. & Laub, C.A. (2016c) Sublethal effect of *Beauveria bassiana* and *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) on *Cyclocephala lurida* (Coleoptera: Scarabaeidae). *Journal of Entomological Science*, 51, 43–53.
- Wu, S., Zhang, Y., Xu, X. & Lei, Z. (2016d) Insight into the feeding behavior of predatory mites on *Beauveria bassiana*, an arthropod pathogen. *Scientific Reports*, 24062.
- Wyckhuys, K.A.G., Kondo, T., Herrera, B.V., Miller, D.R., Naranjo, N., & Hyman, G. (2013) Invasion of exotic arthropods in South America's biodiversity hotspots and agro-production systems. In: Peña, J. (Ed.) *Potential invasive pests of agricultural crops*. CAB International, Wallingford, pp. 373–400.
- Xie, L., Yan, Y. & Zhang, Z.Q. (2018) Development, survival and reproduction of *Stratiolaelaps scimitus* (Acari: Laelapidae) on four diets. *Systematic and Applied Acarology*, 23, 779–794.
- Xie, Z.N., Wu, Z.X., Nettles, W.C., Saldaña, G. & Nordlund, D.A. (1997) In vitro culture of *Trichogramma* spp. on artificial diets containing yeast extract and ultracentrifuged chicken egg yolk but devoid of insect components. *Biological Control*, 8, 107–110.
- Xiong, X., Michaud J.P., Zhen, L., Wu, P., Chu, Y., Zhang, Q. & Liu, X. (2015) Chronic, predator-induced stress alters development and reproductive performance of the cotton bollworm, *Helicoverpa armigera*. *BioControl*, 60, 827–837.
- Xu, H.-Y., Yang, N.-W., Chi, H., Ren, G.-D. & Wan, F.-H. (2018) Comparison of demographic fitness and biocontrol effectiveness of two parasitoids, *Encarsia sophia* and *Eretmocerus hayati* (Hymenoptera: Aphelinidae) against *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science*, 74, 2116–2124.
- Xu, Y. & Zhang, Z.Q. (2015) *Amblydromalus limonicus*: a “new association” predatory mite against an invasive psyllid (*Bactericera cockerelli*) in New Zealand. *Systematic and Applied Acarology*, 20, 375–382.

- Xu, Y.J., Luo, F., Gao, Q., Shang, Y. & Wang, C. (2015) Metabolomics reveals insect metabolic responses associated with fungal infection. *Analytical and Bioanalytical Chemistry*, 407, 4815–4821.
- Yamanaka, T., Morimoto, N., Nishida, G.M., Kiritani, K., Moriya, S. & Liebhold, A.M. (2015) Comparison of insect invasions in North America, Japan and their Islands. *Biological Invasions*, 17, 3049–3061.
- Yang, X.-B. & Liu, T.-X. (2009) Life history and life tables of *Bactericera cockerelli* (Homoptera: Psyllidae) on eggplant and bell pepper. *Environmental entomology*, 38, 1661–1667.
- Yang, X.-B., Zhang, Y.-M., Henne, D.C. & Liu, T.-X. (2013) Life tables of *Bactericera cockerelli* (Hemiptera: Triozidae) on tomato under laboratory and field conditions in Southern Texas. *Florida Entomologist*, 96, 904–913.
- Yao, J., Saenkham, P., Levy, J., Ibanez, F., Noroy, C., Mendoza, A., Huot, O., Meyer, D.F. & Tamborindeguy, C. (2016) Interactions “*Candidatus Liberibacter solanacearum*”—*Bactericera cockerelli*: Haplotype effect on vector fitness and gene expression Analyses. *Frontiers in Cellular and Infection Microbiology*, 6, 1–13.
- Yu, J.-Z., Chi, H. & Chen, B.-H. (2013) Comparison of the life tables and predation rates of *Harmonia dimidiata* (F.) (Coleoptera: Coccinellidae) fed on *Aphis gossypii* Glover (Homoptera: Aphididae) at different temperatures. *Biological Control*, 64, 1–9.
- Yu, J., Chi, H. & Chen, B.-H. (2005) Life table and predation of *Lemnia bipagiata* (Coleoptera: Coccinellidae) fed on *Aphis gossypii* (Homoptera: Aphididae) with a proof on relationship among gross reproduction rate, net reproduction rate, and preadult survivorship. *Annals of the Entomological Society of America*, 98, 475–482.
- Yuan, H., Wu, S. yong, Lei, Z.R., Rondon, S.I. & Gao, Y.L. (2018) Sub-lethal effects of *Beauveria bassiana* (Balsamo) on field populations of the potato tuberworm *Phthorimaea operculella* Zeller in China. *Journal of Integrative Agriculture*, 17, 60345-7.
- Zhang, P., Liu, F., Mu, W., Wang, Q., Li, H. & Chen, C. (2014) Life table study of the effects of sublethal concentrations of thiamethoxam on *Bradysia odoriphaga* Yang and Zhang. *Pesticide Biochemistry and Physiology*, 111, 31–37.
- Zhang, T., Reitz, S.R., Wang, H. & Lei, Z. (2015a) Sublethal effects of *Beauveria bassiana* (Ascomycota: Hypocreales) on life table parameters of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Economic Entomology*, 108, 975–985.

- Zhang, Y.X., Sun, L., Lin, G.Y., Lin, J.Z., Chen, X., Ji, J., Zhang, Z. & Saito, Y. (2015b) A novel use of predatory mites for dissemination of fungal pathogen for insect biocontrol: The case of *Amblyseius swirskii* and *Neoseiulus cucumeris* (Phytoseiidae) as vectors of *Beauveria bassiana* against *Diaphorina citri* (Psyllidae). *Systematic and Applied Acarology*, 20, 177–187.
- Zhang, Z.-Q. (2003) *Mites of greenhouses: identification, biology and control*. CABI, Wallingford, UK, 244 pp.
- Zhao, T.Z. (2005) Damage analysis and loss evaluation after *Hyphantria cunea* (Drury)'s invading China (Master's thesis). Beijing Forestry University, Beijing, China.
- Zhou, F., Ali, S. & Huang, Z. (2010) Influence of the entomopathogenic fungus *Isaria fumosorosea* on *Axinoscymnus cardilobus* (Coleoptera : Coccinellidae) under laboratory conditions. *Biocontrol Science and Technology*, 20, 709–722.